UNIVERSITY OF NAIROBI

RAPID IDENTIFICATION OF EDIBLE OILS MANUFACTURED IN KENYA

BY

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DECLARATION

This research thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this thesis to my late daughter, Natasha Muthoni Wangeci for inspiration and strength to accomplish my goals in life (may she rest in peace) and my mother Gladys and sister Beth for their encouragement and motivation to soldier on and aspire more of life.

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ABSTRACT

The aim of this research was to develop a rapid identification method of edible vegetable oils manufactured in Kenya. Vegetable edible oils processed and refined in Kenya have not been characterized and hence it's impossible to distinguish between them. Sunflower, Corn and Soya bean vegetable oils were analysed for physico-chemical parameters, absorbance in Ultraviolet Visible and in Infrared spectroscopy to establish specific fingerprint. Twenty two samples comprising of nine Sunflower of three different brand type, nine Corn oils of three different brand type and four Soya bean oils of two different brand type were collected from various supermarkets in Nairobi. The samples were transported, prepared and stored in the refrigerator at 4°C.

Physico-chemical parameters analysed include Density, Refractive Index, Peroxide Value, Saponification Value and Total Free Fatty Acids. Ultraviolet/visible spectrophotometer at specific wavelengths was used to establish characteristic absorbance of each type of vegetable oil. The Infrared spectroscopy method was subsequently applied to establish specific absorbance characteristic of each vegetable oil at given wavelength.

Sunflower oil was found to have Density of 0.9205±0.0025 g/cm^3, Refractive index of 1.468 nD ±0.001, Saponification Value of 191±3 mgKOH/g, Peroxide Value of were ≤ 10 mmol/kg and Total Free Fatty Acid of ≤ 0.085%. The Sunflower oil Ultraviolet/ Visible gave absorbance peak of 2.779 ±0.183 at 315.0nm and 2.742 ±0.098 at 300nm wavelength. Corn oil had Density of 0.921±0.004 g/cm^3, Refractive index of 1.4665 nD ±0.0015, Saponification Value of 192±5 mgKOH/g, Peroxide Value of were ≤ 10 mmol/kg and Total Free Fatty Acid of ≤ 0.1%. The Ultraviolet/Visible absorbances for Corn oil were 3.019 ±0.082 at 315nm, 3.0725 ±0.0685 at 330nm and 3.034 ±0.181 at 345nm. Soya bean oil had Density of 0.922 ±0.003 g/cm^3, Refractive index of 1.468 nD ±0.002, Saponification Value of 192 ±3 mgKOH/g, Peroxide Value of were ≥ 10 mmol/kg and Total Free Fatty Acid of ≤ 0.176%. Soya bean oil the Ultraviolet/ Visible absorbance were at 3.1325 ±0.1235 at 300nm, 3.101 at 315nm and 3.087±0.048 at 345nm. The three vegetable oils were found to have distinct infrared fingerprints. Corn oil had specific peaks at 1150-1130 cm^{-1} and 1090 cm^{-1}and Soya bean oils had specific peaks of 1100-1090 cm^{-1} and 900 cm^{-1}. Sunflower oil infrared spectra did not exhibit 1150-1130 cm^{-1}, 1100-1090 cm^{-1}and 900 cm^{-1} specific peaks which were found in corn and soya bean oil. A protocol that can be used to distinctively analyse Sunflower, Corn and Soya bean oils was developed that is relatively cheaper both in time and resource and applicable by oil manufacturers.

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LIST OF ABBREVIATION

I.V – Iodine value

S.V- Saponification value

FFA – Free fatty acids

A V – Acid value

MUFA –Mono unsaturated fatty acids

PUFA- Poly-unsaturated fatty acids

KOH- Potassium hydroxide

mg – milligram

IR – infrared spectroscopy

UV/Vis – Ultraviolet/ Visible spectroscopy

LA- Linoleic Acid

GC-FID – Gas Chromatography -Flame Ionization Detector

CHAPTER ONE: INTRODUCTION

1.1: General overview

Kenya's economy largely depends on the agricultural sector, which account for 27% of the Gross Domestic Product (GDP) in 2011. Other than agro-production the sector boasts a comparatively wide range of manufacturing industries, with food processing being the largest single activity. About 66% of manufacturing sector in Kenya is agro based owing to the country's agricultural economy foundation. The sector has grown by 11.5% since 1999 (Ministry of Agriculture, 2009).

Vegetable oil is one of the key sub-sector of agriculture, currently there are about thirty vegetable oil refiners in the country. The larger companies include Bidco Oil Refineries, Kapa Oil Refiniries, Pwani Oil Refineries, Palmac Oil Refineries and Unilever. These companies engage in production of edible cooking oils and fats of about two hundred thousand metric tonnes in the year 2009. Bidco Oil Refineries Ltd maintained its strong leading position in oils and fats in 2014 with a value share of 46%. Kapa Oil Refineries Ltd followed in second position with a 24% value share in 2014, while Unilever Kenya was third position with a 15% value share. Unilever's Blue Band spreadable oils and fats brand is the leading oils and fats brand overall in Kenya in 2014. Bidco's Elianto corn oil brand is the second biggest brand overall with a projected value share of 9% in 2014. Kapa Oil's Rina vegetable and seed oil brand and Prestige margarine brand are set to account for 8% and 7% of total oils and fats retail value sales respectively in 2014.

Some of the large vegetable refiners are also involved in growing of oilseed crops and supporting small farmers in better farming methods to increase the vegetable oil production in Kenya. Edible

vegetable oils are extracted from plant sources or from seeds like corn, sunflower, soya bean, cashew nut, groundnut, palm, coconut, simsim (sesame) and cottonseed. Vegetable edible oils are important in foods and various other industries e.g. cosmetic, pharmaceuticals and lubricants. They are key diet components and also do provide characteristic flavor and texture to food (Moreal *et al*., 1990).

Edible oils play an important role as carriers of essential fatty acids (EFA) and are important in the maintenance of integrity of cell membrane in our bodies. They are also needed for the synthesis of prostaglandins which have many vital functions to perform in the body. There are three methods for extracting vegetable oils. Vitamin E is a powerful antioxidant and vegetable oils are a major dietary source of this vitamin. Each fatty acid also has its own specific properties (De Deckere and Verschuren, 2000). Linoleic acid is a polyunsaturated fatty acid with cholesterol-lowering properties and a-linolenic acid is also linked to heart health. Sunflower oil, corn oil and soybean oil are rich in linoleic acid (LA or Omega 6), another polyunsaturated acid. Lack of it will slow your growth and will lead to transformations in your skin cells, endocrine gland, mucous membrane and sex organs.

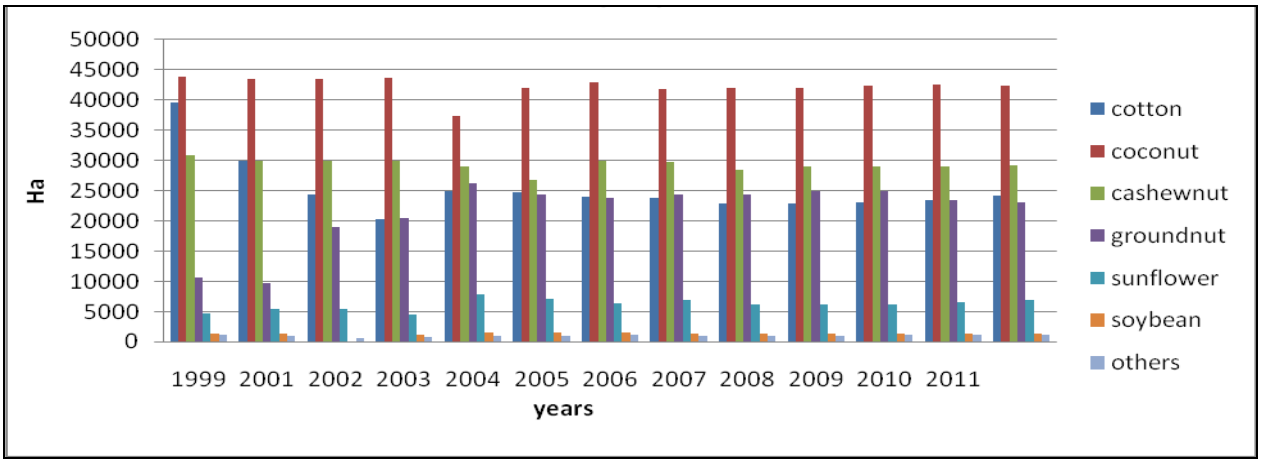
Vegetable oils are generally extracted by distillation, often by using steam. Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, and cold pressing. Extraction and purification operations of oil include heating, distillation and chemical modification which may alter their properties (Cerretai *et al*., 2005). The oil rich part of the plant may be placed under pressure to extract oil, giving expressed oil. Oils may also be extracted from plants by dissolving part of the plants in water or other solvent(s) (Devesh *et al*., 2011). The crude may be separated from the plant material and concentrated, giving extracted or leached oil. The mixture may also be separated by distilling the oil away from the plant material. Vegetable oils often have different

properties and are used more than the pressed or leached vegetable oils (Ministry of Agriculture, 2009).

Consumer demand and their preoccupation for a rational and health diet gave rise to increased consumption of vegetable oils rich in unsaturated fatty acids. This led to an increase in oil imports in Kenya (USDA foreign Agriculture Service, 2009).

1.1.1: Production

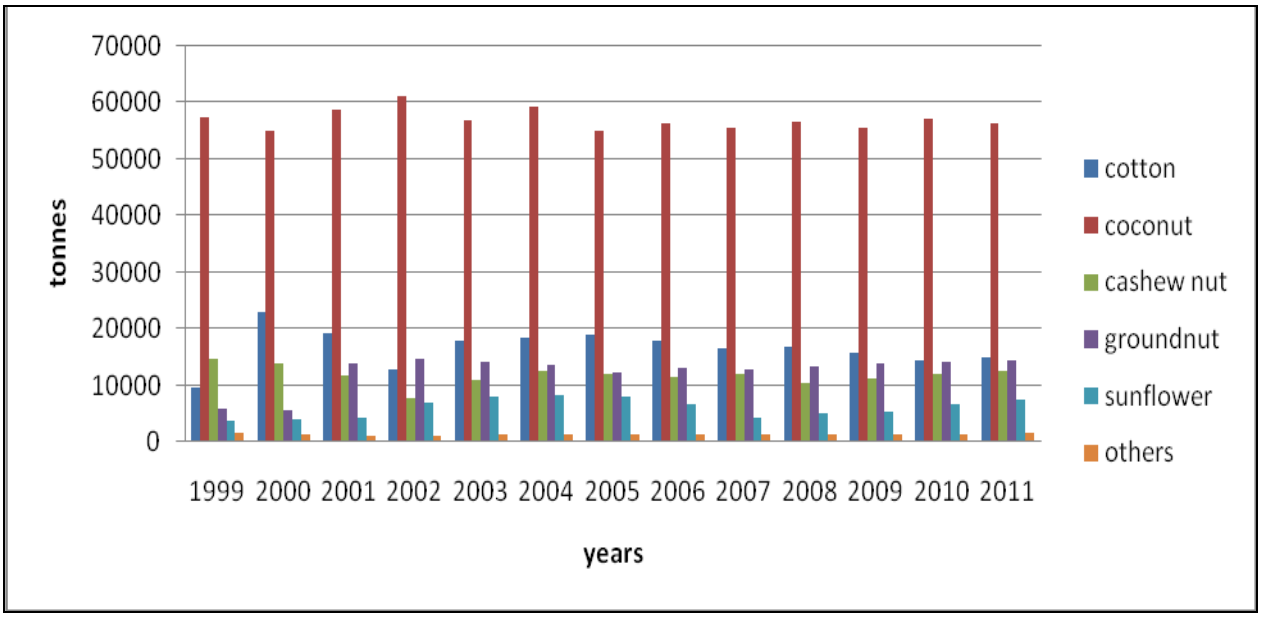
Kenya has increased area under oil crop to one hundred and twenty five thousand hectares and produced just over two hundred thousand tons of oil crops during calendar year 2009. Oil crops include coconut, cashew nut, groundnuts, sunflower, soybeans, palm, simsim (sesame), cottonseed and corn oil (Government of Kenya, 90K). The total area covered by vegetable oil crops has increased from one hundred and twenty thousand, six hundred and sixty seven hectares in 2002 to one hundred and twenty eight thousand, two hundred and one in 2011 as shown in Figure 1.1 (Frank, 1997 and Frank and Aamick, 2004).



Source: Crop Dev Division annual Reports, Ministry Of Agriculture 2009

Figure 1.1: Area under vegetable oil crops in Kenya, 1999-2011 (Ha)

Production of vegetable in Kenya is as shown in Figure 1.2. it indicates that the crop production has remained fairly stable.

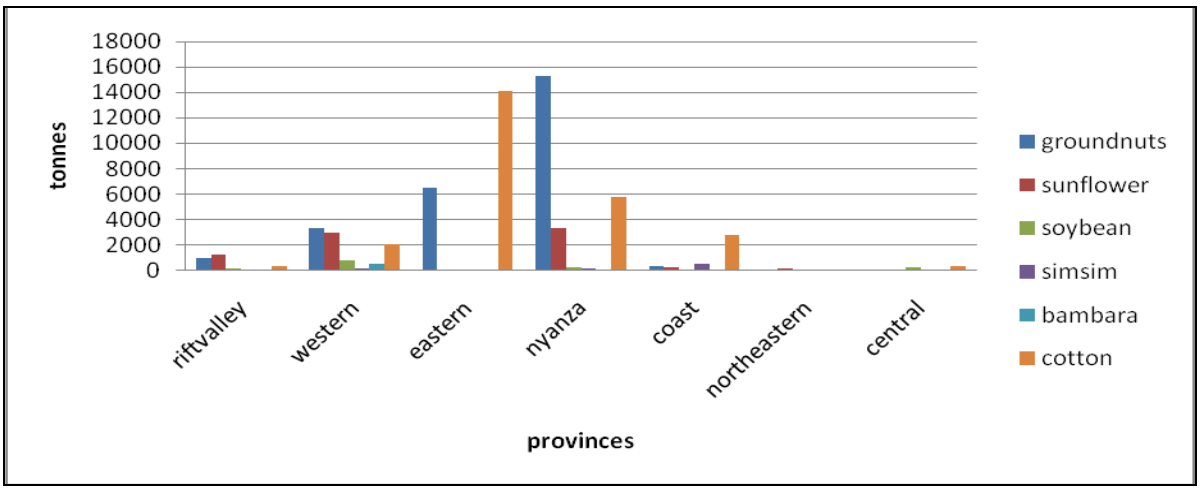


Source: Crop Dev Division annual Reports, Ministry Of Agriculture 2009

Figure 1.2: Production of vegetable oil in Kenya 1999-2011

The local production of oil crops is projected at 20 million metric tonnes by the year 2019, of which over 5 million metric tonnes are expected to come from the Lake Basin region. Cultivation is mostly in four out of eight regions (figure 1.3). Areas under vegetable oil crops remained fairly steady over the years. This has been partly attributed to irregular and unstable weather conditions and inadequate

supply of raw materials hence contributing to increase of import of crude oil (Material safety datasheet, 91/55).



Source: Crop Dev Division annual Reports, Ministry Of Agriculture 2009

Figure 1.3: Area under oil crop production, Kenya 2003 (Ha)

By region, production of oil crops varies with each oil crop. Cultivated area production trend of sunflower and soya bean has remained fairly constant. Further, the Food and Agriculture Organization, (FAO) has initiated farmers-based support since 2003 in oil crop growing areas in western Kenya. They have done this by creating farmer's field school network in conjunction with Kenya Agriculture Research Institute Centre (KARI).

FAO has been exploring the potential of oilseeds in Kenya with industrial producers and its outgrower network of some 60,000 farmers. Processing companies like BIDCO have also actively supported and encouraged local farming of oilseeds. The ministry of agriculture recently began promoting oilseed production by providing seeds through the Kenya Agriculture Research Institute Centre at very modest prices.

Production levels of vegetable oil derivatives such as margarine, pharmaceutical, lubricant and cooking oils have increased during the past 20 years and further increases are expected in the coming years. Annual production and consumption of oils and fats is about 550,000 tones and rose steadily at a rate of 126,000 tons per year (increase of 23%). This requires effort to meet the demand, which also grows at around this rate, partly as a consequence of increasing population (Jaetzold *et al*., 2006).

A growing number of companies have established operations to process vegetable oils in Kenya as indicated in table 1.1

Table 1.1: Vegetable oil processing and refining companies in Kenya

Company name	**Location**	**Activity category**	**Products**
Aberdare oil millers	Nyeri	Miller	Edible oils & fats
Afya Cooking Oil Manufacturers	Kakamega	Refiner	Fats & oils
Arkay Manufacturing plant	Eldoret	Buyers of raw materials	Sunflower, soybean oil
Bidco Oil Refineries Limited	Nairobi	Refiner	Fats, margarine oils, industrial bulk oils
Corn Products Limited	Nairobi	Miller	Corn syrups, corn germ, gluten feed meal, dextrose, dextrin, waxy corn starch
Kapa Oil Refineries Limited	Nairobi	Refiner	Fat, margarine, oils, industrial bulk oils
Kenya Nut Company	Thika	Processor	Macadamia nuts oils
Menegai Oil Refineries Limited	Nakuru	Refiner	Edible oils
Nakuru Oil Mills	Nakuru	Millers	Edible oils

Oil Crop Development Limited	Nakuru	processor	Maize seed, vegetable oil
Oil Extraction Limited	Nakuru	Oil extractor	Edible oils
Palmac Oil Refineries	Nakuru	Refinery	Fats, margarine, oils, industrial bulk oils
Premier Oil Extraction	Nairobi	Extractor	oilseeds
Pwani Valley Product Limited	Mombasa	Refiner	Fats. Margarine, oils, industrial bulk oils
Rift Valley Product Limited	Nakuru	Processor	crude palm oils, sunflower oil, plant extraction
Sansoro Oil Mill	Kisii	Miller and refinery	grains
Unilever Limited	Nairobi	Refiner	Fats, margarine, oils, industrial bulk oils
Voi Industries Limited	Nakuru	Processor	Cotton, sunflower seeds
Western Seed and Grain Company	Kitale	Processor	Seeds and grains

Source: Ministry of Agriculture, 2009

Vegetable Oil Refinery: Processing of crude oil by neutralizing, bleaching, dewaxing , deodorizing, refining, physical & chemical processes are combined to remove undesirable natural as well as environmental- related components from the crude oil (Strecher *et al*., 1996).

Vegetable oil manufacturer: This method, which entails minimal processing, produces edible oil with characteristics that consumers desire such as bland flavour and odour, clear appearance, light colour, stability to oxidation and suitability for cooking and frying needs.

Vegetable oil processer: This involves removal of natural oil from the seeds and creates a final product which oxidizes easily which can be done by highly intensive mechanical and chemical processes to extract the oil from the seeds.

1.1.2: Quality of vegetable oils

To acquire the quality of oil, use certain parameters are used by the food industries to establish the quality of their products. Several factors affect the edible oil quality such as agronomic techniques, seasonal conditions, ripening stage, harvesting and carriage systems, storage and processing method. This is determined by different analytical methods in order to access the stability of oil and to avoid adulterations. Free fatty acids content is one of the most frequently used parameter to assess the quality of oil during oil production, storage and marketing and it is also used to classify the oils (Oil World Annual report, 1999). Other important parameter to consider in oil analysis is the peroxide index. Peroxide Index is indicator of oxidative rancidity in foods. These are the primary oxidation products used as indicators of oil quality and stability (Orthoefer *et al*., 1987).

Physico-chemical parameters of edible oils; density, peroxide value, saponification value, iodine value, free fatty acids content are based on wet chemistry methods but are time consuming and laborious requiring use of solvents in large quantities which may be a potential environmental hazards.

The vegetable oil manufacturers use World Health Organization (WHO) guidelines, CODEX standard and Kenya Bureau of Standard (KEBS) as standard indicated in appendices 2, 3 and 4.

Kenya Oil refiners and manufacturers used physico-chemical parameters to meet the quality requirements. Protection against misleading and false description of vegetable oil is regulated by the Kenya Bureau of Standard (KEBS).

Research to develop versatile analytical methods on the manufacturer needed is a continuous process.

The processing of oils is a highly capital intensive venture, require advanced technology and specialized skills. The refining process produces oil which is used mostly for cooking. Most of the large refineries are situated in Nairobi and Mombasa Cities, Kenya.

1.1.3: Market

A large percent of oil manufactured in in Kenya is marketed locally. Oils and fats is expected to increase in value by 4% in constant 2014 terms over the forecast period. With the increasing use of publicity campaigns raising awareness about the health issues surrounding the use of oils and fats, vegetable and seed oils is bound to continue increasing in popularity throughout the forecast period. Growth in healthier types of oils and fats is expected to offset the declines expected in consumption of oils and fats which are perceived as being less healthy during the forecast period. These changes are expected to emerge gradually as the majority of the Kenyan populations are low-income consumers with little choice about the types of oils and fats they use (Jaetzoid *et al*., 2006).

High prices of vegetable oils like olive oil cause producers and traders to resort to partial or total substitution of these with other cheaper oils (sunflower, soybean and corn oil) (USDA foreign Agriculture Service, 2009).Kenya is making efforts to satisfy the local demand for various oil crops, and inspite of unreliable climatic conditions in recent years, production has remained steady, with commendable growth in some of three crops.

1.1.3.1: Imports

Kenya imports vegetable oils in order to supplement its local production, which is presently inadequate to meet local demand. Vegetable oils imports from Kenya experienced a drop in the 2004 however the imports picked up again from 2005 onward increasing to 700000 metric tons in 2012. This increase was mainly due to expansion to markets in Uganda and Tanzania, among other countries. The trend of domestic imports is detailed in the Figure 1.4.

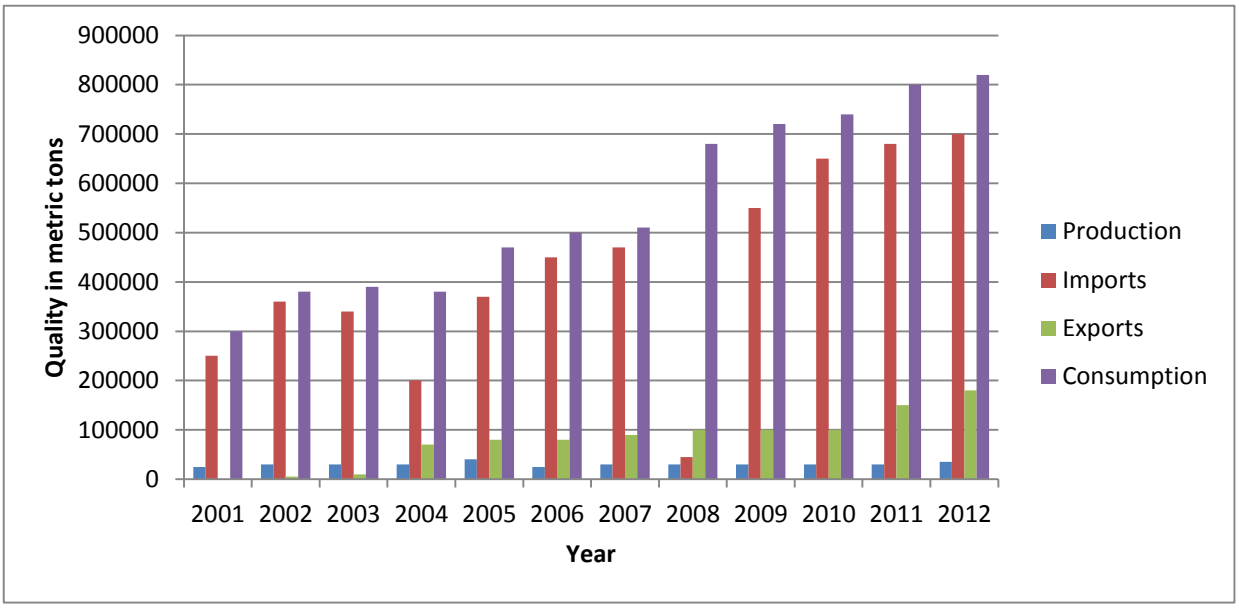
Kenya refiners will likely increase import of crude vegetable oils, because Malaysia and other suppliers sell into the world market at relatively low prices vis-a-vis United State and other European

countries. Malaysia provides over 90% of Kenya's vegetable oil import. Brazil, Indonesia, Singapore, India and Italy supply the remaining balance. Soybean oil from the U.S costs $950 per ton compared to $753 from `Malaysia which keeps U.S exports out of our market. (Ministry of Agriculture, 2009) Kenya appears to have increased its vegetable oil consumptions to about 8 billion Kshs since 1999. Every year, Kenya uses Sh50 billion to import 600,000 metric tonnes of vegetable oil.

1.1.3.2: Exports

Kenya exports processed vegetable oil to East and Horn of African countries, as well as Europe and United State of America (U.S.A). Kenya is ranked 15th in World Exports of vegetable oils (Material safety datasheet, 91/55).

The COMESA region is the principal market of Kenya's vegetable oil products; these include Uganda, Tanzania, Zimbabwe, Zambia, Zaire, Rwanda and Burundi. One of the leading companies in Kenya, Bidco recently opened processing plants in Uganda to further increase and develops their market in the region. Kenya's key export value destinations for oilseeds include Netherlands, the UK, and Germany where export values stood at Kshs 10million, 6million and 1.4 million respectively which lead to increase of vegetable oil exports(Export processing zones authority under Ministry of Industrialization and Enterprise Development).



Sources: Ministry of Agriculture, Kenya National Bureau of Statistics, Global Trade Atlas, and FAS estimates.

Figure 1.4: Vegetable Oil Production, Consumption and Trade

1.1.4: Uses

Oils are used for both food applications and industrial uses. They are consumed in butter, shortening, margarine, salad oils, and cooking oils, as well as in animal feeds, fatty acids, soaps, personal care products, biodiesel, paints (made from alkyd resins), lubricants, and greases (Prota foundation, 2008). Food applications account for the major share (about three-fourths) of the Kenya consumption of fats and oils. However, there has been a continued shift from food to industrial consumption, particularly in biodiesel. Around 14% of current oil production is used as starting material in manufacturing industry and around 6% are used as animal feed (indirectly) as shown in figure 1.5. The remaining 80% is used for human food preparation as spreads, frying oils, salads and cooking oils.

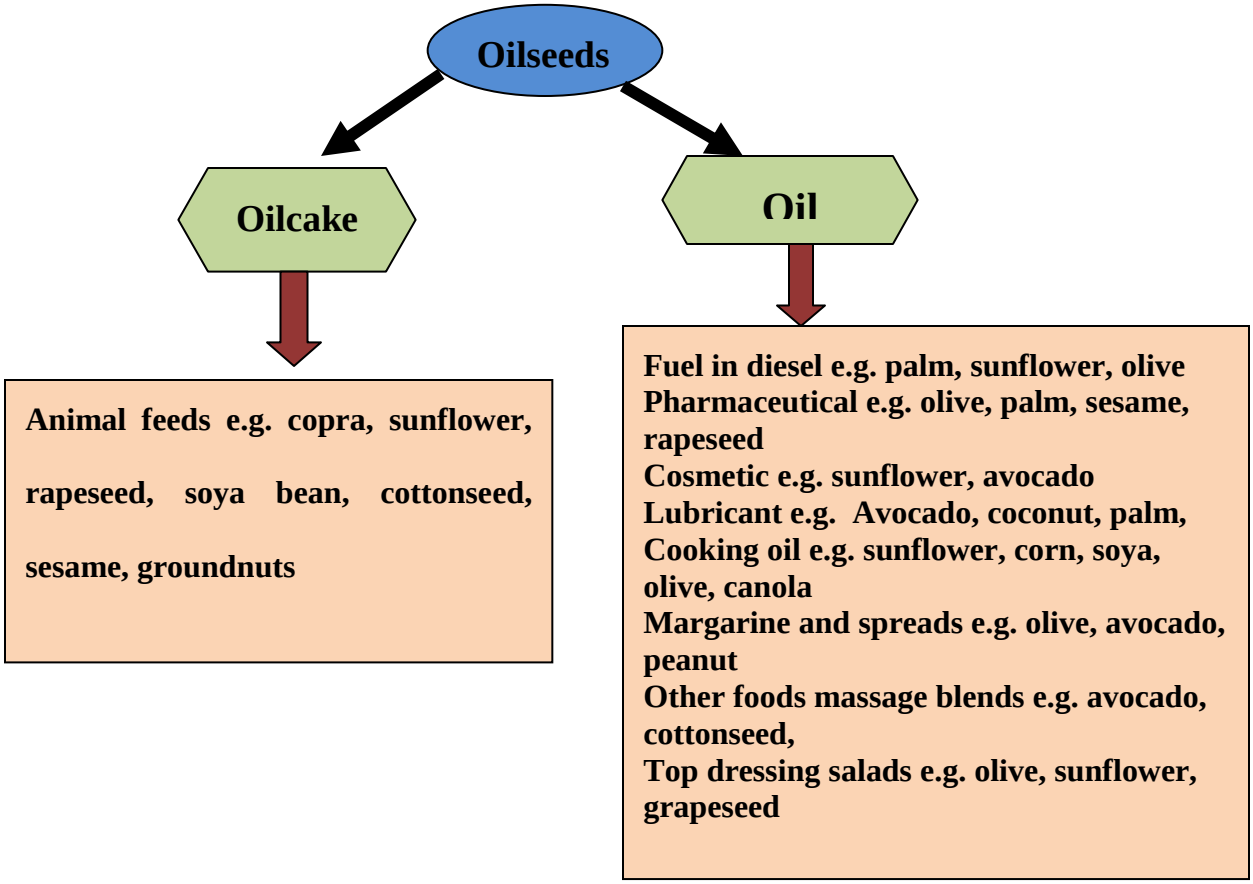


Figure 1.5: Uses of vegetable oils in Kenya

1.2: Problem statement

Oil manufacturing companies in Kenya do not categorically identify their products scientifically. There lacks data to support the oil products quality and one is not able to clearly state the type of oil being offered in the market.

Physico-chemical parameters data does not differentiate sunflower oil from corn oil or soya bean oils. The advance methods available in research and high end laboratories such as Gas chromatography – Mass spectroscopy is not only expensive, lengthy but also not accessible to most manufacturers or routine analysis in a quality control laboratory.

Authentication of vegetable oils can be carried out by a variety of classical physico-chemical methods. Use of physico-chemical methods only may lead to misrepresentation and likely have the vegetable oils to escape detection. Physico-chemical parameters do not distinguish between the oils.

Gas chromatography –mass spectroscopy/ FID used to identify and differentiate vegetable edible oils cannot be the manufacture's choice of analysis because it's expensive, preparation of the sampler for analysis is time consuming, reagents used are expensive and not readily available.

1.3: Justification of the study

Science and technology have played an important role in the vegetable oil industries in ensuring the society's need for safe and good quality edible vegetable oil. Commercial malpractices may include edible vegetable oils adulteration, whereby some of the ingredients that do not comply with standard requirements are added. Some of the manufacturers or vendors use substandard raw material in their products because they are relatively cheap and easily available. The manufacturers of edible vegetable oil are mostly interested and focused in their financial gain than the standard quality of their products. Hence they do not install advanced analytical methods for qualitative and quantitative analysis for their edible vegetable oils.

This project envisaged to provide a protocol that is rapid, in expensive for analysis and identification of vegetable oils in Kenya.

1.4: Objectives

1.4.1: Main objective;

- ➢ Establish a rapid method for identification of sunflower, corn and soya bean oils manufactured in Kenya.

1.4.2: Specific objectives;

i. Determine the physico-chemical parameters i.e. density, refractive index, saponification value, peroxide value and free fatty acid values for sunflower oil, corn oil and soya bean oil.

ii. Identify absorption peaks in the Ultraviolet / Visible region for sunflower oil, corn oil and soya bean oil.

iii. Identify IR fingerprint regions of sunflower oil, corn oil and soya bean oil manufactured in Kenya.

iv. Developing an analytical protocol for analysis of the three edible vegetable oils.

CHAPTER TWO

2.1: Literature review

Physico-chemical properties are important in determining the overall quality and stability of the oils. A number of factors have been reported to affect oil quality and include pre-processed factors such as growing season, soil fertility, post-harvest storage conditions such as temperature and post process factors such as heat-thermal degradation and air contact (Turner, 2010). A number of researchers have highlighted other factors affecting quality of oil. The quality of vegetable oil has also been reported to be dictated by several physical and chemical parameters that are dependent on the source of oil, processing and storage conditions (Shahidi and Spurvey, 1996). Furthermore, some of the parameters used to evaluate the quality of the oils according to (Chabiri *et al.*, 2009) has been outlined and include moisture content, smoke point, saponification value, acid value, iodide value and peroxide value among other parameters. Edible vegetable oils are "food stuff" which is composed primarily of glycerides of fatty acids being obtained only from the vegetable sources (Codex, 1999). They may contain small amount of other lipids such as phosphatides of unsaponifiable constituents and of free fatty acids naturally present in the oil (Downey and Davies, 2002).

The common vegetable edible oils manufactured Kenya include sunflower oil, corn oil and soya bean. These were the vegetable edible oils considered in this project.

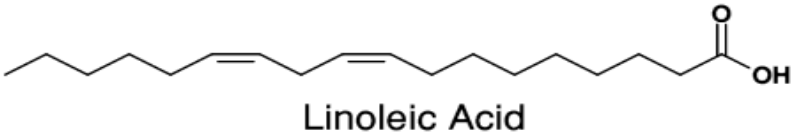
2.2: Sunflower oil

Sunflowers are botanically classified as *Helianthus annuls*. They are a large plant and are grown throughout the world because of their relatively short growing season. Domesticated sunflowers typically have a single stalk topped by a large flower. This is significantly different from the smaller, multiply branched wild sunflower (Ozdemir and Ozturk, 2007). During the growing period, the individual flowers are each pollinated. Seed development then begins moving from the outer rim of the flower toward the center. It generally takes 30 days after the last flower is pollinated for the plant to mature. Sunflower plants reach various heights, but most are from 1.52–2.1 m tall. The diameter of the flower heads is relatively large, typically between 7.62 and 15.24 cm, although some can measure more than 30 cm. An exception is the dwarf varieties, which are only 0.91–1.22 m high and have smaller flower heads. A common characteristic of sunflower is a tendency for their flowering heads to follow the movement of the sun during the day. This phenomenon, called heliotropism, has the benefit of reducing damage from birds and preventing the development of disease.

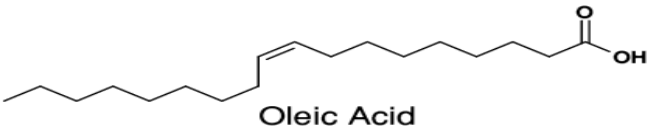
Figure 2.1: Sunflower plant and seeds

2.2.1: Oil Composition

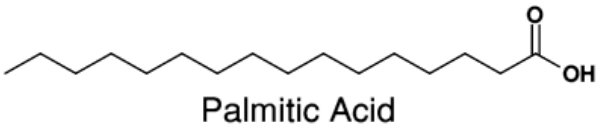
Sunflower oil contains predominantly linoleic (48–7%), oleic (14–40%), palmitic (4–9%) and stearitic acids (1–7%). There are several types of sunflower oils produced, classified in terms of their acids content i.e. high linoleic, high oleic and mid oleic content. High linoleic sunflower oil typically has at least 69% linoleic acid. High oleic sunflower oil has at least 82% oleic acid.



Linoleic Acid



Oleic Acid



Palmitic Acid

The variation in the unsaturated fatty acids profile is strongly influenced by both genetics and climate. In the last decade, high stearic of sunflower oil have been developed to avoid the use of hydrogenated vegetable oils in the food industry. The conventional sunflower oil (high linoleic) is used for home cooking oil, margarine and for industrial use such as in paint industry. The high oleic sunflower oil is used for cosmetics and gasoline blend and other purposes (Wrolstad *et al*., 2005). Sunflower oil also contains lecithin, tocopherols, carotenoids and waxes. Sunflower oil's properties are typical of vegetable triglyceride oil. It is light in

taste, appearance and has high vitamin E content. The refined oil is clear and slightly amber-coloured with a slightly fatty odour.

Contents from open pollinated and hybrid sunflowers based on a study conducted by the United States Department of Agriculture

Table 2.1: Sunflower oil composition

Content	Yield
Oil	44 – 51%
Protein	17 – 19%
Hull	20 – 22%
Fiber residue	15 – 20%
Ash	0.4%

Source: USDA foreign Agriculture Services, Gain report Global Agriculture Information Network, 2009

2.2.2: Benefits

Some of the health benefits of sunflower oil include its ability to improve heart health, boost energy, strengthen the immune system, improve your skin health, prevent cancer, lower cholesterol, protect against asthma, and reduce inflammation (Dorrell and Vicky, 1997).

- **Heart Health:** Sunflower oil is rich in Vitamin E and low in saturated fat. Besides, it is rich in certain phytochemicals like choline and phenolic acid that are beneficial for your heart. It also contains monounsaturated and polyunsaturated fats along with vitamins that makes it one of the healthiest oils for consumption. High oleic sunflower oils contain 80% or more monounsaturated fats hence helps in lowering cardiovascular diseases and chance of heart attack.

- **Skin Health:** Sunflower oil is rich in vitamin E, which acts as an antioxidant in the body. It directly connected to preventing heart disease and boosting your immune system. Vitamin E is related to improving skin health and regenerating cells. The skin is better protected against damage from the sun, as well as the natural degradation of age that occurs when free radicals are present in the body. Antioxidants like vitamin E neutralize free radicals, keeping them from destroying or damaging healthy cells. This is why sunflower oil is commonly used in cosmetic applications. Sunflower oil also contains proteins, which are vital for building and repairing tissues and the production of hormones and enzymes. Our body requires high amounts of proteins. Since the body does not store proteins, they have to be consumed, and sunflower oil fulfils this requirement.
- **Energy Booster:** The fatty acid content in sunflower oil is also connected to energy levels in the body. Saturated fats can make you feel sluggish, while unsaturated fats, of which sunflower oil has many, can keep one feeling energized.

- **Cancer Prevention:** Sunflower oil is rich in antioxidants and substances that act as antioxidants. Vitamin E, which has varieties known as tocopherols which are powerful antioxidants that can eliminate free radicals before they can mutate healthy cells into cancerous cells. The carotenoids found in sunflower oil help in the prevention of uterine, lung and skin cancers. In addition, they provide a good source of Vitamin A and aid in preventing cataracts.
- Anti-Inflammatory Capacity: Sunflower oil has been correlated with a lower amount and severity of asthma attacks because of its anti-inflammatory qualities, which are derived from its vitamin content, as well as the beneficial fatty acids sunflower oil contains. Along with

asthma, sunflower oil has also been linked to a reduction in severity of arthritis, which is an inflammatory disease (Downey and Davies, 2002).

- **Immune System Health:** Sunflower oil protects the skin by strengthening the membrane barriers, thereby making it harder for bacteria and viruses to enter the body. In infants, sunflower oil is highly recommended because it can protect the babies from infections, particularly when they are born premature and are highly susceptible to infections. This same benefit is extended to adults.

2.3: Corn oil

2.3.1: Composition

Unlike many vegetable oils, corn oil (maize oil) is obtained from seeds (kernel). The germ, which constitutes 8-14% of the total weight of the corn grain, contains 84-86% of the total oil content of the corn. The germ is the primary product for making corn oil and one of the most important ingredients of animal feed (Orthoefer *et al*., 1987). The germ oil contains unsaturated fatty acids (linoleic 56%, oleic 30%, linolenic 0.7%) and 14% of saturated fatty acids. Its protein part contains about 37% globulins, 51% glutelin, 5% zein, and 7% of non-soluble oils. Maize germ also carries a wide range of amino acids and is rich in vitamin E (tocopherol), beta-sitostiron and phytin (Moreal *et al*., 1990). Production of corn oil is costly due to low level of oils in the kernel. A process of wet milling from corn was developed to isolate pure starch efficiently from corn kernels (List *et al*., 1977). Oil is usually obtained from dry milled corn germ by applying pressure.

Figure 2.2: Corn plant and seeds

2.3.2: Benefits

- **Cholesterol Lowering Effect:** Corn oil is good for health of cardovascular system because it's rich in linoelic acids and other PUFAs (polyunsaturated fatty acids). PUFAs also have LDL cholesterol lowering activity. This contributes to the earlier cholesterol lowering effect caused by phytosterols (Lowell, 2006).

- **High Blood Pressure**: Corn oil specifically is able to lower blood pressure levels by about 10 % because it contains polyunsaturated fatty acid that lowers high blood pressure.

- **Skin**: Corn oil can be applied on skin as massage oil. It improves skin functioning because of the presence of linoleic acid and vitamin E in it. Corn oil is a gentle oil and one can use it as base oil for household products like lip balms, salves, creams and night oils. Corn oil is about 59 % linoleic acid and because of it, this oil penetrates quickly into the skin (Chabiri *et al*., 2009)

- **Hair**: Corn oil is used as a hot oil treatment. It helps dry and undernourished hair. It conditions the hair and makes it smooth.

- **Pets**: Corn oil is also used on animals. It is gentle and great for their skin care. Massaging corn oil on a dog's hair makes the coat healthier. Corn oil is also fed to horses to treat dull coat conditions.

2.4: Soya bean oil

The shape of the soya bean seed varies from almost spherical to elongated. The industrial varieties grown for oil production are nearly spherical while the elongated varieties are used as vegetable oil directly (Woerfel, 1995). The colour of the seed may be yellow, green, brown or black. Industrial varieties are yellow and the presence of seeds of other colours in a lot is considered a defect. Seed size is expressed as the number of seeds per unit volume or weight. Industrial soya-beans weigh 18-20 grams per 100 beans. The seeds of "vegetable" varieties are considerably larger.

2.4.1: Composition

Seed structure consists of the seed coat (hull) and two cotyledons, plus two additional structures of lesser weight: the hypocotyl and plumule. The cotyledon represents 90% of the seed weight and contains practically all the oil and protein in its palisade-like cells. Microscopic examination of these cells reveals the presence of protein bodies (also known as aleuron grains) and lipid bodies (or spherosomes) which constitute storage bodies for proteins and oil, respectively. Protein bodies measure, on the average, 10 microns while the lipid bodies have, typically, 0.2 to 0.5 microns in diameter.

Figure 2.3: Soya bean plant and seeds

The hull, which accounts for roughly 8% of the seed weight, holds the two cotyledons together and provides an effective protective layer. It can be removed from the seed by cracking followed by aspiration, as in the process of mechanical de-hulling prior to solvent extraction.

It consists primarily of neutral lipids, which include tri- , di- and monoacylglycerols, free fatty acids and polar lipids such as phospholipids (Frank, 2002). It contains a minor amount of unsaponificable matter that includes phytosterols and tocopherols. The composition of soya beans may vary somewhat according to variety and growing conditions. Through plant breeding it has been possible to obtain protein levels of between 40% and 45%, and lipid levels of between 18 and 20%. Usually, an increase of 1% in protein content is accompanied by a decrease of 0.5% in oil. Incidentally, this negative correlation between protein and oil is one of reasons for the lack of interest in high-protein varieties of soya bean since the production of these varieties does not result in increased income per hectare cultivated (El-abassy *et al*., 2009).

Soya bean oil has a high content of linoleic acids and a lower level of linolenic acid. Triacyglycerols (TAG) are primarily neutral lipids in soya bean oil. Due to high concentration of unsaturated fatty acid in soya bean oil, nearly all the TAG molecules contain at least two unsaturated fatty acids, and di- and tri- saturated are essensially absent (List *et al*., 1977).

2.4.2: Benefits

- **Cholesterol Control:** Omega-3 fatty acids in soya bean oil can reduce dangerous cholesterol levels and counteract the negative types. The fatty acid composition as well as the powerful plant sterols, such as β-sitosterol can actually cause a reduction in cholesterol storage in the gut by 10-15%. Basically, soya bean oil can seriously decrease you chance of atherosclerosis and other heart conditions, such as heart attacks and strokes.

- **Cognitive Impact:** Alzheimer's disease is a terrible affliction that affects millions of people around the world. It results in the cognitive deterioration of a person's brain as neural connection fail and die, thereby making everything from remembering the past to performing simple tasks a challenge. Soya bean oil has an impressively high level of vitamin K, which has been consistently connected with improving the symptoms of Alzheimer's, and even reversing the effects in some cases. The vitamin K acts as an antioxidant against free radicals, keeping them from damaging the neural cells (Frank, 2002).

- **Bone Health:** Another important function of vitamin K is its osteotrophic potential, which means that it can stimulate the regrowth or increased healing of bone. While this is often associated with calcium, vitamin K, of which soya bean oil has a lot, can also stimulate bone development in a very positive way, so make sure to switch to soya bean oil if you want to

prevent certain conditions like osteoporosis, which is often a natural result of the aging process.

- **Eye and Skin Health:** Omega-3 fatty acids, which make up 7% of the total fatty acid content in soybean oil, are integral to protecting the cardiovascular system in the role of scraping our "bad" cholesterol, but it also protects cell membranes. This includes the very fragile and dangerous areas of the skin and eyes, both of which are common entrance points for bacteria and other foreign materials. These omega-3s also promote healthier vision by acting as antioxidants and neutralizing free radicals that can cause macular degeneration and cataracts.

- **Antioxidant Potential:** The high vitamin E content in soybean oil also acts as a powerful antioxidant while similarly protecting the skin from the damage of free radicals. Vitamin E is directly associated with improving the appearance of blemishes, reducing acne scarring, protecting the skin against sunburn, and stimulating the regrowth of new skin cells to promote healing. Vitamin E is also associated with general antioxidant activity in the rest of the body, which boosts the immune system and helps to eliminate free radicals that cause certain conditions like cancer, premature aging, cognitive disorders, and heart diseases.

2.5: Physical and chemical properties of edible oils

The physical properties of oil depend upon its chemicals composition. Table 2.2 below gives a summary of edible oil parameters that are important in assessing quality (Wood, 1978 ; Moreal *et al*., 1990).

Table 2.2: Quality parameters for edible oils

PARAMETER	UNITS
Fatty acid composition and distribution	%
Relative density (20° C or 40°C)	Kg/m^3
Relative index (40°C)	(n_D^{25})
viscosity (25°C)	centiposes
color	Visual, lovibond or colorimeter
Turbidity	Visual or instrumental
Odor and taste	Sensory evaluation
Iodine value (IV)	g iodine/ 100g sample (WiJs method)
Saponification value	mg KOH/g
Acid value (AV)	Mg KOH/g
Peroxide value (PV)	Meq oxygen/100g sample
Volatile matter (105°C)	%
Specific heat at (19.7 °C)	Cal/g
Heat of combustion	Cal/g
Smoke point/ flash point/ fire point	°C

Density, saponification value, iodine value, acid value and peroxide value are some of the important parameters in oil chemistry.

2.5.1: Physical properties of edible oils

2.5.1.1: Color and appearance

Most vegetable oils are yellow –red and amber liquids t room temperature. The color is from the presence of chlorophylls and carotenoids which may be removed during the blenching process; often lighter color has been associated with better quality oils,

especially for salads and shortenings. The presence of chlorophyll not only renders a green color to products, but also acts as sensitizers for oil oxidation. Carotenoids are present in edible oils at different quantities levels (Barbara, 2004).

2.5.1.2: Density (ρ)

Density is defined as the mass per unit volume of a substance (Equation 2.1), and it is a physical property of matter (Paquot, 1979). A physical property can be measured without changing the chemical identity of the substance.

$$\text{Density} = \text{mass} / \text{volume} \quad \text{.....Equation 2.1}$$

The unit of density is expressed as g/ml or $g/cm^{-3}$for liquid according to AOAC (2002). It is carried out using a pycnometer at the temperature of 298.15 ± 0.05K, the pycnometer of capacity of $25cm^3$ being calibrated with water. The relative error in the density, $đρ / ρ$, was estimated by the expression in Equation 2.2

$$đ\rho / \rho = đ m/ m + đ m°/ m° + đ m \rho / m \rho \quad \text{.....Equation 2.2}$$

Where m, m° and m_p are the mass of the pycnometer when filled with oil, water and air, respectively and $đ m = đ m_o = đ m_p = 0.0001g$ are the uncertainties. The relative error in the density of water, $đ \rho_o / \rho$, was considered to be a negligible quality in comparison with others.

Density decreases lineally with increases in temperature: $\rho = b + mT$

Where ρ is density, T is temperature, b and m are constants. These constants are different for different oils. A widely used method for density predication of vegetable oil was

developed by Lund and discussed by Halvorsen and co-workers (1993). The Lund relationship (equation2.3) is

$$\text{Specific gravity} = 0.8475 + 0.00030\,SV + 0.00014\,IV \quad \text{.....Equation 2.3}$$

SV - saponification number

IV- iodide value

A generalized method of estimated density, which was developed by Rodenbush and co-workers was also extended to predict oil viscosity, thereby relating these two key physical properties (Oremusova and Vojtekova, 2005).

2.5.1.3: The refractive index.

Refractive index relates molecular weight, fatty acid length, degree of unsaturation, and degree of conjugation. A mathematical relationship between refractive index and iodine value (IV) has been described in Equation 2.4 .

$$n_D^{25} = 1.45765 + 0.0001164IV \quad \text{.....Equation 2.4}$$

In optics the refractive index or index of refraction n of a substance (optical medium) is a dimensionless number that describes how light, or any other radiation, propagates through that medium. It is defined as Equation 2.5

$$n=frac\{c\}/\{v\}, \quad \text{.....Equation 2.5}$$

Where c is the speed of light in a vacuum and v is the speed of light in the substance. For example, the refractive index of water is 1.33, meaning that light travels 1.33 times slower in water than it does in vacuum as in Figure 2:4

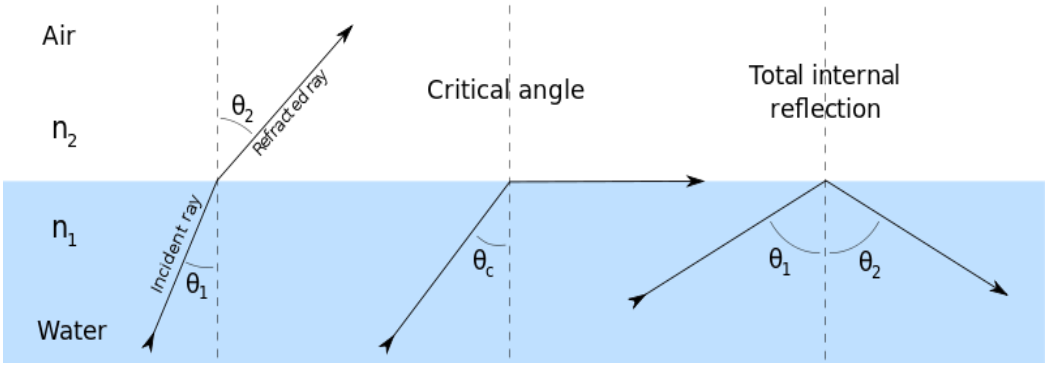


Figure 2.4: Typical values for different materials

The historically first occurrence of the refractive index was in Snell's law of refraction, $n_1 \sin\theta_1 = n_2 \sin\theta_2$, where θ_1 and θ_2 are the angles of incidence of a ray crossing the interface between two media with refractive indices n_1 and n_2 (Lide, 1991).

Instrumentation

The relative index of the sample is measured at 298±0.05k with a Carl Zeiss Abbe refractometer (32-G 110e) (figure 2.5) with a precision of 1x 10^{-4} at the wavelength of 589nm.

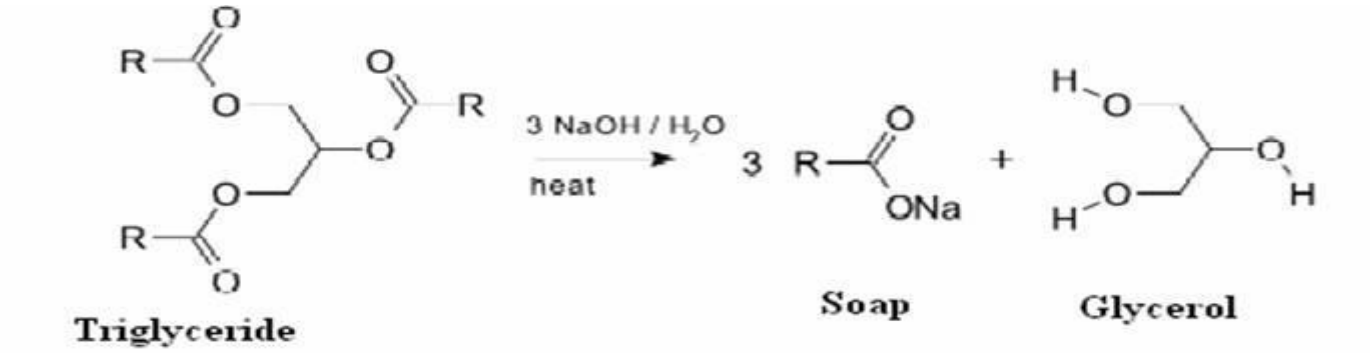
Source: High tech pharmaceutical Limited

Figure 2.5: Carl Zeiss Abbe refractometer

2.5.2: Chemical properties

2.5.2.1: Saponification number:

Saponification is the hydrolysis of fats or oils under basic conditions to afford glycerol and the salt of the corresponding fatty acid.



It is important to the vegetable oil manufacture to know the amount of free fatty acid present, since this determines to a great extent in large measure the refining loss. The amount of free fatty acid is estimated by determining the quantity of alkali that must be added to the fat to render it neutral. This is done by warming a known amount of the fat with strong aqueous caustic soda solution, which converts the free fatty acid into soap (Mowlah *et al*., 1990).

The saponification number is the number of milligrams of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat (Equation 2.6). It gives information concerning the character of the fatty acids of the fat- the longer the carbon chain; the less acid is liberated per gram of fat hydrolysed (Chindo *et al.*, 2010). It is also considered as a measure of the average molecular weight (or chain length) of all the fatty acids present. The long chain fatty acids found in fats have low saponification value because they have relatively fewer number of carboxylic

functional groups per unit mass of the fat and therefore high molecular weight (European pharmacopoeia, 2004).

$$\mathbf{I_s} = \frac{28.08\,(n_2 - n_1)}{m} \quad \text{……Equation 2.6}$$

I_S – Saponification value

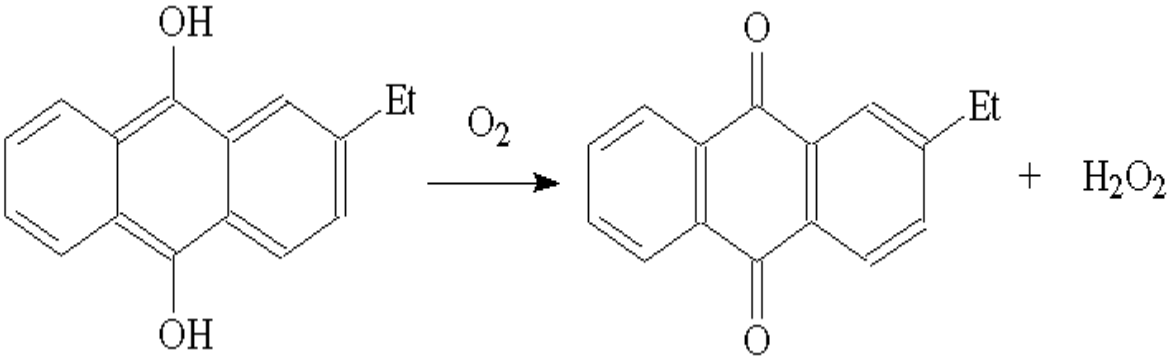
n_1 – ml of 0.5M hydrochloric acid

n_2 – ml of 0.5M hydrochloric acid (blank)

m- mass of the sample

2.5.2.2: Peroxide value:

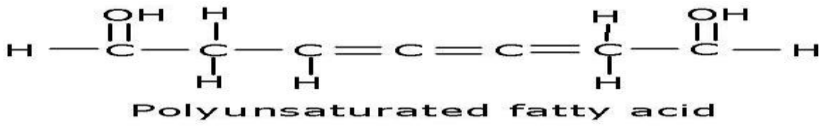
Peroxide value is the most commonly used measurement of lipid oxidation. The peroxide Ip is the number that expresses in milli equivalents of active oxygen the quality of peroxide contained in 1000g of the substance (European pharmacopoeia, 2004).



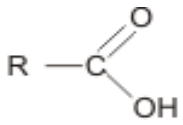
The peroxide value is a useful measure for samples with low levels of oxidation and when the hydroperoxides are not decomposed (Marfil *et al*., 2011). During prolonged oxidation, a maximum peroxide value of 10 mmol/kg is reached (Kamau and Nanua, 2008).

2.5.2.3: Free fatty acids:

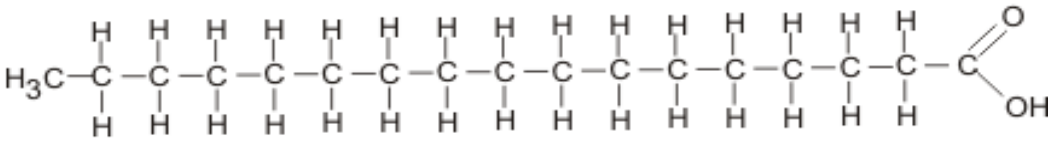
Fatty acids are aliphatic monocarboxylic acids derived from or contained in esterified form. Long-chain fatty acids (LC-FA) are organic compounds in which the hydrocarbon chain length may vary from 10 to 30 carbons (Markley, 1990). The hydrocarbon chain can be saturated or unsaturated (contains one or more double bonds). Based on the number of double bonds, unsaturated fatty acids are classified into the following group:(i) monounsaturated fatty acids (monoenoic acids, MUFA), containing one double bond, for example, oleic acid, (ii) polyunsaturated fatty acids (polyenoic acids, PUFA), having two or more double bonds, for example,γ-linolenic acid,(iii)eicosanoids, which are derived from polyenoic fatty acids, for example, prostaglandins (Eder, 1995).



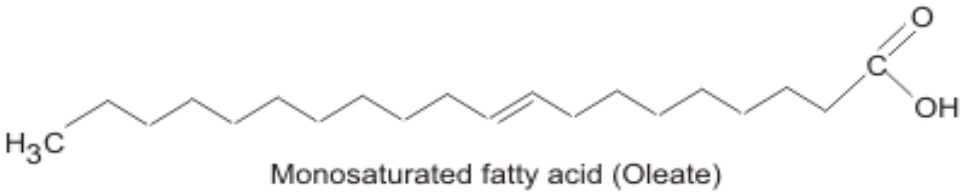
Polyunsaturated fatty acid



Fatty Acid - General formular



Saturated Fatty Acid (Palmitate)



Monosaturated fatty acid (Oleate)

Fatty Acids F.B. 2009

2.6: Instrumentation

2.6.1: Gas chromatography

2.6.1.1: Principle

Chromatographic techniques for the detection and identification of fatty acids in oils have undergone major changes in recent years due to improvements of analysis time, detection limit and separation characteristics. GC analysis of fatty acids is the first chromatographic method known in fatty acids analysis for 60 years (S. Casal and B. Oliveira, 2010). GC for analysis for fatty acid methyl esters (FAME) are the most convenient and accurate method. Gas chromatography is used to simultaneous determination of total amounts of triclyglycerides, diacylycerides, monoacylgycerides and fatty acid methyl ester in alcoholysis of different oil. In gas chromatography of fatty acids, a variety of columns with different properties such as polar and nonpolar are available. Of all the most frequently applied in fatty acids analyses are the polar columns. The fused silica columns allow improvements in the separation of PUFA, for example, from fish oil samples. Another type of stationary phases used for fatty acids analysis is polar polyesters such as Carbowax column, for example, PEG (polyethylene glycol) which is commercially available as Carbowax-20 M, CP-Wax 52CB, DB-Wax. Next example of polar stationary phase used in GC is cyanopropyl polysiloxane such as commercially available HP-88, CP-Sil88, BPX70, SP-2340, and SP-2560. As it was described in Casal and Oliveira report, the high polarity of the cyanosilicone phases allows the separation of geometrical isomers (cis and trans) of mono- and polyunsaturated fatty acids. Among them the most popular is Chromosorb series (Eder, 1995). Others, which belong to polar stationary phases, are organosilicon polyesters (EGSS-X), butanediol succinate (BDS), and diethylene glycol

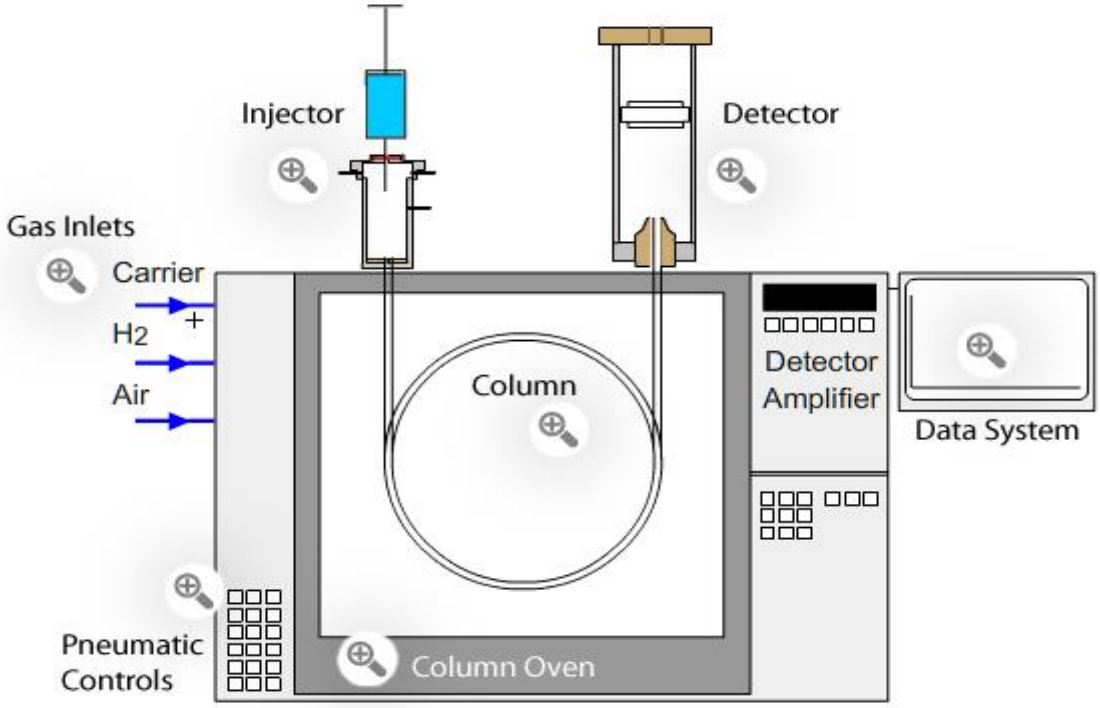
succinate (DECS) (J. L. Guil-Guerrero *et al* , 2003). Conventional GC for analysis for FAME uses a slightly polar column using polyglycol carbowax-20 because natural oils have majority cis form of double bonds. Separation of trans/cis configuration requires more polar stationary phase including SP-2560, SP-2340, OV-275, BPX-70 or CP-SIL-88 using highly polar cyanosilicone. American Oil Chemist society (AOCS) approved official method Ce 1h-05 for the determination of cis, trans- saturated, mono-saturated and polyunsaturated fatty acids in vegetable or non-ruminant animals oils and fats by capillary GC with a flame Ionization Detector (AOCS,2009).

2.6.1.2: GC-FID Analysis (Gas Chromatography Coupled with Flame Ionization Detector) of Fatty Acids

The current research shows that flame ionization mode of detection is the most common analytical method used in routine quantitative analysis of fatty acids in various matrices, because it is robust and of low cost in comparison with mass spectrometry (S. Casal and B. Oliveira, 2010). The studies conducted by Schreiner confirmed the accuracy and robustness of GC-FID technique for quantification of LC-PUFA (M. Schreiner, 2005). In order to check the robustness of gas chromatographic method in combination with FID for quantification of PUFA, various chromatographic systems were used by Schreiner. Thus, this work is a guideline which explains how to achieve the accuracy 17 and robustness of GC-FID method. Novel possibilities in GC-FID analysis are to use this technique for determining of medium and long-chain polyunsaturated fatty acids in clinical (parenteral) formulations (Z. Xu, K. Harvey *et al*., 2010). The current research indicates the usefulness of GC-FID in quantification of some mono- and polyunsaturated fatty acids (e.g., oleic, linoleic, and linolenic) in oils of wheat germ and seeds produced as by-products (M. M. M. Hassanein and A. G. Abedel-Razek,

2009). The content of long-chain polyunsaturated fatty acids like eicosapentaenoic acid and docosahexaenoic acid in different assortments of smoked Atlantic mackerel and Baltic sprats was determined by the use of gas chromatograph equipped with FID (A. Stołyhwo, I. Kołodziejska, and Z. E. Sikorski, 2006).

2.6.1.2: Components of GC-FID



Source: LC GC's chromacedemy, Crawford scientific

Figure 2.6: Schematic diagram of Gas Chromatography Coupled with Flame Ionization Detector

Instrumental components

I. **Carrier gas:** The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependant

upon the type of detector which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities.

II. **Sample injection port:** For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour - slow injection of large samples causes band broadening and loss of resolution. The most common injection method is where a micro syringe is used to inject sample through a rubber septum into a flash vapouriser port at the head of the column. The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample. For packed columns, sample size ranges from tenths of a microliter up to 20 microliters. Capillary columns, on the other hand, need much less sample, typically around 10^{-3} μL. For capillary GC, split/splitless injection is used. Have a look at this diagram of a split/splitless injector;

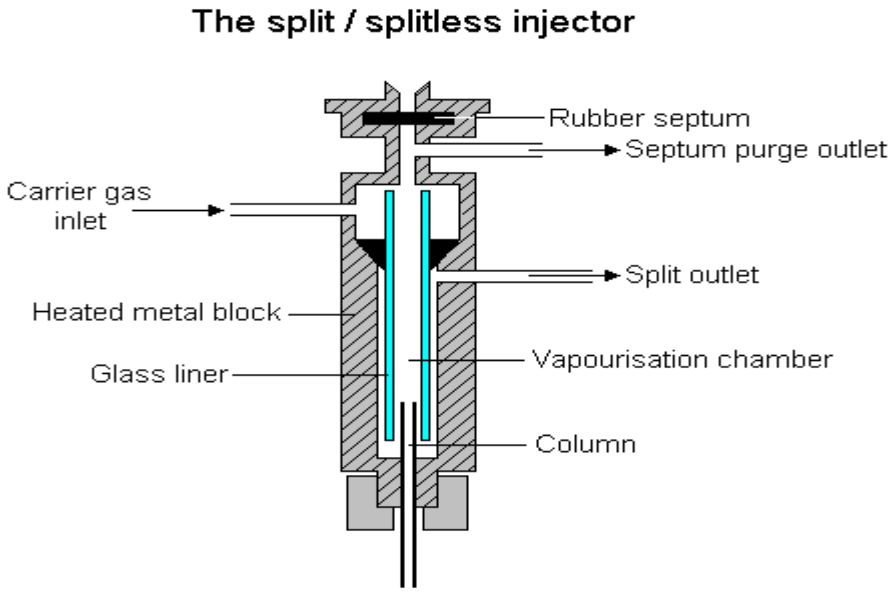


Figure 2.7: Diagram of Sample injection port Gas Chromatography

The injector can be used in one of two modes; split or splitless. The injector contains a heated chamber containing a glass liner into which the sample is injected through the

septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vapourises to form a mixture of carrier gas, vapourised solvent and vapourised solutes. A proportion of this mixture passes onto the column, but most exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column (Frank, 1997).

III. Columns: There are two general types of column, *packed* and *capillary* (also known as *open tubular*). Packed columns contain a finely divided, inert, solid support material (commonly based on *diatomaceous earth*) coated with liquid stationary phase. Most packed columns are 1.5 - 10m in length and have an internal diameter of 2 - 4mm.

Capillary columns have an internal diameter of a few tenths of a millimeter. They can be one of two types; *wall-coated open tubular* (WCOT) or *support-coated open tubular* (SCOT). Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase. In support-coated columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns (Elder, 1995). In 1979, a new type of WCOT column was devised - the *Fused Silica Open Tubular* (FSOT) column;

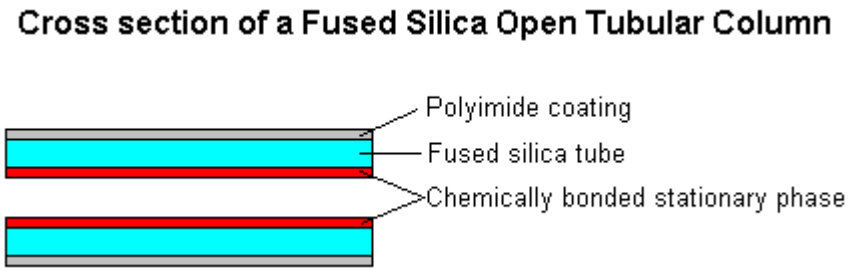


Figure 2.8: Diagram of tubular column for Gas Chromatography

These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating. These columns are flexible and can be wound into coils. They have the advantages of physical strength, flexibility and low reactivity.

IV. **Pneumatic controls:** For precise work, column temperature must be controlled to within tenths of a degree. The optimum column temperature is dependant upon the boiling point of the sample. As a rule of thumb, a temperature slightly above the average boiling point of the sample results in an elution time of 2 - 30 minutes. Minimal temperatures give good resolution, but increase elution times. If a sample has a wide boiling range, then temperature programming can be useful. The column temperature is increased (either continuously or in steps) as separation proceeds (Frank and Aamick, 2004).

V. Detectors: The effluent from the column is mixed with hydrogen and air, and ignited. Organic compounds burning in the flame produce ions and electrons which can conduct electricity through the flame. A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame. The current resulting from the pyrolysis of any organic compounds is measured. FIDs are mass sensitive rather than concentration sensitive; this gives the advantage that changes in mobile phase flow rate do not affect the detector's response. The FID is a useful general detector for the analysis of organic compounds; it has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but unfortunately, it destroys the sample.

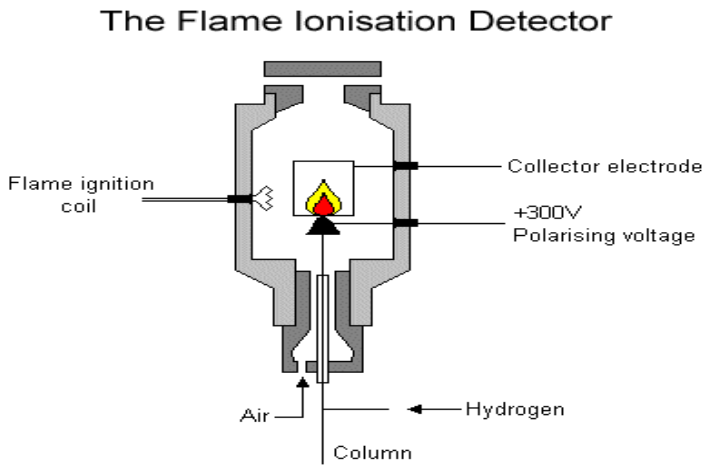


Figure 2.9: Diagram of Flame Ionisation Detector (FID)

2.6.2: Ultraviolet/visible spectroscopy

2.6.2.1: Introduction

Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance. Spectrophotometry is one of the most useful methods of quantitative analysis in various fields such as chemistry, physics, biochemistry, material and chemical engineering and clinical applications. Ultraviolet/Visible (UV/Vis) spectrophotometer uses light in UV and Visible part of the electromagnetic spectrum.

Ultraviolet-visible spectroscopy (UV = 200-400 nm, visible = 400-800 nm) corresponds to electronic excitations between the energy levels that correspond to the molecular orbitals of the systems. In particular, transitions involving π orbitals and lone pairs (n = non-bonding)

are important and so UV-Vis spectroscopy is of most use for identifying conjugated systems which tend to have stronger absorptions (Kruzlicova *et al*., 2008).

Compounds which are coloured, absorb radiation from 400nm to 800nm. But for compounds which are colourless absorption of radiation is in the UV region. In both UV and Visible spectroscopy, only the valence electrons absorb the energy, hence the molecule undergoes transition from ground state to excited state.

2.6.2.2: Principle

The lowest energy transition is that between the **h**ighest **o**ccupied **m**olecular **o**rbital (HOMO) and the **l**owest **u**noccupied **m**olecular **o**rbital (LUMO) in the ground state of a molecule. The absorption of the electromagnetic radiation excites an electron to the LUMO and creates an excited state. The more highly conjugated the system, the smaller the HOMO-LUMO gap, and therefore the lower the frequency and longer the wavelength, as indicated in figure 2.6. The colours we see in inks, dyes, flowers *excreta* are typically due to highly conjugated organic molecules. The unit of the molecule that is responsible for the absorption is called the **chromophore**, of which the most common are C=C (π to π^*) and C=O (n to π^*) systems (Jimenez, 2003).

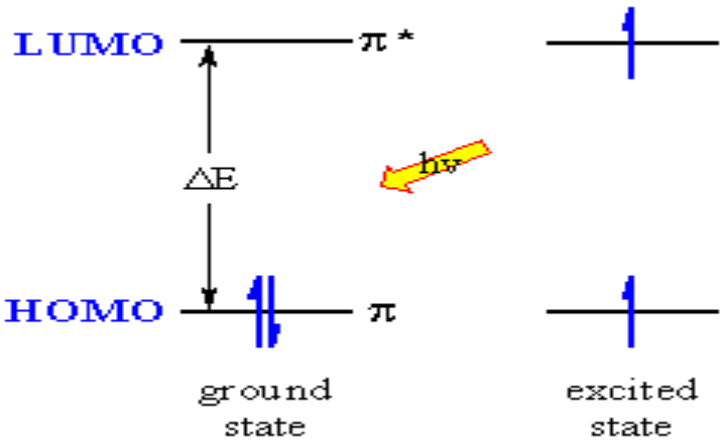


Figure 2.10: Energy transition in UV spectroscopy

If the sample compound does not absorb light (**I**) at a given wavelength the emitted light ($\mathbf{I_0}$)will be of same value, **I** = $\mathbf{I_0}$. However, if the sample compound absorbs light then **I** is less than $\mathbf{I_0}$, and this difference may be plotted on a graph versus wavelength, as shown on the right (Kruzlicova *et al*., 2008). Absorption may be presented as **transmittance** (**T** = **I**/$\mathbf{I_0}$) or **absorbance** (***A***= log$\mathbf{I_0}$/**I**). If no absorption has occurred, T = 1.0 and *A*= 0. Most spectrometers display absorbance on the vertical axis, and the commonly observed range is from 0 (100% transmittance) to 2 (1% transmittance). The wavelength of maximum absorbance is a characteristic value, designated as $\boldsymbol{\lambda_{max}}$.

UV spectroscopy obeys the Beer-Lambert law, which states that: when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution (Frank, 1997).

The expression of Beer-Lambert law is-

$$A = \log (I_0/I) = \varepsilon cl$$

Where, A = absorbance

I_0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

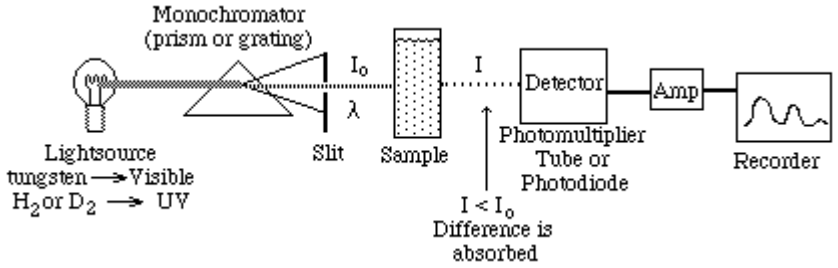
c = molar concentration of solute

l = length of sample cell (cm.)

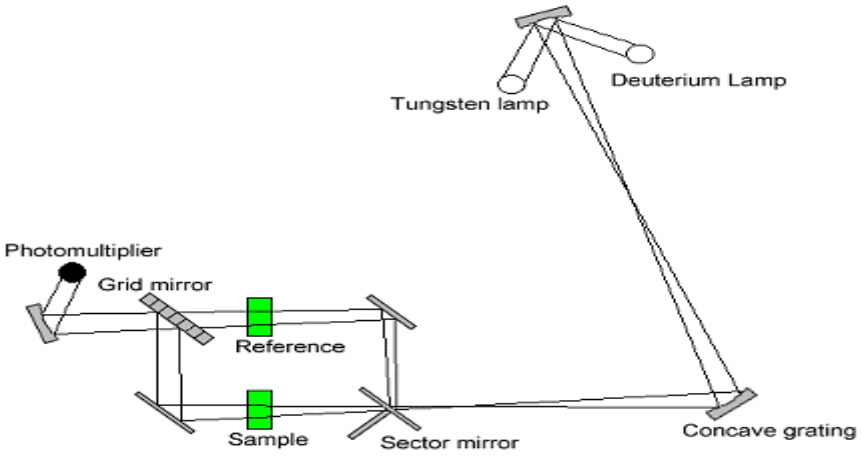
ε = molar absorptivity

From the Beer-Lambert law it is clear that greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption.

2.6.2.3: Components of ultraviolet/visible spectroscopy



(a)



(b)

Figure 2.11: Schematic diagram of (a) single beam UV/Vis spectroscopy (b) Double beam UV/vis spectroscopy

I. **Source of light:** Tungsten filament lamps and Hydrogen-Deuterium lamps are most widely used and suitable light source as they cover the whole UV region. Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen-Deuterium lamps falls below 375 nm.

II. **Monochromator:** Monochromator are also now as wavelength selector which generally composed of prisms and slits. The most of the spectrophotometers are double beam spectrophotometers. Polychromatic radiation (radiation of more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated, and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit. All monochromators contain the following component parts;

- An entrance slit
- A collimating lens
- A dispersing device (usually a prism or a grating)
- A focusing lens
- An exit slit

III. **Sample cells:** The commonly used cells are made of quartz or fused silica. These are readily available even in matched pairs where sample cell is almost identical to the reference cell. The path lengths of the cells are 10mm or 1cm long.

IV. **Detectors:** There are three types of detectors widely used in UV/Vis spectrometers; barrier layer cell, photocell and photomultiplier tube. Generally two photocells serve the purpose of detector in UV spectroscopy. One of the photocell receives the beam from sample cell and second detector receives the beam from the reference. The intensity of the radiation from the reference cell is stronger than the beam of sample cell. This results in the generation of pulsating or alternating currents in the photocells.

V. **Recording system:** The signal from the detector is finally received by the recording system. The readings are displayed on the screen.

Figure 2.12: UV/Vis spectrophotometer: model UV-Vis UV 1800 Shimadzu

2.6.3: Infrared spectroscopy

2.6.3.1: Principle

Infrared spectroscopy is a vibrational method in the wavelength region of 1 to 100 µm. Infrared radiation excites vibrational and rotational motions in molecules (Guillen and Cabo, 1998). Infrared radiation is absorbed by organic molecules and converted into energy of molecular

vibration. When the radiant energy is equal to the energy of a specific molecular vibration, absorption occurs (Guillen and Cabo, 1997). Except for the differences in the energy transfer from the radiation to the molecule, the principles of IR spectroscopy are the same as those of UV/VIS spectroscopy or other spectroscopic techniques and characterized by the Bouger Lambert-Beer Law. Infrared spectra are presented by a plot of the percentage of transmission vs the wavenumber in cm^{-1}. The typical IR absorption range for covalent bonds is 600 (lower limit) - 4000 cm^{-1} (upper limit). Transitions between a vibrational level and the next higher vibrational level ($v \Rightarrow v+1$) are strong IR spectroscopy based on the solution to the Schrödinger equation 2.7 of the Harmonic Oscillator.

$$E_v = (v + ½)\, h\nu_0, \qquad \text{where } 2\pi\nu_0 = k\,(\mu)^{½} \quad \text{……..Equation 2.7}$$

Frequency (γ) is the number of wavelength that passes through a point in one second and is measured in Hz where 1Hz = 1cycle /seconds. Wavelength (λ) lambda is the length of one complete wave cycle which is measured in cm. Wavelength and frequency is inversely related (Frank and Aamick, 2004). Energy is related to wavelength and frequency by the following Equation 2.7.

$$E = h\gamma = hc / \lambda \quad \text{……Equation 2.7}$$

Where the

h- Plank's constant (6.6×10^{-34} joules/sec)

c- speed of light (2.99792458×10^{8} m/s)

Wavenumber, $\overline{\gamma} = 4.12\,(K / M)^{½}$

K – Force constant

M – Reduced mass (m_1m_2 / $m_1 + m_2$)

More complex molecules have many bonds, and vibrations can be conjugated, leading to IR absorptions at characteristic frequencies that may be related to chemical functional group (Guillen and Cabo, 1997).

The IR region is commonly divided into smaller areas.

a) ***Near IR*** region: The absorbance observed in the near IR region (13000 – 4000cm^{-1}) are overtones or combinations of the fundamental stretching bands which occur in the 3000 – 1700cm^{-1} region (Yang *et al*., 2005). The bands that are usually involved are usually due to C-H, N-H or O-H stretching. The resulting bands in the NIR are usually weak in intensity and the intensity generally decreases by the factor of 10 from one overtone to the next. The bands in the near IR are often overlapping making them less useful than the MIR region qualitative analysis (Chen *et al*., 1999).

b) ***Mid IR:*** The spectrum 4000 – 400cm^{-1} can be divided into 5 regions and the nature of a group frequency may generally be determined by the region in which it is located (Woodcook *et al*., 2005). The regions are generalized as follows: the X-H stretching region (4000-2500cm^{-1}), the triple bond region (2500 – 200cm^{-1}), the double band region (2000 – 1500cm^{-1}) and fingerprint region (1500 – 600cm^{-1}). The fundamental vibrations in the 4000 – 2500cm^{-1} region are generally due to O-H, C-H and N-H stretching. O-H stretching produces a broad band that occurs in the range 3700 – 3600 cm^{-1} (Yang *et al*., 2005). By comparison N-H stretching is usually observed between 3400 and 3300cm^{-1}. The absorption is much sharper for O-H stretching than C-H stretching for aliphatic compounds, in the range of 3400 – 2850cm^{-1}(Armanta *et al*., 2007). If the C-H bond is adjacent to a double bond or aromatic ring, the C-H

stretching wavenumber increases and absorbs between 3100 and 3000cm^{-1}. Triple bond stretching 2500 – 2000 cm-1 region. C ≡ C bonds absorbs between 2300 and 2050cm^{-1} (Guillen and Cabo, 1998).

c) ***Far IR:*** The far infrared region is defined as the region between 400 – 100cm^{-1}. The region is more limited than the mid infrared for spectra structure correlations, but does provide information regarding the vibrations (Alexa *et al*., 2007). Intramolecular stretching modes involving heavy atoms can be helpful for characterizing compounds containing halogen atoms, organometallic compounds and inorganic compounds (Diem, 1993). Skeletal bending modes involving an entire molecule occur in the far IR for molecules containing heavier atoms because bending modes are usually no more than one – half of the wavenumber of the corresponding stretching mode (Buning, 2003).

2.6.3.2: Components of infrared spectrophotometer

A common IR spectrophotometer consists of a source, sample compartment and detector. A light source emits polychromatic IR light, which is focused on a sample. The light is partially absorbed by the sample when it is passing through it. Molecules in the sample interact with the light, they take up energy and use this energy to vibrate, with the condition that the dipole moment changes. A detector registers how much light is transmitted through the sample. The result is a characteristic spectrum showing the transmittance (absorbance) of electromagnetic radiation as function of wavelength (wavenumber). Diagram 2.3 shows how the light passes through to the detector.

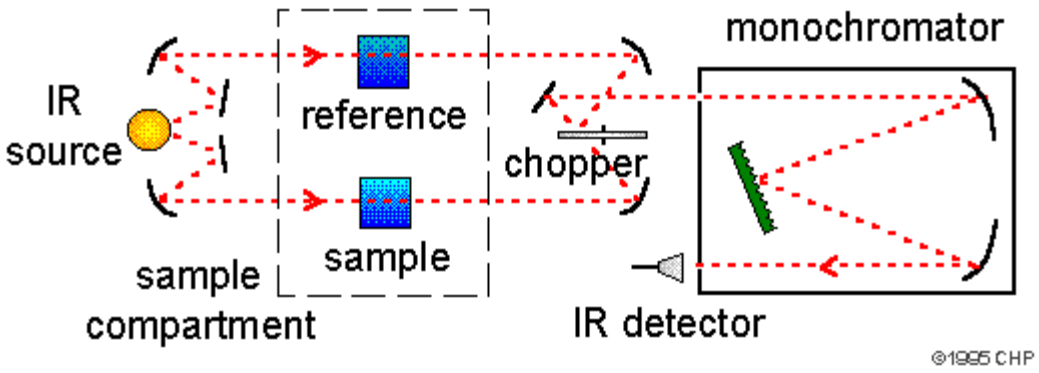


Figure 2.13: Schematic diagram of infrared spectrophotometer

I. **Source**: Infrared energy is emitted from a glowing black-body source. This beam passes through an aperture which controls the amount of energy presented to the sample. The various popular sources of IR radiations are incandescent lamp, Nernst lamp, globar source and mercury arc lamp (Frank, 1997 ; Lide, 1991).

II. **Sample cells**: Infrared spectroscopy can analyse sample in three forms: solid, liquid or gas samples. The material containing the sample must be transparent to IR radiation. This condition restricts our selection to only certain salts like NaCl or KBr. A final choice of salt will depend on the wavelength range to be studied (Frank and Aamick, 2004).

III. **Monochromators**: The sample in IR spectrometer absorbs only at certain frequencies from the radiation source so it becomes necessary to select desired frequencies from the radiation source and reject the radiation of other frequencies. The selection is achieved by means of monochromator which are of two types: prisms and grating monochromator.

IV. **Detector**: This is to measure the intensity of the unabsorbed infrared radiation. Detectors like thermocouple, golay cell, bolometers, thermistors and pyroelectric are used.

Figure 2.14: Infrared spectrophotometer

CHAPTER THREE

3.0: MATERIALS AND METHODS

3.1: Apparatus and equipment

- Analytical balance (model: BL-3200 HL, Shimadzu, Japan)
- Refractometer (model: Carl Zeiss Abbe refractometer)
- Infrared spectrometer (model: Perkin-Elmer 467 infrared spectrophotometer)
- Ultraviolet-visible (UV-Vis) (model: UV-Vis UV 1800 Shimadzu, ENG240V, SOFT)
- Gas –Chromatography / Flame Ionization Detector

3.2: Reagents

- Potassium hydroxide Sigma-Aldrich ACS reagent 93.5% w/v
- Hydrochloric acid; Baker analyzed ACS reagent 36.5 – 38.0% v/v
- Phenolphthalein solution; Baker analyzed ACS reagent 90%
- Acetic acid (glacial); specified reagent for general laboratory 99.9% v/v assay
- Chloroform ≥ 99% assay; specified laboratory reagent v/v
- starch indicator solution
- Potassium dichromate $K_2Cr_2O_7$ ≥ Baker analyzed ACS reagent 99.9% w/v assay
- Sodium thiosulphate $Na_2S_2O_3$; Sigma-Aldrich ACS reagent 98% w/v assay

- Potassium iodide, Sigma-Aldrich 99.9% w/v
- TMS-DM (Fluka Chemicals 97% GC)
- $NaOCH_3$ (Alltech Associates 99%)
- Methanol (Sigma–Aldrich 99.93% ACS HPLC grade)
- Ethanol (Sigma–Aldrich 95%)
- Toluene (Sigma–Aldrich 99.8% HPLC grade)
- Hexane (Sigma–Aldrich 95 + % anhydrous)
- Calcium chloride (EM Science, anhydrous powder)
- Hydrochloric acid (trace metal grade) was purchased from Fisher Scientific and used as received.
- Standards: Palmitic (C16:0), Palmitoleic (C16:1), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2), Linoleic (C18:3) and Erucic (C22:0) acid, all at 98 + % purity (Sigma–Aldrich).

3.2.1: Preparation of reagents

Preparation of 0.5M alcoholic potassium hydroxide

- 28.05g of potassium hydroxide was weighed and dissolved in 20ml of water and the final volume made to 1 litre using ethanol

Preparation of 0.1M potassium hydroxide

- 5.61g of potassium hydroxide was weighed and placed into 100ml volumetric flask and dissolved in 80ml of distilled water then stirred till it fully dissolved. It was then topped to the mark.

Preparation of 0.01M potassium dichromate

- 2.94g of potassium dichromate was dissolved in distilled water and diluted to 1000ml mark in a volumetric flask.

Preparation of 0.5M hydrochloric acid

- Approximately 300ml of distilled water was placed into 500ml volumetric flask. 21ml of concentrated hydrochloric acid was added and water added to the mark.

Preparation of phenolphthalein

- 1g of phenolphthalein was dissolved in 50ml of absolute ethanol and water was added to 100ml mark

Preparation of 1% starch indicator solution

- 10g of soluble 1%starch was dissolved and topped with distilled water to 1000ml mark.

Preparation of saturated potassium iodide

- 50g of potassium iodide was dissolved in 500ml of distilled water and stirred.

Preparation of 0.01M sodium thiosulphate

- 2.45g of sodium thiosulphate and 0.02g of sodium carbonate was dissolved in distilled water and topped to 1000ml mark.

Hydrochloric acid solutions

- Hydrochloric acid solutions were prepared in methanol at 0.25, 0.5, 1, 2, 5, 10, 15 and 20% (v/v) concentrations and the weight percent of acid in each alcohol solution was determined by titration with a 0.1M KOH solution that was standardized according to AOCS Official Method H 12-52. The mass of HCl was determined by titration of the acid solution, and not based on the assumption that HCl concentration is 32% HCl by weight. A 5% (w/w) solution of calcium chloride in deionized water was prepared as needed.

3.3: Sampling

Three types of Sunflower and Corn oils and two types of Soya bean oils were sampled. Out of each brand, 3 different batches were sampled at different supermarket. A total sample collected was 22 of 500ml size. Samples of vegetable edible oil: Sunflower oils (Table 3.1), Corn oils (Table 3:2) and Soya bean oils (Table 3:3) were collected from local supermarkets within Nairobi CBD during the period of October 2012 and February 2013. The population of interest included all supermarkets within Nairobi Central Business District (CBD) within a radius of 50km Nairobi City with more than 2 branches. The set of the supermarkets was randomly selected based on monthly sales of more than Ksh. 150,000 information obtained from Beiersdorf (EA) ltd current customer database (App I). The supermarkets were selected by the population that shop there. The top supermarkets in Nairobi CBD that have the largest population on costumers are Nakumatt, Tuskys and Naivas hence they were target sampling areas. For Tuskys supermarkets are most pronounced chains store found in within Nairobi city centre shopped mostly by the middle class population while for Nakumatt supermarket is based

in upper region of CBD. For Woolmatt and Naivas have gained entry into the market long with Tuskys and Nakumatt which is shopped mostly by lower income population. This mode of sampling was able to target all the supermarkets shopped by the whole population of all the levels.

The samples were transferred into clean plastic laboratory containers with lids. The oil samples were stored at room temperature, dry place, well-sealed and out of direct sunlight. The vegetable oils once opened were refrigerated to sustain its shelf life. During sampling expiry and manufacturing date were factors for the analysis plan matrix.

Tables 3.1, 3.2 and 3.3 contains the information about each type of samples, date of sampling, location of sampling, lab code, manufacture date, expiry date and the batch number

Table 3.1: Sunflower

Lab code	Type of sample	Supermarket	Date of sampling	Batch No.	Expiry date
RS	Rinsun sunflower oil	Nakumatt	14/10/2012	6793913A	09/2014
		Naivas	07/11/2012	9612548C	12/2014
		Tuskys	13/01/2013	1253321F	01/2015
SS	Sungold sunflower oil	Tuskys	25/12/2012	02-34034	12/2014
		Naivas	07/11/2012	01-21650	08/2014
		Nakumatt	05/02/2013	05-25194	02/2014
ES	Equatorial naturals sunflower oil	Woolmatt	21/11/2012	1054A04T	10/2015
		Nakumatt	14/10/2012	1154G23L	06/2014
		Naivas	07/11/2012	851A24H	04/2015

The three most common sunflower oils used by a large population were rinsun, sungold and equatorial naturals oils. Three samples of each brand were sampled from different points as indicated in Table 3.1 above.

Table 3.2: Corn oil

Lab code	Type of sample	Supermarket	Date of sampling	Batch No.	Expiry date
EC	Elianto corn oil	Woolmatt	21/11/2012	01-31018	06/2014
		Naivas	07/11/2012	01-26210	04/2014
		Tuskys	13/01/2013	03-15412	02/2013
CC	Captain corn oil	Nakumatt	14/10/2012	3515113A	12/2014
		Woolmatt	25/12/2012	4521627M	07/2014
		Tuskys	13/01/2013	54101225R	01/2015
DD	Chef corn oil	Tuskys	25/12/2012	A01/21524	07/2014
		Naivas	07/11/2012	A01/15133	09/2014
		Nakumatt	14/10/2012	A01/15467	02/2015

The three most common corn oils used by a large population were elianto, captain and chef oils. Three samples of each brand were sampled from different points as indicated in Table 3.2 above.

Table 3.3: Soya bean oil

Lab code	Type of sample	Supermarket	Date of sampling	Batch No.	Expiry date
SG	Soyagold oil	Woolmatt	21/11/2012	01-36638	06/2014
		Naivas	07/11/2012	01-26542	04/2014
HS	Hawaya soya bean oil	Tuskys	05/02/2013	B125045L	05/2015
		Woolmatt	21/11/2012	N16523J	03/2014

The two most common soya bean oils used by the population were soyagold and hawaya oils. Three samples of each brand were sampled from different points as indicated in Table 3.3 above.

3.4: Analysis of physico-chemical properties

3.4.1: Density

The weight of empty pycnometer and stopper (M_o) was taken and density recorded. The pycnometer of capacity 50 cm^3 was used after calibration with distilled water. The pycnometer was then filled with distilled water and the overflow from the capillary hole wiped. The weight of the pycnometer with water and the stopper was weighted (M_1) and recorded. This was done in triplicate. The procedure was repeated using sunflower oil sample RS and weight with the stopper (M_2) was read and recorded in triplicate. The temperature (t) of the laboratory was measured. The same procedure 3.4.1 was used for all other oil samples.

Calibration of pycnometer was done using distilled water

Weight of pycnometer (M_o)

Volume of the pycnometer (M_1)

Weight of water- { M_1 - Mo = $M_{H2O.}$ }

The weight of the sample was calculated by M_2 - Mo =M_L. Using M_L and M_{H2O} the density was determined using the equation 4.1.

Density (ρ) =(M_L / M_{H2O}) x ρ H_2O…….Equation 3.1

Weight of oil sample – M_2

3.4.2: Refractive Index

The instrument was calibrated with a glass prism using water. The Refractive Index of distilled water is 1.3330 at 20 ^oC and 1.3306 at 40 ^oC. The relative index of the sunflower oil sample RS was measured at 298±0.05K with a Carl Zeiss Abbe refractometer (32-G 110e) with a

precision of 1x 10^{-4} at the wavelength of 589nm. With the help of a screw head the double prism was opened and a drop of each sample was placed on the prism. The prism was tightly screwed. Water was circulated through the instrument. And the instrument was left to stand for a few minutes before taking the readings so that the temperature of the instrument and the sample were the same. The prism was cleaned between readings by wiping off the oil with cotton pad moistened with ethyl alcohol and let to dry. The oil was filtered through a filter paper to remove impurities and traces of moisture. Circulation of water through the refractometer to desired temperature (25^{o}C) was done. The prisms were cleaned and dried. A few drops of the sample was placed on the prism. The prism was screwed and allowed to stand for 1-2 minutes. The instrument was adjusted to obtain the desired readings in triplicates. The procedure 3.4.2 was used for all the other oil samples.

Temperature correction

Refractive Index is corrected when necessary using the Equation 3.2

Relative Index (nD) = $R^1 + K^1 (T^1 - T)$……Equation 3.2

R- Reading of the refractometer reduced to specified temperature T ^{o}C

R^1 – Reading at T^1

k- Constant 0.000385 for oils at 40^{o}C

T^1- temperature at which the reading R_1 is taken

T-specified temperature (40^{o}C)

3.4.3: Saponification number.

2gm of Sunflower oil sample RS was weight into a clean and dry conical flask. 25ml alcoholic KOH was added. The conical flask was then whirled vigorously with a magnetic stirrer and boiled for half hour. This was titrated immediately after adding 5 drops of phenolphthalein with 0.5M HCl until the color changed from red, back to the original oil color (i.e color before adding phenolphthalein). A blank solution was prepared (25ml of alcoholic KOH and 5 drops of phenolphthalein with 0.5M HCL). This procedure 3.4.3 was used for all the other oil samples.

The equation 3.3 was used to calculate the Saponification value

$$\textbf{mg KOH / gram oil} = \frac{\textbf{D x F x M}}{\textbf{A(g)}} \quad \text{.....Equation 3.3}$$

D- Difference between the sample and the blank titration volumes

A is the weight of the sample.

M = 0.5M HCl

F- Equivalent of KOH (56.11 mg/mol)

3.4.4: Peroxide Value

3g of Sunflower oil sample RS to be examined was placed into 250ml conical flask fitted with a glass stopper. 10ml of chloroform was added and swirled to dissolve the oil. 15ml of acetic acid and 1.0ml potassium iodide was added and mixed. This was left for 5 minutes in a dark place. 30ml distilled water and 1ml starch indicator was added and the solution titrated with sodium thiosulphate. It was shaken to dissolve the oil sample and 0.5ml of saturated potassium iodide solution was added. It was then shaken

for exactly 1 min and 1ml of starch solution added and the titration was continued while shaking vigorously, until the color was discharged (n_1= amount of 0.01M sodium thiosulphate). A blank test was put under the same conditions ($n_{2=}$ amount of 0.01M sodium thiosulphate). The blank was prepared by mixing 10ml chloroform, 15ml acetic acid, 1.0 ml KI and 30ml H_2O. The volume of 0.01M sodium thiosulphate used in the blank titration did not exceed 0.01ml. This procedure (3.4.4) was used for all the other oil samples.

Peroxide Value was calculated using Equation 3.4

$$\mathbf{PV = (V_1 - V_o) \times T \times 1000/m} \quad \text{.....Equation 3.4}$$

V_1 – volume of sodium thiosulphate solution required to titrate the sample

V_o – volume of sodium thiosulphate solution required to titrate the blank determination (ml)

T – titre of the sodium thiosulphate

m- Mass of the sample

3.4.4.1: Standardisation of Sodium Thiosulphate Solution

210g was weight from the primary standard of potassium dichromate. The potassium dichromate was dissolved in 100ml of water in a glass-stopped, 500ml flask. The solution was swirled for 10 minutes to completely dissolve the solid. The stopper was removed and the inner walls of the flask with water and liberated iodide titrated with sodium thiosulphate until the solution turned yellowish green in colour. Then 3ml of starch was added to the solution and the titration was continued until the blue colour was discharged.

3.4.5: Acid value and free fatty acids

5.0g of sunflower oil sample RS was placed in a dried flask and 25ml of absolute ethanol and 2-3 drops of phenolphthalein added. The sample was heated while shaking in water bath (60^{o} C) for 10 minutes till the sample dissolved, then cooled. The solution was titrated against 0.1M KOH until pink colour appeared (end point).The acid value was obtained then used to calculate free fatty acid (FFA). This procedure (3.4.5) was used for all the other oil samples.

Using the equation 4.6 the free fatty acid was calculated using the acid value Equation 3.5

$$\mathbf{AV = \frac{(S - B)\ x\ M\ x\ 56.1\ g\ KOH/\ mol}{W}} \quad \text{.....Equation 3.5}$$

AV- acid value

W - weight of the sample.

S – Volume of KOH for sample titration

B- Volume of KOH for the blank titration

M – molarity of KOH (0.1M)

$$\mathbf{\%\ FFA\ (free\ fatty\ acid) = AV\ x\ 0.503} \quad \text{.....Equation 3.6}$$

3.5: Fatty acid analysis by Gas Chromatography with a Flame Ionization Detector

3.5.1: Preparation of standards

The individual fatty acid methyl ester standards (FAMEs): Palmitic (C16:0), Palmitoleic (C16:1), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2), Linoleic (C18:3) and Erucic (C22:0)acid were purchased from Sigma (Sigma-Aldrich, Germany) as representatives of saturated FA, cis and trans of moo- unsaturated FA and cis and trans of poly- unsaturated FA,

respectively, was used to prepare the stock solution (standard 1). Individual FAMEs standards were used for preparation of stock standard mixture (50 mg/ml) from which six working standards (0.5 – 30.0 mg/ml) were prepared by diluting with n-hexane. Furthermore, from these six working standards calibration curves were produced. For construction of the calibration curve, the aforementioned working standards were analyzed in triplicate. Identification and contents of the fatty acids were carried out by comparing sample FAME peak retention times and peak area with those obtained for FAME mix standard. The linearity of the method was estimated by performing of 3 replicates of FAME mix standard solution in a range from 0.5 to 30.0 mg/ml at six concentration levels.

3.5.2: Preparation of FAMES

Each of the vegetable oil of the samples was transferred to a screw –cap test tube and a known concentration of internal standard was added. FAMEs were prepared by using 1ml of 2M $NAOCH_3$ in water bath at 60 °C. Drops of concentrated glacial acetic were added to neutralize the NaOH. After the solvent was removed under nitrogen gas flow, the samples were re-dissolved in 1ml of methanol: toluene (2:1, vol.), and 100µl of TMS-DM (2M) in n-hexane was added at 60 °C for 5 minutes. Drops of glacial acetic acid were added until the yellow colour disappeared to remove unreacted TMS-DM and the reaction mixture was diluted with 1 ml of 0.5% Nacl solution. Methyl esters were extracted twice in hexane containing the FAMEs were pooled and dried under nitrogen gas before stored at - 20° C until GC analysis.

3.5.3: GC analysis of FAMEs

FAMEs in all the test tube samples were re-dissolved in 100µl hexane and 1 µl volume of each sample was injected five times into GC-FID (Hewlett-Packard 6890 gas chromatograph - Agilent Technologies) for separation and quantification of FAMES. The analysis was carried out using a SP-2560 column (100 m × 0.25 mm ID, 0.20 µm film) from Supelco (Bellefonte, PA, USA). The run was under an optimized temperature programme as follows: initial column temperature 100 °C for 4 minutes, programmed to increase at the rate of 10 °C min^{-1} up to 160 °C and then at 3 °C min-1 up to 220 °C. This temperature was maintained for 5 minutes, then at 10 °C min-1 up to final temperature of 260 °C and held for 5 minutes. Injector and detector temperature were at 260 °C and 280 °C, respectively. Helium was used as the carrier gas at a flow of 1 ml nim-1 with a split ration of 30:1

3.5.4: Validation procedure

A guideline for validation of chromatographic methods was used for validation of the method (Taverniers and Bockstaele, 2004). Within the validation procedure linearity, precision and recovery, limit of detection (LOD) and limit of quantification (LOQ) were investigated.

3.6: Ultra-violet/Visible spectroscopy analysis

The sunflower oil sample RS was filtered (22µm filter) and 5ml of sample was transferred in a clean centrifuge tube with a cap. The sample was run neat to obtain a full scan spectrum. Zeroing of the instrument was done using an empty quartz cell. The quartz cells were filled with 4ml distilled water and the absorption read. The maximum allowable background absorbance is 0.01cm^{-1}. If 0.01 cm^{-1} ultraviolet absorbance for the water is exceeded, the cause was identified and any determined source of contamination was eliminated. Initial zeroing

check-up was done after 10 samples had been read. The quartz cells were rinsed with distilled water followed by small amount of vegetable oil sample to be analysed. Refilling of the quartz cells with 4ml vegetable oil was carefully done by use of pipetting in the spectrophotometer cells and the cell windows were wiped using a soft cloth and placed in the spectrophotometer holder cell. Samples were run on the UV/Vis spectrophotometer and the ultraviolet absorption recorded. All the measurements were performed at 20ºC and at ambient pressure. This procedure (3.6) was used for all the other oil samples.

3.7: Infrared spectroscopy analysis

The samples were filtered and analyzed. Firstly a small drop of the sunflower oil sample RS was smeared on the face of a highly polished NaCl salt plate. A second plate was placed on top of the first plate so as to spread the liquid in a thin layer between the plates. Finally, vegetable oil on the edge of plate was wiped off. The sandwich plate was mounted onto the sample holder. After the experiment, the plates were cleared with isopropanol and rinsed with the next vegetable oil to be analyzed. The wavelength range was 4000 – 600cm^{-1}. This procedure (3.7) was used for all the other oil samples.

3.8: Establishment of analytical protocol

Physicochemical properties such as density, refractive index, saponification value (SV), peroxide value (PV) and free fatty acids of sunflower oil sample was studied to evaluate the compositional quality of oils. The first step to determine the density and refractive index as physical parameters and evaluated using the working ranges. Second step is to determine the chemical parameter; saponification value, peroxide value and free fatty acids and verified. Then sample was quantified using UV/Vis spectroscopy for colour discrimination using the

wavelength. Lastly the oil sample was identified using IR spectroscopy in identification of oil sample. This procedure (3.8) was used for all the other oil samples. The protocol was verified for ease of performance, flow of activities and discrimination of vegetable oils.

CHAPTER FOUR

4.0: RESULTS AND DICSUSSION

4.1: Physico-chemical properties

4.1.1: Density measurements

Sunflower and Corn oil had similar densities. Soya bean oil has a higher density than both corn and Sunflower oils as shown in table 4.1 below.

Table 4.1: Density for sunflower, corn and soya bean oils

	Sample lab code	**DENSITY (g/cm^3)**	**AVERAGE DENSITY (g/cm^3)**	**STANDARD DEVIATION**
Sunflower oil	RS	0.919	0.920	0.0012
	SS	0.921		
	ES	0.919		
Corn oil	EC	0.919	0.918	0.001
	CC	0.918		
	DC	0.917		
Soya bean oil	SG	1.067	1.0355	0.045
	HS	1.004		

Sunflower oil samples RS had an average density of 0.919 g/cm^3 the SS had average density of 0.921 g/cm^{-3} and ES had average density of 0.919 g/cm^{-3} as well Corn oil EC, CC snd DC samples had average density of 0.919, 0.918 and 0.918 g/cm^{-3} respectively. Soya bean oil samples SG and HS had of 1.067and 1.004 g $/cm^{-3}$ respectively. Sunflower, Corn and Soya bean oil average densities of 0.920, 0.918 and 1.0355g/cm^{-3} respectively as indicated in table 4:1. Density of any oil at a given temperature

is known to increase as the mean molecular weight diminishes (i.e. with higher saponification number), and also as the degree of unsaturated increases (i.e. with higher iodine value).

4.1.2 Refractive Index

Table 4.2: Relative Index for Sunflower, Corn and Soya bean oils

	Sample lab code	RELATIVE INDEX (nD at 40^{o} C)	AVERAGE RELATIVE INDEX (nD at 40^{o} C)	STANDARD DEVIATION
Sunflower oil	RS	1.466	1.463	0.003
	SS	1.466		
	ES	1.464		
Corn oil	EC	1.465	1.464	0.0006
	CC	1.464		
	DC	1.464		
Soya bean oil	SG	1.469	1.471	0.002
	HS	1.472		

Sunflower oil samples had average Relative Index of 1.463 at 40°C. Corn and Soya bean oil samples had average relative index of 1.464 and 1.471 at 40°C respectively. Refractive Index of oil reflects its degree of unsaturation. The higher the refractive index the greater the degree of unsaturation or conjugation hence Soya bean oil has the highest refractive index with the highest degree of unsaturation.

4.1.3 Saponification number

Blank value = 12.8ml, 12.6ml, 12.7ml

Average blank value= 12.7 (ml)

Table 4.3: Saponification value of sunflower, corn and soya bean oils

	Sample lab code	S V (mg KOH / g sample)	AVERAGE S V (mg KOH / g sample)	STANDARD DEVIATION
Sunflower oil	RS	190.35	188.013	3.05
	SS	184.56		
	ES	189.13		
Corn oil	EC	190.35	187.467	2.89
	CC	184.56		
	DC	187.10		
Soya bean oil	SG	191.15	188.32	4.00
	HS	185.49		

Average saponification value for the sunflower, Corn and Soya bean oil samples was 188.013, 187.467 and 188.32 mg $_{KOH}$/g respectively. Corn oil had the lowest average saponification value of 187.467 mg $_{KOH}$/g. Soya bean oil samples had the highest value of 188.32 mg $_{KOH}$/g. The lower value of saponification value suggests that the mean molecular weight of fatty acids is lower. These imply that the fat molecules weight of fat molecules did not interact with each other.

4.1.4 Peroxide Value

Table 4.4: Peroxide Value of oils

	Sample lab code	PEROXIDE VALUE (mmol peroxide/ Kg sample)	AVERAGE PEROXIDE VALUE (mmol peroxide/ Kg sample)	STANDARD DEVIATION
Sunflower oil	RS	6.665	6.776	0.295
	SS	6.553		
	ES	7.111		

Corn oil	EC	1.083	1.418	0.335
	CC	1.418		
	DC	1.753		
Soya bean oil	SG	11.913	11.188	1.025
	HS	10.463		

The average Peroxide Values for Sunflower and Corn oil samples was 6.776 and 1.418 mmol/kg respectively as indicated in table 4.4. Soya bean oil sample recorded average peroxide value for SG and HS as 11.913 mmol/kg. The Peroxide Value is greatly reduced by the refining process used, vary in different seed oils depending on the extraction methods, storage conditions and sample varieties. Higher Peroxide Value can be caused by amongst others, the storage method of the oils psychotropic organisms secreting oxidative enzymes can grow at temperatures as low as 5°C or even below. Peroxide Value is used as measure of the extent to which rancidity reactions have occurred during storage. It also indicates of quality and stability of oils. It could also be a reflection of high levels of oxidative rancidity of oils, the absence or low levels of antioxidants. It has been shown in Corn oil that the exact peroxide value at which organoleptic rancidity sets in depends upon conditions such as the amount of oxygen available, the temperature and the amount of surface exposed. So, it is possible that some of these factors may have led as there existed some air spaces in the storage bottles even and the blowing of nitrogen in the air spaces before storage might not have been that efficient to expel all the oxygen available. Peroxide Value 2.5 to 5 mmol/kg indicates a relatively good quality and stability of the oil. The higher the Peroxide Value the less stability hence shorter life span.

4.1.5: Total free fatty acid content

Table 4.5: Total Free Fatty Acids of oils

	Sample lab code	%FREE FATTY ACID	AVERAGE % FREE FATTY ACID	STANDARD DEVIATION
Sunflower oil	RS	0.094	0.556	0.817
	SS	0.150		
	ES	0.075		
Corn oil	EC	0.266	0.145	0.105
	CC	0.094		
	DC	0.075		
Soya bean oil	SG	0.113	0.651	0.760
	HS	0.188		

Average total free fatty acids for Sunflower, Corn, Soya bean oil samples were 0.556, 0.145 and 0.651% respectively. Results for all the oil samples ranged from 0.075 to 0.226 %. The recommended free fatty acid value by CODEX-STAN and KEBS are 0.085% and 0.25% respectively. The percentage of fatty acids for corn oil samples had the lowest value of 0.145% which was below the KEBS recommended value. Geographical and climatic differences could be attributed to this apart from the method of extraction which has been previously shown to have an effect on the oil yields. Free fatty acid levels is an index of the quality of fats and oils and among the parameters that have been studied and monitored periodically (in terms of days and months) under certain conditions alongside Peroxide Value by several researchers on their respective works. In all these different studies, both the free acid level and peroxide values rises with an increase in the number of storage period of the oils.

4.2: GC-FID analysis of FAMEs

Table 4.6 indicates the retention time and coefficient of correlation (*r*2) for fatty acids.

Table 4.6: Linearity of the method

Fatty acids	**Retention time (min)**	**Coefficient of correlation (r2)**
Palmitic (C16:0)	22.759	0.99994
Palmitoleic (C16:1)	23.500	0.99990
Stearic (C18:0)	25.794	0.09987
Oleic (C18:1)	26.575	0.99994
Linoleic (C18:2)	27.320	0.99877
Linoleic (C18:3)	27.796	0.99886
Erucic (C22:0)	29.330	0.99992

About the data obtained from the examination of the fatty acid profile, arithmetic mean (X), standard deviation (SD) and coefficient of variation (CV) were calculated.

Table 4.7: Limit of detection and limit of quantification

Fatty acids	LOD (µg/ml)	LOQ (µg/ml)
Palmitic (C16:0)	0.05	0.21
Palmitoleic (C16:1)	0.09	0.24

Stearic (C18:0)	0.07	0.24
Oleic (C18:1)	0.09	0.31
Linoleic (C18:2)	0.08	0.24
Linoleic (C18:3)	0.06	0.28
Erucic (C22:0)	0.04	0.23

Table 4.8: Repeatability, reproducibility and accuracy of the method

Fatty acids	Repeatability RSD %			Reproducibility RDS %			Recovery %
	Oil sample			Oil sample			
	Sunflower n=9	Corn n=9	Soy bean n=6	Sunflower n=9	Corn n=9	Soy bean n=6	
Palmitic (C16:0)	1.26	1.28	1.74	2.19	3.55	4.23	98.21
Palmitoleic (C16:1)	1.57	1.49	1.59	2.47	3.91	3.92	98.95
Stearic (C18:0)	0.96	1.36	1.27	3.05	3.26	2.48	97.22
Oleic (C18:1)	1.98	1.89	1.84	3.14	2.09	2.91	96.83
Linoleic (C18:2)	1.79	1.25	1.28	2.91	2.74	2.65	102.26
Linoleic (C18:3)	1.26	1.06	1.57	2.15	2.75	3.49	100.13
Erucic (C22:0)	1.59	1.89	0.98	3.49	3.09	3.97	97.26

The individual fatty acid methyl ester standards (FAMEs Palmitic (C16:0), Palmitoleic (C16:1), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2), Linoleic (C18:3) and Erucic (C22:0) acid were dissolved in n-hexane and added to the vegetable oil to the concentration of 1,000 ppm (w/v) and. Fatty acids were derivatized to fatty acid methyl esters (FAME) using BF3/MeOH (14% boron trifluoride) and analyzed by GC according to AOAC 969.33 (16) with some modification. FAME was analyzed by Hewlett-Packard 6890 gas chromatograph (Agilent Technologies) with a FID, and a SP-2560 column (100 m × 0.25 mm ID, 0.20 μm film) from Supelco (Bellefonte, PA, USA). The oven temperature started at 100oC for 4 min, increased to 225oC at 3oC/min, and held at 225oC for 20 min. The temperatures of injector and detector were 225 and 285oC, respectively. The flow rate of helium carrier gas was 0.75 mL/min, the injection volume was 1 μL, and the split ratio was 1:200. Peaks of GC chromatograms were identified comparing the retention times of a mixture of standard fatty acid methyl esters. Each peak of fatty acid was quantified using an equivalent of the concentration of the internal standard. Samples were separately analyzed in triplicate.

The precision of the method was evaluated through repeatability and reproducibility and the results are expressed as the relative standard deviation (RSD, %) (Table 4.9). Repeatability of the method was established by six fold analyses of three different samples, while the reproducibility was established by three fold analyses of three different samples in three consecutive days.

The recovery (%) of the method was established by spiking a sample with a standard working solution at one concentration level (10.0 mg/ml), and assaying it in triplicate (Table 4.7). Accuracy of the method was verified through the recovery. The fatty acid composition of the vegetable oil samples are presented in Table 4.9.

Table 4.9: Fatty acid profiles for sunflower, corn and soya bean oils

Fatty acids	Samples											
	Sunflower				Corn				Soya bean			
		X	SD	CV		X	SD	CV		X	SD	CV
Palmitic (C16:0)	7.1	4.7	1.0	21.27	11.7	10	2.0	20.0	10.2	9.0	2.0	22.22
Palmitoleic (C16:1)	6.08	8.8	0.8	9.09	11.67	25.1	1.8	7.17	11.75	13.5	0.93	6.89
Stearic (C18:0)	3.26	2.0	0.4	20.0	1.85	3.5	1.5	42.86	3.7	4.0	0.9	22.5
Oleic (C18:1)	16.93	31.5	4.5	14.29	25.16	26.8	1.2	4.48	20.8	31.5	1.2	3.81
Linoleic (C18:2)	73.73	59.5	7.5	12.61	60.60	48	4.5	9.38	55.53	49.5	6.5	13.13
Linoleic (C18:3)	1.0	32.5	4.5	13.85	0.48	26.8	1.2	4.48	9.3	28.5	1.2	4.21
Erucic (C22:0)	0.9	59.5	7.5	12.61	0.3	48	4.5	9.38	0.2	57.5	2.2	3.83

Mean (X), standard deviation (SD) and coefficient of variation (CV) for fatty acid composition (% by weight of total fatty acids present)

The GC method was used for identification and quantification of FAs and the results obtained was able to identify of the samples as Sunflower oil, Corn oil and Soya bean oil.

4.3: Ultraviolet/ Visible spectra

4.3.1 A UV/VIS spectra for sunflower oil sample RS

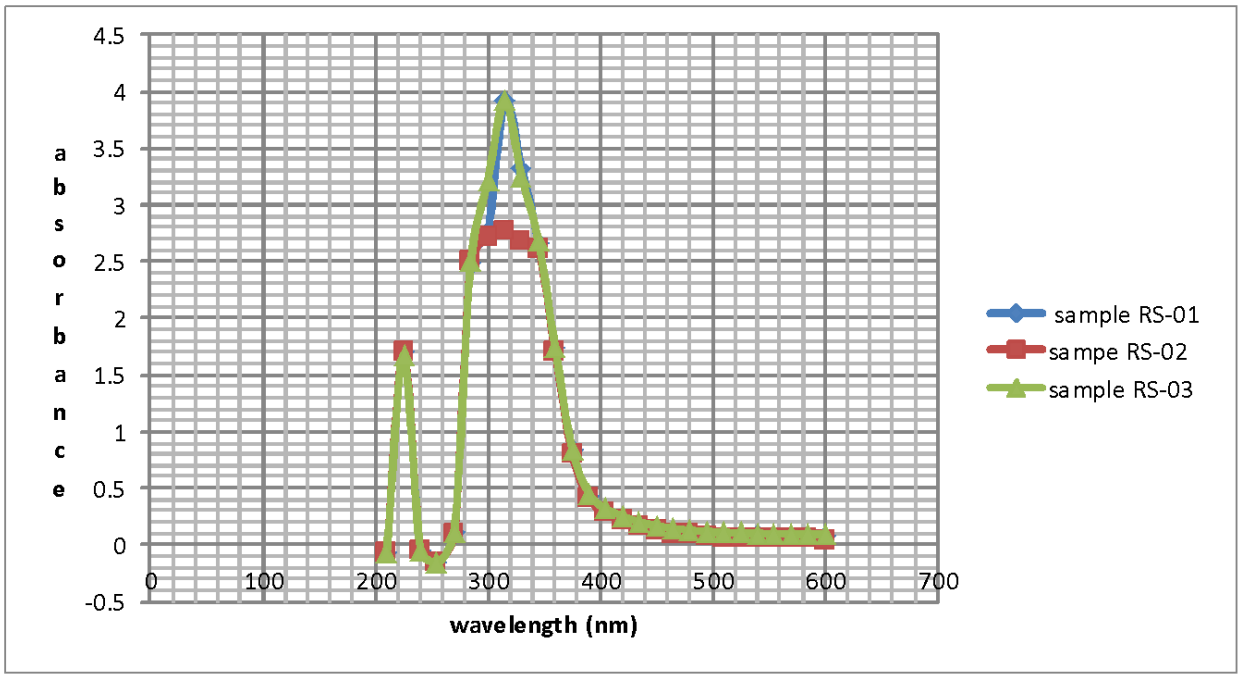


Figure 4.1: UV spectrum of Sunflower oil sample RS

All Sunflower oil samples for RS-01, RS-02 and RS-03 had consistently peaks at λ_{max} of 225nm and the same absorption of 1.699. For the second peak Sunflower oil sample RS-02 varied from Sunflower oil sample RS-01 and RS-03 in both λ_{max} and absorbance. The RS-01 Sunflower oil peak had a λ_{max} of 300nm with absorbance of 2.723. The RS-02 and RS-03 Sunflower oil samples had similar λ_{max} of 315nm with same absorbance of 3.913.

Table 4.10: Absorbance for sunflower oils RS

Samples	Maximum Wavelength	Absorbance
RS-01	300.0 225.0	2.723 1.699
RS-02	315.0 225.0	3.913 1.699
RS-03	315.0 225.0	3.913 1.699

4.3.2: A UV/VIS spectra for sunflower oil sample SS

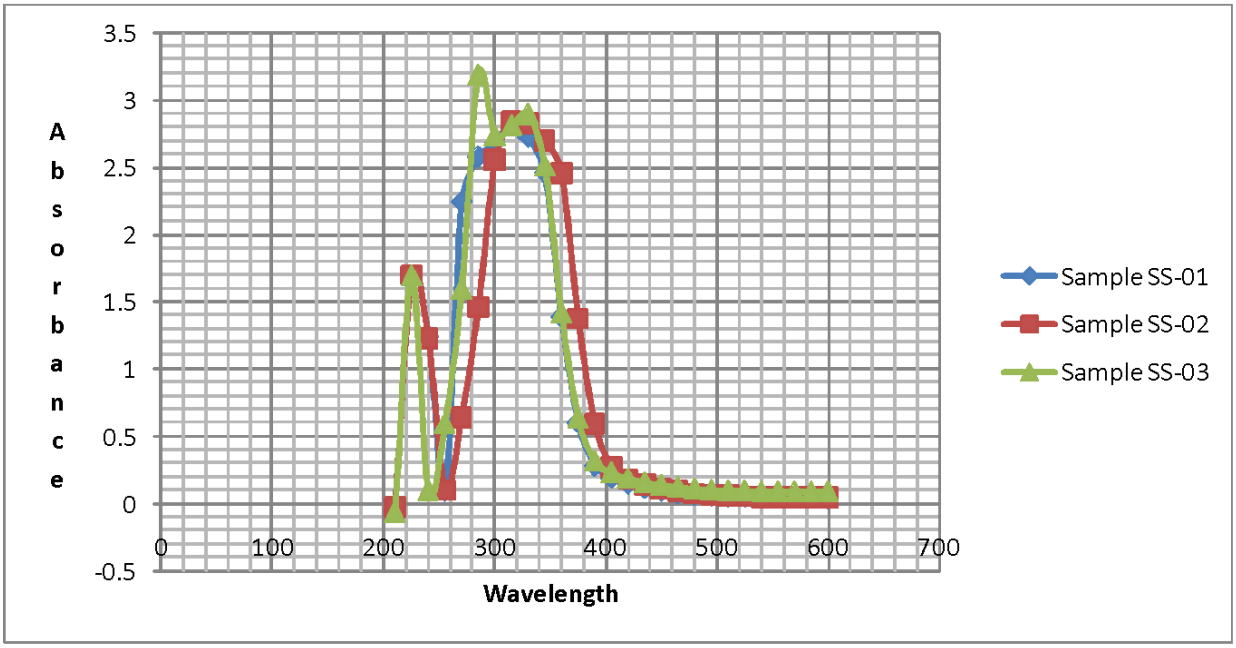


Figure 4.2: A UV/Vis spectrum of sunflower oil sample SS

Sunflower oil samples for SS-01, SS-02 and SS-03 had absorbance of 1.699 at λ_{max} of 225nm. The SS-01 and SS-02 Sunflower oil samples had similar second peak at absorbance of 2.840 and at the same

λ_{max} of 315nm. The SS-03 Sunflower oil sample had λ_{max} at 330nm and 285nm with absorbance of 2.897 and 3.250 respectively. The SS-03 Sunflower oil sample peak had the highest absorbance of 3.190 at λ_{max} of 285nm. The SS-03 had three peaks due to high levels of carotenoid pigments present in the sample.

Table 4.11: Absorbance for Sunflower oils SS

Samples	Maximum Wavelength	Absorbance
SS-01	300.0 225.0	2.840 1.699
SS-02	330.0 225.0	2.840 1.699
SS-03	315.0 285.0 225.0	2.897 3.250 1.699

4.3.3: A UV/VIS spectra for Sunflower oil sample ES

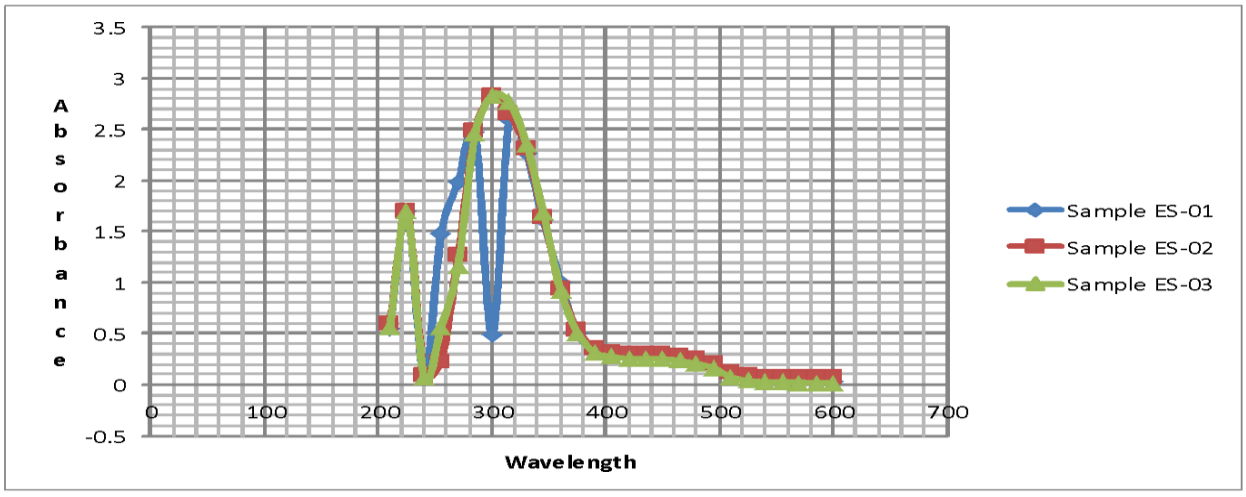


Figure 4.3: A UV/Vis spectrum of Sunflower oil sample ES

Sunflower oil samples ES-01, ES-02 and ES-03 had similar absorbance of 1.699 at λ_{max} of 225nm. The ES-01 Sunflower oil sample consisted of three peaks with λ_{max} of 315nm, 285nm and 225nm with absorbance of 2.596, 2.469 and 1.699 respectively. For the ES-02 and ES-03 Sunflower oil samples have similar peaks at λ_{max} of 300nm with similar absorbance of 2.840. The production process of ES-01 leaves high levels of carotenoid pigments compared to ES-02 and ES-03 hence the manufacture cannot reproduce the same colour intensity.

Table 4.12: Absorbance for sunflower oils ES

Samples	Maximum Wavelength	Absorbance
ES-01	315.0 285.0 225.0	2.596 2.469 1.699
ES-02	300.0 225.0	2.840 1.699
ES-03	300.0 225.0	2.840 1.699

4.3.4: A UV/VIS spectra for Corn oil sample EC

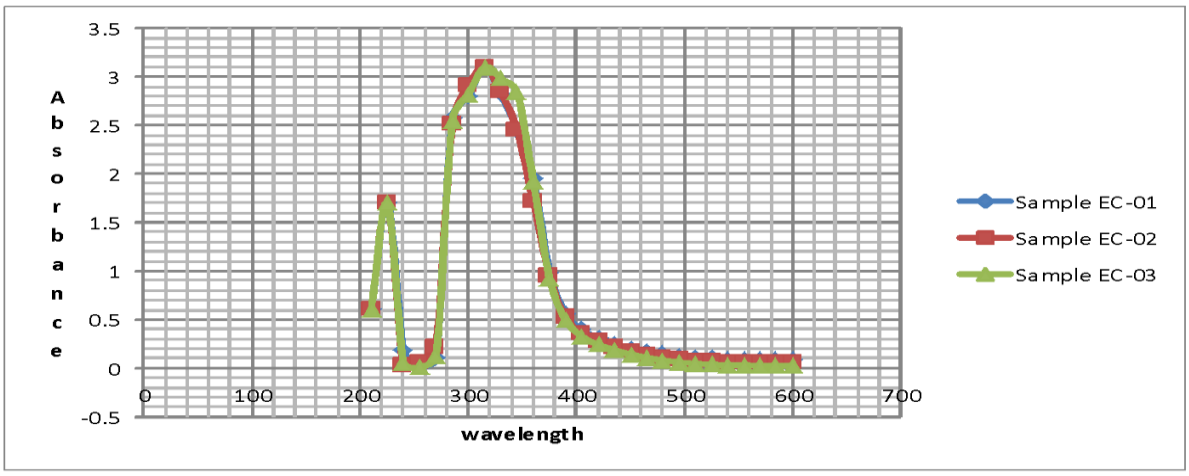


Figure 4.4: A UV/Vis spectrum of Corn oil sample EC

Corn oil samples EC-01, EC-02 and EC-03 the λ_{max} and the absorbance were perfectly similar to each other. The Corn oil samples had two well defined peaks of λ_{max} of 315nm and 225nm with absorbance of 3.101 and 1.699 respectively.

Table 4.13: Absorbance for corn oils EC

samples	Maximum Wavelength	Absorbance
EC-01	315.0 225.0	3.101 1.699
EC-02	315.0 225.0	3.101 1.699
EC-03	315.0 225.0	3.101 1.699

4.3.5: A UV/VIS spectra for Corn oil sample CC

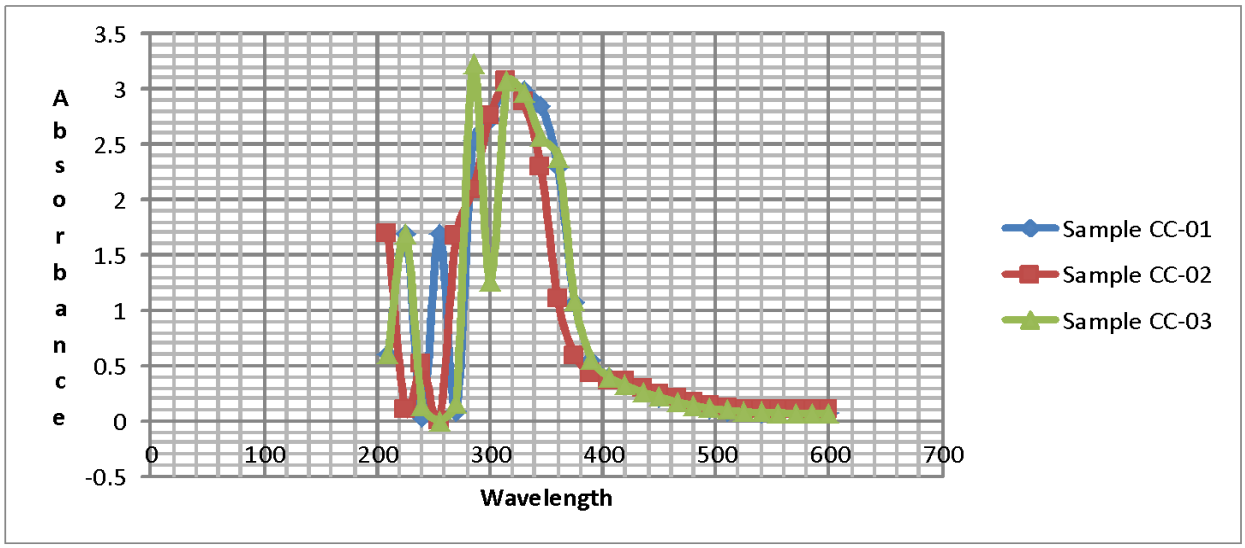


Figure 4.5: A UV/Vis spectrum of corn oil sample CC

Three Corn oil samples CC-01, CC-02 and CC-03 had λ_{max} of 315nm with absorbance of 3.068. The CC-03 Corn oil sample consisted of three peaks where λ_{max} were at 315nm, 285nm and 225nm with absorbance of 3.223, 3.068 and 1.699 respectively. The CC-01 and CC-02 corn oil samples had similar peaks with λ_{max} of 351nm and 225nm with absorbance of 3.068 and 1.699 respectively.

Table 4.14: Absorbance for Corn oils CC

samples	Maximum Wavelength	Absorbance
CC-01	315.0 225.0	3.068 1.699
CC-02	315.0 225.0	3.068 1.699
CC-03	315.0 285.0 225.0	3.068 3.223 1.699

4.3.6: A UV/VIS spectra for Corn oil sample DC

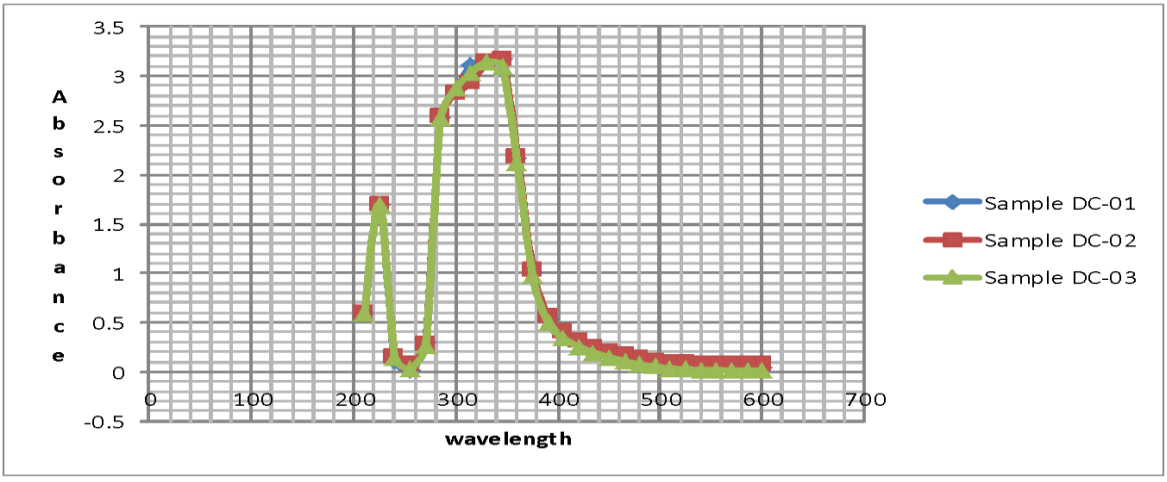


Figure 4.6: A UV/Vis spectrum of Corn oil samples DC

Corn oil samples DC-01, DC-02 and DC-03 had peaks at the same λ_{max}, 330nm and 225nm with absorbance of 3.141 and 1.699 respectively.

Table 4.15: Absorbance for corn oils DC

Samples	Maximum Wavelength	Absorbance
DC-01	330.0 225.0	3.141 1.699
DC-02	330.0 225.0	3.141 1.699
DC-03	330.0 225.0	3.141 1.699

4.3.7: A UV/VIS spectra for Soya bean oil sample SG

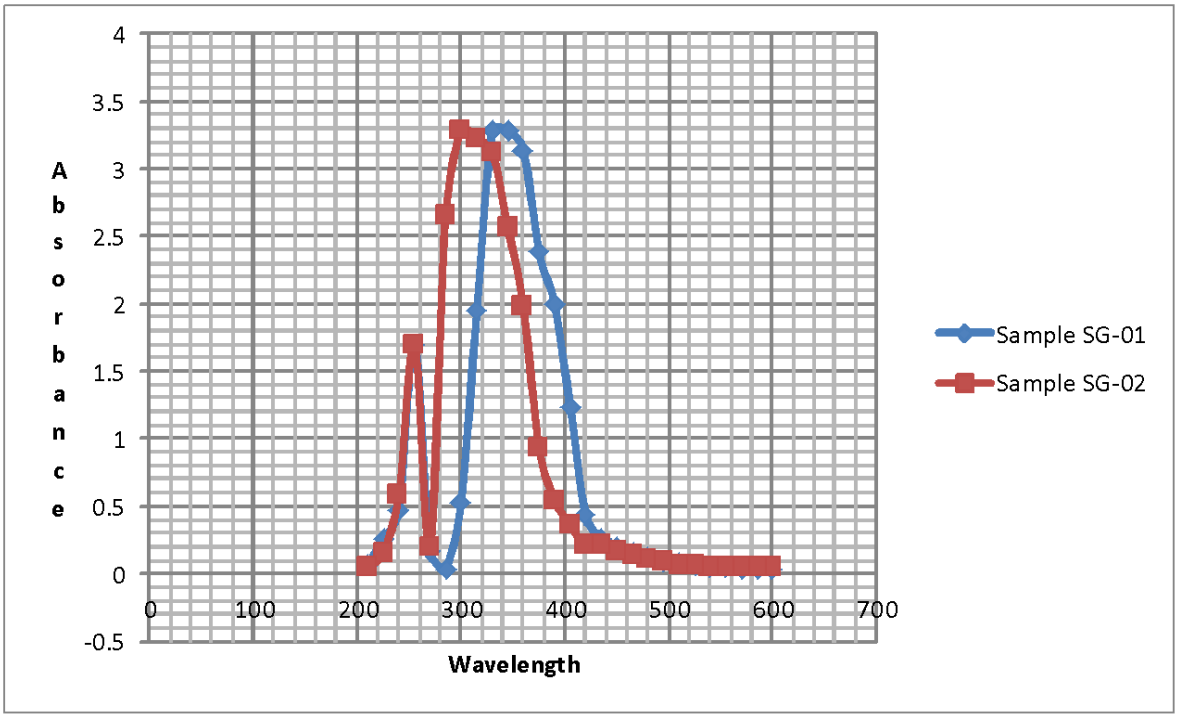


Figure 4.7: A UV/Vis spectrum of Soya oil sample SG

Soya bean oil samples of SG-01 and SG-02 had two well defined peaks with the same λ_{max} and absorbance. The λ_{max} recorded was 330nm and 225nm with absorbance of 3.286 and 1.699 respectively.

Table 4.16: Absorbance for Soya bean oils SG

Samples	Maximum Wavelength	Absorbance
SG-01	330.0 225.0	3.141 1.699
SG-02	330.0 225.0	3.286 1.699

4.3.8: A UV/VIS spectra for Soya bean oil sample HS

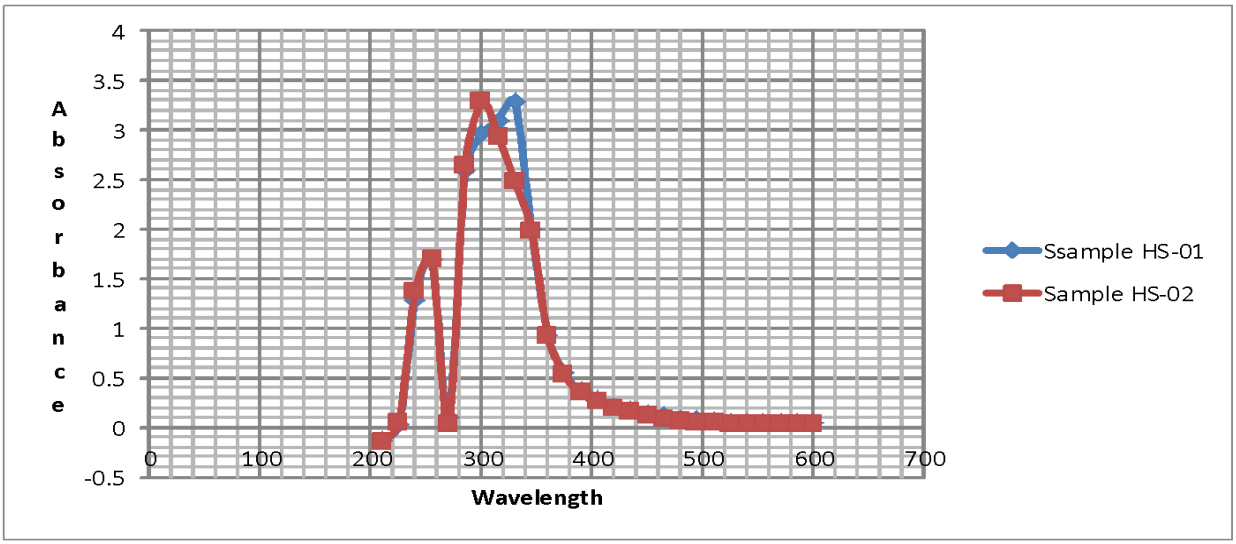


Figure 4.8: A UV/Vis spectrum of soya oil sample HS

Soya bean oil samples HS-01 and HS-02 had the same λ_{max} at 225nm with absorbance of 1.699. The HS-01sample HS sample second peak at λ_{max} 330nm had absorbance 3.286 and HS-02 sample had λ_{max} of 300nm with absorbance 3.286.

Table 4.17: Absorbance for Soya bean oils HS

Samples	**Maximum Wavelength**	**Absorbance**
HS-01	330.0 225.0	3.286 1.699
HS-02	300.0 225.0	3.286 1.699

Table 4.18: Specific maximum wavelength for oil samples

samples	**Maximum wavelength (nm)**	**Average Absorption range**
Sunflower oil samples		
RS	**315.0** **225.0**	**3.913** **1.699**
SS	**300.0** **315.0** **330.0** **225.0**	**2.840** **2.897** **2.840** **1.699**
ES	**300.0** **225.0**	**2.840** **1.699**
Corn oil samples		
EC	**315.0** **225.0**	**3.101** **1.699**

CC	**315.0** **225.0**	**3.068** **1.699**
DC	**330.0** **225.0**	**3.141** **1.699**
Soya bean oil samples **SG**	**330.0** **225.0**	**3.214** **1.699**
HS	**300.0** **330.0** **225.0**	**3.286** **3.286** **1.699**

Sunflower oil samples ES had absorbance an additional peak λ_{max} at 300.0nm with high absorbance of 2.644 to 2.840 and for the Corn oil samples DC also had an addition peak λ_{max} at 330.0 with high absorption (3.004 to 3.141). By looking at this two oil samples physical appearance using bare eyes one can identify that there colour is intense than the other in the sample category. This can be attributed to the method of extraction process, the type of seeds used and time of harvesting the seeds.

4.4: INFRARED SPECTROSCOPY

Below are the infrared spectroscopy spectra obtained for all the oil samples.

4.4.1: Infrared spectra for Sunflower oil sample

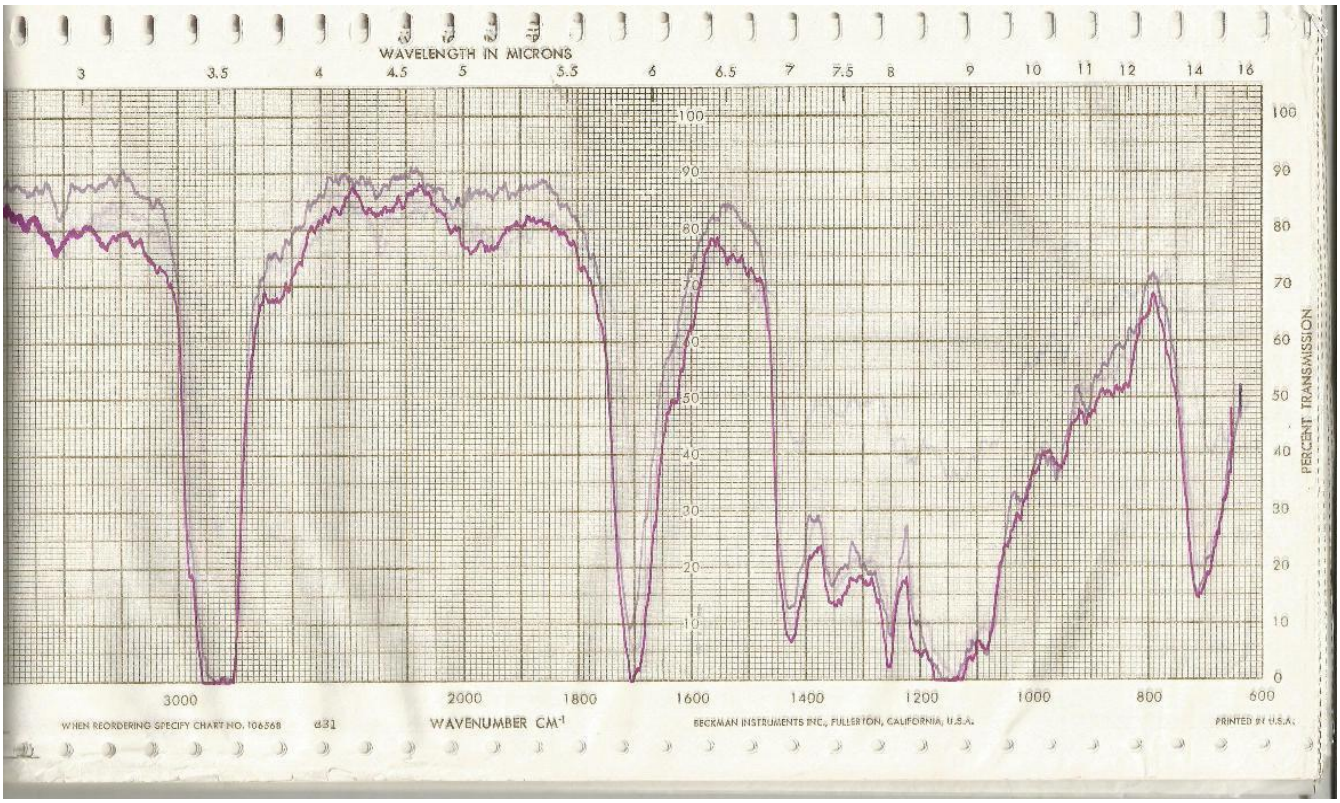


Figure 4.9: Infrared spectra for Sunflower oil samples

The infrared spectrum for Sunflower oil sample had peaks at the wave numbers; 3420cm^{-1}, 2925-2800cm^{-1}, 1710 cm^{-1}, 1430 cm^{-1}, 1350 cm^{-1}, 1250 cm^{-1},1080 cm^{-1},960 cm^{-1}, 910 cm^{-1} and 710 cm^{-1}.

4.4.2: Infrared spectrum for Corn oil sample

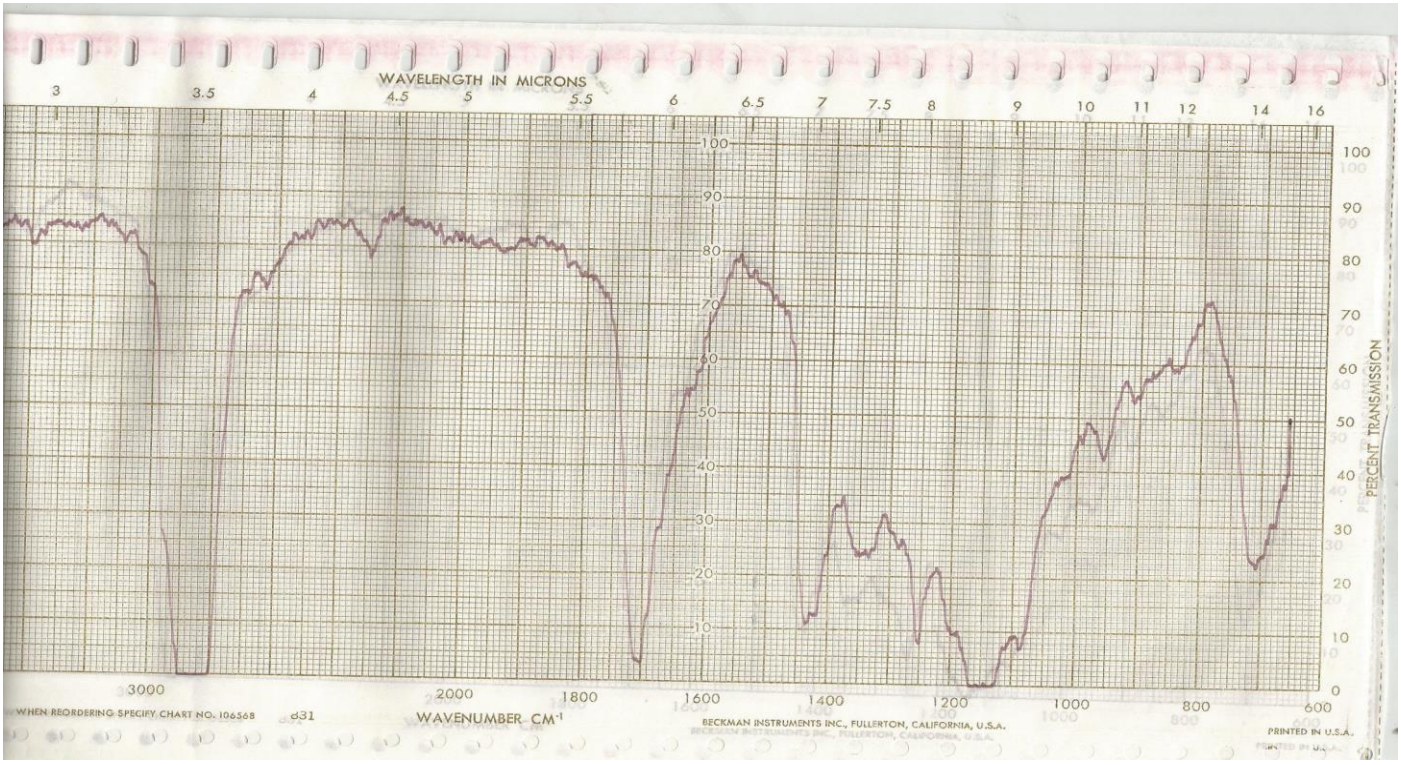


Figure 4.38: Infrared spectrum for Corn oil sample

The infrared spectrum for the Corn oil sample which had peaks at the following wave numbers; 3400 cm^{-1}, 2900-2800cm^{-1}, 2300 cm^{-1}, 1700 cm^{-1}, 1630 cm^{-1}, 1430 cm^{-1}, 1350 cm^{-1} , 1250 cm^{-1}, 1150cm^{-1}, 1080 cm^{-1}, 960 cm^{-1}, 900 cm^{-1},850 cm^{-1} and 700 cm^{-1}

4.4.3: Infrared spectrum for Soya bean oil sample

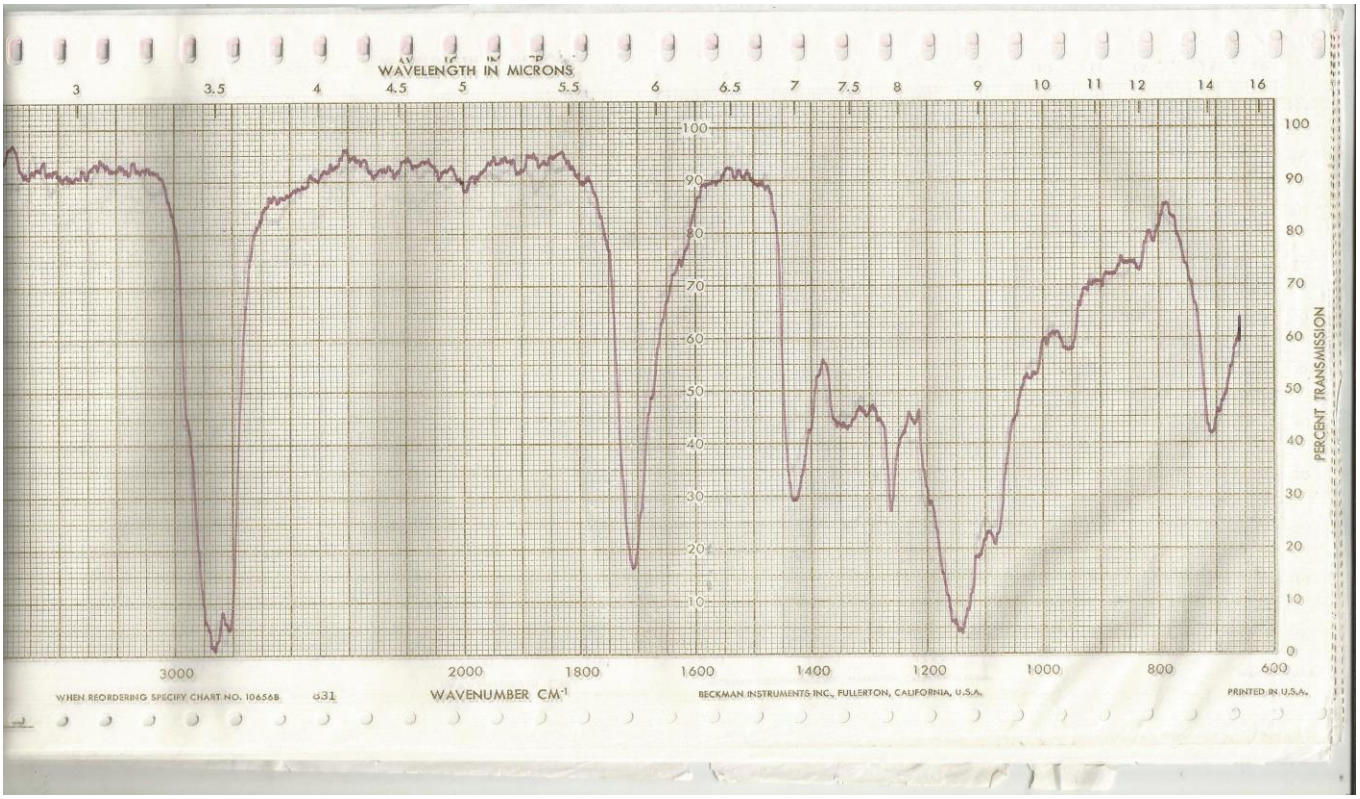


Figure 4.31: Infrared spectrum for Soya bean oil

The infrared spectrum for the Soya bean oil sample SG-01 which had peaks at the following wave numbers; 2880-2800cm^{-1}, 1700 cm^{-1} , 1430 cm^{-1}, 1350 cm^{-1} , 1270 cm^{-1}, 1150 cm^{-1}, 1080 cm^{-1}, 950 cm^{-1}, 850 cm^{-1} and 710 cm^{-1}.

Table 4.19: Summary of Infrared spectra

	SUNFLOWER OIL	CORN OIL	SOYA BEAN OIL
1	**3420 -3400 cm^{-1}**	**3420 -3400 cm^{-1}**	**2925 - 2850 cm^{-1}**
2	**2925 - 2800 cm^{-1}**	**2925 - 2800 cm^{-1}**	**1710 - 1700cm^{-1}**
3	**1710 – 1700cm^{-1}**	**1710 – 1700cm^{-1}**	**1430-1440 cm^{-1}**
4	**1430 -1420cm-1**	**1430 – 1420cm^{-1}**	**1360 – 1350cm^{-1}**
5	**1350-1300cm^{-1}**	**1350 – 1360 cm^{-1}**	**1270 -1260 cm^{-1}**
6	**1260-1240cm^{-1}**	**1270 -1250cm-1**	**1100-1090 cm^{-1}**
7	**960 -950cm^{-1}**	**1150-1130cm-1**	**950 cm^{-1}**
8	**710 - 700cm^{-1}**	**1090 - 1080cm^{-1}**	**900 cm^{-1}**
9		**950 - 960cm^{-1}**	**720-710 cm^{-1}**
10		**710-700cm^{-1}**	

Maximum absorption frequency of the major molecules of vegetable oils is presented in table 4.21. The IR spectra of vegetable oils present a series of bands with different in the absorption (appendix 15- 27). The spectrum interpretation was done by comparing the obtained spectrums with a series of preliminary data presented. The IR spectra of vegetable oils present a series of bands with different intensities and forms. Some regions of the spectra present a very good signal/noise ratio, which corresponds to various types of vibration characteristic to different types of atoms. Based on the absorption at different wavelengths are divided into six intervals spectra from 4000cm^{-1} to 500cm^{-1}.

It was evident that the interval of 4000-3100 cm^{-1} that corresponds first region where the vegetable oils did not have infrared absorption. Maximum absorption frequency of the major molecules of

vegetable oils is presented in table 4.15. The investigated sunflower oil sample absorbance was at 3420 – 3400cm^{-1} while for corn oil and soya bean oil samples they had no absorbance in this region of 4000 – 3100cm^{-1}.

In the second interval spectra of 3100-2800cm^{-1}from the IR spectra presents the absorption bands in the vicinity of frequencies 2925, 2900, 2850 and 2800 cm^{-1}. These absorptions characteristics to symmetrical and asymmetrical vibrations v(C-H) of the CH_2 and CH_3 aliphatic groups from the alkyl rest of the triglycerides, which are found in large quantities in vegetable oils. Absorption near 2900

cm^{-1} represents a significant index of the degree of unsaturated oil and it can be used in the identification of vegetable oils forgery. By measuring the absorption intensity near 2900cm^{-1}, one can make classification of vegetable oils and also identify the additional foreign oil in pure oil. For Sunflower oil (table 4.15) one observes that the absorption at 2925-2800 cm^{-1}, specific frequency of the methyl –oleate. Sunflower oil presents higher intensity of absorption 2950 cm^{-1}and lower one at 2800cm^{-1} due to increase content of linoleic acid in the composition of triglycerides. This absorbance was assigned to the symmetrical and asymmetrical C-H stretching vibration of CH_2 and CH_3 groups from the alkyl of the triglycerides, which are found in quantities in vegetable oils. Band around 3006cm^{-1} assigned to CH stretching vibration of Cis-double bond (=CH). Strong band absorption were observed in the region 3000- 2853 cm^{-1} respectively. The stretching vibration of methylene and methyl groups can also be seen at the 2922 and 2853 cm^{-1} respectively. Methylene and methyl groups are also observed at 1465cm^{-1} and 1377cm^{-1} due to their bending vibrations. A band shift observed at 3009 cm^{-1} was assigned to the C-H stretching vibration of Cis-double bond, which allows for the determination of oils. The differences in the specific spectral bands (3050- 2800 cm^{-1}) and 1745 cm^{-1}

showed that there extinguishable differences in the band around 3006cm^{-1} assigned to the C-H stretching vibration of the Cis- double bond (CH).

In the 3050-2800cm^{-1} intervals there are not differences in the absorption of samples of Sunflower, Corn and Soya bean oil, because of a similar content in unsaturated fatty acids. Sunflower, Corn and Soya bean oil samples at 2950 and 2850cm^{-1}, bands assigned to oleic and linoleic acids.

The third correspond to interval 1800-1600cm^{-1} and has two major bands near 1730 and 1690 cm^{-1}. The absorption at 1730 cm-1 is characteristic of short carbohydrate chain, does not show significant variations by modifying the content of sunflower oil, because of low content of saturated acids in both Corn and Soya bean oil samples. This spectral band near to 1700-1720 cm^{-1} corresponds to the double C-O double bond stretching vibration and C=C link and correlated with the content of polysaturated fatty acids in the molecule, which were determined by GC-FID. At 1700 cm^{-1} the frequency is assigned to the cis isomers of the molecule, and because the intensity of the absorption at this frequency is higher, the content of cis isomers is greater. It is also interesting to follow the spectral changes in the C-O region (~ 1700 cm^{-1}). This observation due to production of saturated aldehyde functional group or other secondary oxidation products that cause an absorbance at 1728cm^{-1} which overlaps with the stretching vibration at 1740cm^{-1} of the ester carbonyl functional group of the triglycerides.

The fourth interval corresponds to 1600 – 1390 cm^{-1} comprises a single spectral band near 1420-1445 cm^{-1} proper to the vibrations of deformation β (CH). This band can be used to determine the total unsaturation. In the 1600- 1390 cm^{-1} region, the IR spectrum of sunflower oils had a maximum at 1430 cm^{-1}, but its frequency doesn't have a linear variation with the modification of the sunflower oil content. all the samples of sunflower oil, corn oil and soybean oil have absorbance peak at this region.

Deformation and bending of C-H and stretching vibration of C-O result in peaks in the 1500-700cm^{-1} region.

The fifth interval corresponds to1390-1200 cm^{-1} includes two bands corresponds to the deformation vibration in the phase of methylene group, while the second band corresponds to deformation vibration in the plan of group =CH, from the double links cis unconjugated. The region has two maximum at 1360 cm-1 and 1270 cm^{-1}. Two bands close to 1350-1300cm^{-1} (assigned to deformation vibrations of methylene group) and 1250-1270cm^{-1} (specific to deformation vibration in-plane of =CH from the unconjugated as double bonds. The 1350-1300cm-1 absorbance band was observed in the sunflower oil samples and corn oil samples and none for the soybean oil samples. For the 1250-1260 cm^{-1} absorbance band was observed in all the oil samples.

The sixth interval corresponds to 1200-700 cm^{-1} contains bands characteristics to the C-C links and to the vibration links C=O. carbohydrates chain is characterized by a series of bands of vibrations due to the –C links at 1100-1000cm^{-1} and 900-800cm^{-1}. Carbohydrate chain vibrations of deformation were detected in the region 450-150 cm^{-1} too. Absorption in the domain 710-720 cm^{-1} is similar intensity for the samples of Sunflower oil and Corn oil; this is due to the carbohydrate radical from the triglyceride structure of oils.

The Infrared Spectra 2000 – 1000cm^{-1} shows differences in the peaks areas and peaks heights of absorption bands 1130 -1150cm^{-1} (assigned to C-O stretching and C-H bending) and at 1100- 1090 cm^{-1} (assigned to C-O stretching).

The average density for Sunflower oil and Corn oil samples 0.92 g/cm^3 and for Soya bean oil sample was 1.036 g/cm^3. The average refractive index for Sunflower and Corn oils samples were 1.465 and 1.467 nD respectively and Soya bean oil samples was 1.467 nD where they were within the accepted range of KEBS of 1.467-1.469. The average peroxide value for Sunflower, Corn and Soya bean oil was 6.667, 1.418 and 11.188 mmol/kg respectively. Sunflower, Corn and Soya bean oil sample had an average Saponification Value of 188.01, 187.34 and 188.32 mg KOH/g respectively. Specific IR spectra regions gives clear differences between Sunflower, Corn and Soya bean oils and occurred in the infrared middle spectra regions (1500-600cm^{-1}) and at 3007 -2853 cm^{-1}. The Sunflower oil consisted of eight distinct peaks while from Corn oil consisted of 10 distinct peaks. Soya bean oil had nine distinct peaks. Corn oil sample can be distinguished Soya bean oil and sunflower oil by the presence of 1150-1130 cm^{-1} and 1090-1080 cm^{-1} peaks. Soya bean oil can be distinguished from sunflower and Corn oil by the presence 1100-1090 cm^{-1} and 900 cm^{-1}. Soya bean oil also did not contain 3420-3400 cm^{-1}.

The protocol this research was to capture, understand, and model the process to be used in analysis of vegetable oils. Relating several physico-chemical parameters and spectroscopic techniques as shown in the schematic diagram 5.1. This research has synthesized physico-chemical and spectroscopic methods into a standard research protocol, from which we developed a procedural model that describes the process of conducting a quality assessment and identification of vegetable oils. The model protocol consists of a series of seven individual steps, each of which specifies detail for the type of analysis, how and why it is conducted, the tools used, the data input and output, and the interpretation of the results. The data obtained in analysis research will provides a rich high-level view of analytical information while providing a detailed analysis at the task level. In this article we concentrate on the latter.

4.5: A schematic protocol diagram for characteristics and development of quality of Sunflower, Corn and Soya bean oils

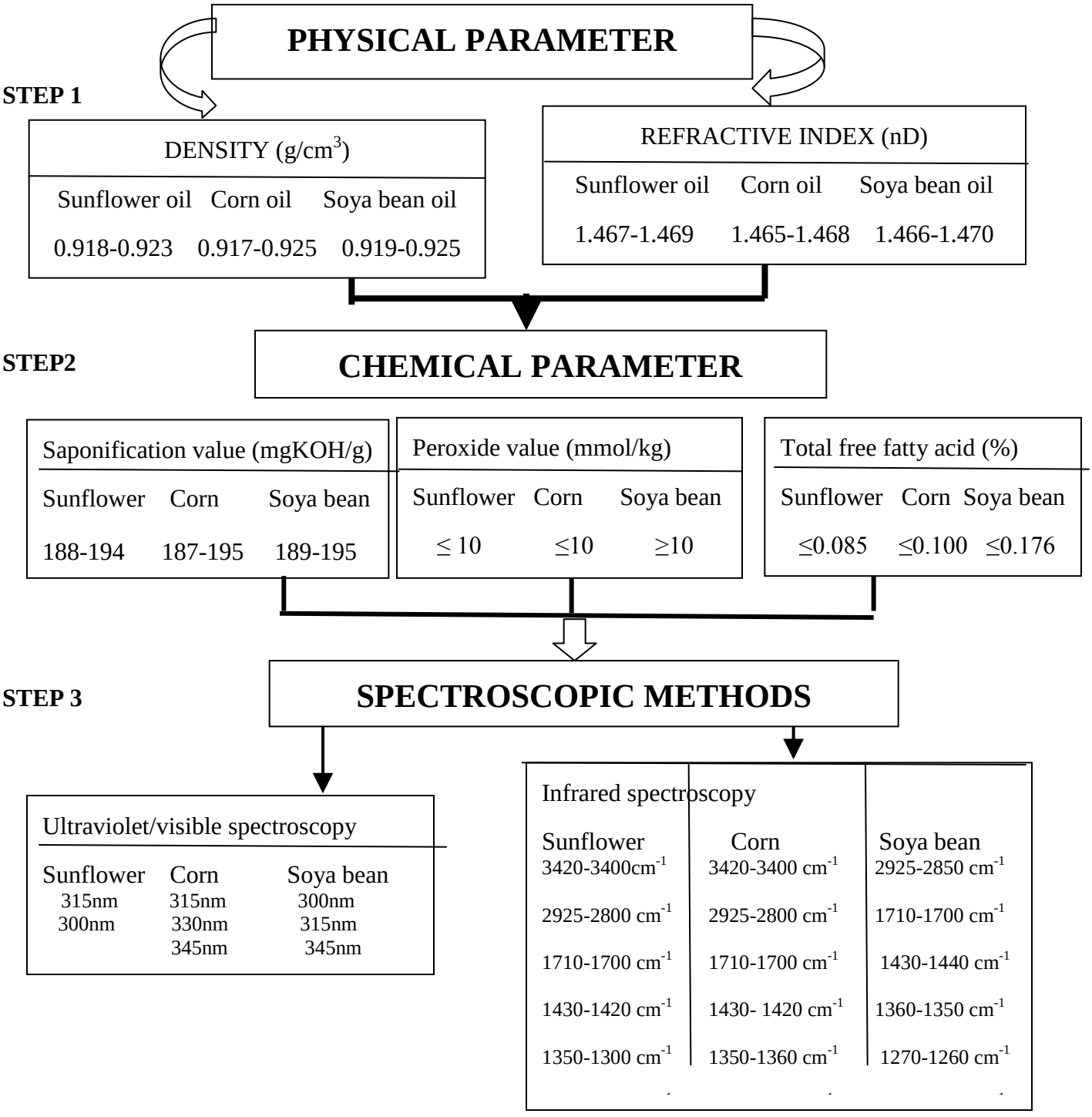


Figure 4.32: Layout protocol for characteristics and development of quality edible oils.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1: CONCLUSIONS

The density of Sunflower oils, Corn oil and Soya bean oil were within value of 0.918 -0.923 g/cm^3. Soya bean had the highest average density. The density of Sunflower and Corn oil had similar values. This implies density cannot be used to distinguish Sunflower oil from Corn oil. The Refractive Index for Sunflower, Corn and Soya bean oil were within the KEBS recommended value but for the Soya bean oil the Refractive Index was higher. In relation with density Soya bean oil has both density and Refractive Index parameters higher than Sunflower and Corn oil. For physical properties density and refractive index cannot be used to differentiate Sunflower, Corn and Soya bean oils due to similarity in their values.

The Saponification Value of Sunflower and soya bean oil were within the CODEX Standard recommended value. Corn oils had saponification values below their respective standards. Corn oil had the lowest average saponification value (187.34 mg KOH/g) which implies that Corn oil has short carbon chain of fatty acids. High values in Sunflower oil and Soya bean oils are attributed to the presence of high free fatty acids content. The results of Peroxide Value indicates that the characteristic of Sunflower and Corn oils are as per KEBS recommendation values apart from Soya bean oil that was higher which has Peroxide Value of 11.188 mmol/kg. Quality evaluation through total free fatty acid percentage value sample and the Peroxide Value confirmed that the quality of these oils was satisfactory. Freshly deodorized oil should have zero Peroxide Value, but in most cases, for the product to have acceptable storage stability the PV of oils for domestic use be less than 10.The free fatty acids percentage values for Sunflower and Corn oil were below as per KEBS recommended values. Soya bean oil was within the recommended value and had the highest free fatty acids

percentage value. The highest free fatty acids content for Sunflower and Corn oil which was contributed by variation in moisture contents, refining and deodorization process used. Chemical properties peroxide value, saponification value and free fatty acids cannot differentiate Sunflower oil, Corn oil and Soya bean oil.

In spectroscopic methods, the oils were analyzed by means of UV/Vis and IR spectroscopy. UV/Vis analysis was able to discriminate oils by differentiating in their λmax absorbance. IR analysis revealed that each vegetable oil could be discriminated by looking at the pattern of each individual spectra.

UV/Vis spectroscopy was used to discriminate oils by the intensity of their λmax. Sunflower, Corn and Soya bean oil had maximum wavelength as per Table 4.18. Sunflower oil , Corn oil and Soya bean oil samples showed similar values of maximum wavelength hence this disqualify out ultraviolet/visible spectroscopy for identification of different oil samples.

The results indicate that specific IR spectra regions prove to be very useful for identification of Sunflower, Corn and Soya bean oils. Vegetable oils contain the same type of fatty acids especially those C16 and C18 and triglycerides content is similar (C50, C52, C54), however, subtle spectral differences exist in Sunflower, Corn and Soya bean oils, so it is possible to identify the addition of foreign. The IR spectra for sunflower, corn and soya bean oil sample were distinguished by specific fingerprint. The use of infrared for analysis of edible vegetable oils is rapid because no sample preparation. Infrared spectroscopy can be used at the differentiation and classification stages of vegetable oils. This technique also allow for classification as well as determination of purity and authenticity of vegetable oils, such as corn, soya bean and sunflower oils

For this project we derive a protocol for analysis of edible oil in manufacturing industries. Infrared spectroscopy remains relatively inexpensive when compared to equivalent technologies.

5.2: RECOMMENDATION

1. Manufacturers are encouraged to use of infrared spectroscopy for identification of vegetable edible oils for routine analysis in quality assessment. Advantages of near infrared spectroscopy include minimal sample preparation (may be performed in situ in many instances), rapid analysis and sample preservation

2. Expansion of scope in analysis of vegetable oils using IR to establish fingerprints for each vegetable oil to be used as a standard references.

3. Recommend to use the protocol for analysis of edible vegetable oils by Kenya manufacturers for differentiating and discrimination between these vegetable oils.

4. To create awareness to vegetable manufacturers in Kenya on quality assessment of vegetable oils and use of infrared technology as a method to identify both raw material and finished products.

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APPENDICES

Appendix 1: Beiersdorf (EA LTD Customer data base-2011).

Data from beiersdorf (EA) Ltd customer data base file 2011		
Supermarket within Nairobi with more than two branches		
OUTLET	AREA	BRANCHES
Armed forces canteen organization	Nairobi	10
Nakumatt Holdings	Nairobi	10
Tuskys Ltd	Nairobi	10
Naivas Ltd	Nairobi	7
Ukwala s/market (PD)	Nairobi	5
Chandarana s/market (PD)	Nairobi	5
Woolmatt Ltd	Nairobi	3
Eastleigh matresses (PD)	Nairobi	4
Home choice s/market (PD)	Pangani	4

Appendix 2: physicochemical characteristic standards of edible oils by World Health Organization (WHO).

	SUNFLOWER	**CORN**	**SOYA BEAN**
Refractive index (nD^{25}) at 40°C	1.467-1.469	1.466	1.466-1.470
Specific density (g/cm3)	0.9188	0.91875	0.9260
Saponification value (mg KOH/g)	188-194	187-193	189-195
Free fatty acids (FFA %)	0.085	0.100	0.176
Acid value (mg (KOH/g)	≤ 0.6	≤ 0.6	≤ 0.6
Peroxide value Meq O_2/100g sample	≤ 10	≤ 10	≤ 10
Iodine value (g iodine/100g sample)	110-143	127-133	120-143

Appendix 3: Recommended (standards) physicochemical characteristic of edible oils by KENYA BUREAU OF STANDARDS (KEBS)

CHARACTERISTICS	REQUIREMENTS
Acid value, (mg/KOH/g (max.)	0.6
Free fatty acid, % (max.), as oleic	0.25
Peroxide value, (mEq oxygen/kg (max.))	10

Reference: KS 2435 (2012) (English): Blended Edible Oils – Specification (Draft Standard)

Appendix 4: Recommended (standards) physicochemical characteristic of edible oils by Codex Standard for Named Vegetable Oils (CODEX-STAN 210 - 1999)

	SUNFLOWER	**CORN**	**SOYA BEAN**
Refractive index (nD^{25}) at 40°C	1.461-1.468	1.465-1.468	1.466-1.470
Specific density (g/cm3) x=20ºC	0.918-0.923	0.917-0.925	0.919-0.925
Saponification value (mg KOH/g)	188-194	187-195	189-195
Iodine value (g iodine/100g sample)	118-141	103-135	124-139

Appendix 5: codex standards for fatty acids of vegetable oils

Fatty acid	Soybean oil		Cottonseed oil		Sunflower-seed oil	
	1981	1993	1981	1993	1981	1993
C14:0	< 0.5	< 0.2	0.4-2	0.6-1	< 0.5	< 0.2
C16:0	7-14	8-13.3	17-31	21.4-26.4	3-10	5.6-7.6
C16:1	< 0.5	< 0.2	0.5-2	0-1.2	< 1	< 0.3
C18:0	1.4-5.5	2.4-5.4	1-4	2.1-3.3	1-10	2.7-6.5
C18:1	19-30	17.7-26.1	13-44	14.7-21.7	14-65	14-39.4
C18:2	44-62	49.8-57.1	33-59	46.7-58.2	20-75	48.3-74
C18:3	4-11	5.5-9.5	0.1-2.1	0-0.4	0-0.7	0-0.2
C20:0	<1	0.1-0.6	0-0.7	0.2-0.5	0-1.5	0.2-0.4
C20:1	<1	<0.3	0-0.5	0-0.1	0-0.5	0-0.2
C22:0	< 0.5	0.3-0.7	0-0.5	0-0.6	0-1	0.5-1.3
C22:1	-	< 0.3	0-0.5	0-0.3	0-0.5	0-0.2
C22:2	-	-	-	-	-	0-0.3
024:0	-	< 0.4	0-0.5	0-0.1	0-0.5	0.2-0.3
C24:1	-	-	-	-	< 0.5	-

Sources: Codex Alimentarius Commission, 1983,1993.

Appendix 6: Characteristic IR Absorption Frequencies of Organic Functional Groups

Functional Group	Type of Vibration	Characteristic Absorptions (cm^{-1})	Intensity
Alcohol			
O-H	(stretch, H-bonded)	3200-3600	strong, broad
O-H	(stretch, free)	3500-3700	strong, sharp
C-O	(stretch)	1050-1150	strong
Alkane			
C-H	stretch	2850-3000	strong
-C-H	bending	1350-1480	variable
Alkene			
=C-H	stretch	3010-3100	medium
=C-H	bending	675-1000	strong
C=C	stretch	1620-1680	variable
Alkyl Halide			
C-F	stretch	1000-1400	strong
C-Cl	stretch	600-800	strong
C-Br	stretch	500-600	strong
C-I	stretch	500	strong
Alkyne			
C-H	stretch	3300	strong,sharp
–C≡C–	stretch	2100-2260	variable, not present in symmetrical alkynes
Amine			
N-H	stretch	3300-3500	medium (primary amines have two bands; secondary have one band, often very weak)
C-N	stretch	1080-1360	medium-weak
N-H	bending	1600	medium
Aromatic			

C-H	stretch	3000-3100	medium
C=C	stretch	1400-1600	medium-weak, multiple bands
Analysis of C-H out-of-plane bending can often distinguish substitution patterns			
Carbonyl	Detailed Information on Carbonyl IR		
C=O	stretch	1670-1820	strong
(conjugation moves absorptions to lower wave numbers)			
Ether			
C-O	stretch	1000-1300 (1070-1150)	strong
Nitrile			
CN	stretch	2210-2260	medium
Nitro			
N-O	stretch	1515-1560 & 1345-1385	strong, two bands

Appendix 7: Characteristic IR Absorptions

frequency, cm–1	*bond*	*functional group*
3640–3610 (s, sh)	O–H stretch, free hydroxyl	alcohols, phenols
3500–3200 (s,b)	O–H stretch, H–bonded	alcohols, phenols
3400–3250 (m)	N–H stretch	primary, secondary amines, amides
3300–2500 (m)	O–H stretch	carboxylic acids
3330–3270 (n, s)	–C(triple bond)C–H: C–H stretch	alkynes (terminal)
3100–3000 (s)	C–H stretch	aromatics
3100–3000 (m)	=C–H stretch	alkenes
3000–2850 (m)	C–H stretch	alkanes
2830–2695 (m)	H–C=O: C–H stretch	aldehydes
2260–2210 (v)	C(triple bond)N stretch	nitriles
2260–2100 (w)	–C(triple bond)C– stretch	alkynes
1760–1665 (s	C=O stretch	carbonyls (general)
1760–1690 (s)	C=O stretch	carboxylic acids

1750–1735 (s)	C=O stretch	esters, saturated aliphatic
1740–1720 (s)	C=O stretch	aldehydes, saturated aliphatic
1730–1715 (s)	C=O stretch	alpha,beta–unsaturated esters
1715 (s)	C=O stretch	ketones, saturated aliphatic
1710–1665 (s)	C=O stretch	alpha,beta–unsaturated aldehydes, ketones
1680–1640 (m)	–C=C– stretch	alkenes
1650–1580 (m)	N–H bend	primary amines
1600–1585 (m)	C–C stretch (in–ring)	aromatics
1550–1475 (s)	N–O asymmetric stretch	nitro compounds
1500–1400 (m)	C–C stretch (in–ring)	aromatics
1470–1450 (m)	C–H bend	alkanes
1370–1350 (m)	C–H rock	alkanes
1360–1290 (m)	N–O symmetric stretch	nitro compounds
1335–1250 (s)	C–N stretch	aromatic amines
1320–1000 (s)	C–O stretch	alcohols, carboxylic acids, esters, ethers
1300–1150 (m)	C–H wag (–CH_2X)	alkyl halides
1300–1150 (m)	C–H wag (–CH2X)	alkyl halides
1250–1020 (m)	C–N stretch	aliphatic amines
1000–650 (s)	=C–H bend	alkenes
950–910 (m)	O–H bend	carboxylic acids
910–665 (s, b)	N–H wag	primary, secondary amines
900–675 (s)	C–H "oop"	aromatics
850–550 (m)	C–Cl stretch	alkyl halides
725–720 (m)	C–H rock	alkanes
700–610 (b, s)	–C(triple bond)C–H: C–H bend	alkynes
690–515 (m)	C–Br stretch	alkyl halides

Appendix 8: Average density for Sunflower, Corn and Soya bean oil samples

Sample lab code	Temperature of distilled water	Pycnometer with sample g (M_1)	Weight of sample (g) (M1-Mo=M_L)	DENSITY (g/cm^3)	AVERAGE DENSITY (g/cm^3)
SUNFLOWER					
RS	24	66.090	45.981	0.919	**0.919**
	25	66.092	45.983	0.919	
	25	66.091	45.982	0.919	
SS	25	66.165	46.056	0.921	**0.921**
	24	66.163	46.054	0.921	
	25	66.164	46.055	0.921	
ES	25	66.097	45.988	0.919	**0.919**
	25	66.096	45.987	0.919	
	25	66.097	45.988	0.919	
CORN OIL					
EC-01	24	66.075	45.966	0.919	**0.919**
	25	66.078	45.969	0.919	
	25	66.076	45.967	0.919	
CC	25	66.004	45.895	0.917	**0.918**
	25	66.011	45.902	0.918	
	24	66.009	45.900	0.918	
DC	25	65.989	45.880	0.917	**0.917**
	25	65.992	45.883	0.917	
	25	65.990	46.881	0.917	
SOYA BEAN OIL					
SG	25	73.479	53.371	1.067	**1.067**
	25	73.504	53.395	1.067	
HS	25	70.294	50.185	1.003	**1.004**
	25	70.319	50.210	1.004	

Appendix 9: Average Relative Index for Sunflower oil samples

Sample lab code	Temperature T^1 (oC)	nD	Average nD	RELATIVE INDEX (nD at 40^o C)	
RS-01	22	1.4728 1.4729 1.4729	1.47283	**1.466**	**1.466**
	25	1.4722 1.4721 1.4724	1.47223	**1.466**	
	21	1.4731 1.4730 14733	1.47313	**1.466**	
SS	22	1.4736 1.4736 1.4734	1.47353	**1.466**	**1.466**
	23	1.4734 1.4735 1.4734	1.47343	**1.466**	
	21	1.4737 1.4736 1.4736	1.47363	**1.466**	
ES	22	1.4730 1.4731 1.4731	1.473067	**1.466**	**1.464**
	22	1.4731 1.4731 1.4731	1.4731	**1.466**	
	21	1.4733 1.4735 1.4733	1.47337	**1.460**	

Appendix 10: Average Relative Index for Corn oil samples

Sample lab code	Temperature (oC)	nD	Average nD	RELATIVE INDEX (nD at 40^{o} C)	
EC-01	22	1.4724 1.4723 1.4721	1.472267	**1.465**	**1.465**
	25	1.4722 1.4720 1.4721	1.4721	**1.466**	
	21	1.4727 1.4728 1.4727	1.472733	**1.465**	
CC	22	1.4713 1.4710 1.4711	1.47113	**1.464**	**1.464**
	24	1.4707 1.4707 1.4706	1.47073	**1.465**	
	22	1.4715 1.4713 1.4714	1.4714	**1.464**	
DC	22	1.4713 1.4711 1.4711	1.471164	**1.464**	**1.464**
	24	1.4706 1.4707 1.4706	1.47063	**1.464**	
	23	1.4709 1.4709 1.4710	1.470933	**1.464**	

Appendix 11: Average Relative Index for Soya bean oil samples

Sample lab code	Temperature (oC)	nD	Average nD	RELATIVE INDEX (nD at 40^{o} C)	
SG	23	1.4623 1.4622 1.4623	1.4623	1.469	1.469
	23	1.4621 1.4623 1.4623	1.4622	1.469	
HS	23	1.4652 1.4651 1.4651	1.4651	1.472	1.472
	23	1.4653 1.4653 1.4653	1.4653	1.472	

Appendix 12: Average Saponification Value for Sunflower oil samples

SAMPLES	AVARAGE WEIGHT OF SAMPLE (g)	AVARAGE TITRE VOLUME (ml)	AVERAGE VOLUME (ml)	AVERAGE DIFFERENCE	AVERAGE SAPONIFICATION VALUE (mg $_{KOH}$ / g sample)	
Sunflower oil						
RS	2.000g	26.3ml	26.23ml	13.53	**189.79**	**190.35**
	2.001g	26.5ml	26.43ml	13.73	**192.50**	
	2.002g	26.3ml	26.17	13.47	**188.76**	
SS	2.000g	26.1ml	26.03ml	13.33	**186.99**	**184.56**
	2.002g	25.9ml	25.83ml	13.13	**183.99**	
	2.001g	25.8ml	25.733ml	13.03	**182.69**	
ES	2.000g	26.4ml	26.33	13.63	**191.19**	**189.13**
	2.001g	26.2ml	26.07	13.37	**187.45**	
	2.002g	26.17ml	26.17	13.47	**188.75**	

Appendix 13: Average Saponification Value for Corn oil samples

SAMPLES	WEIGHT OF SAMPLE (g)	TITRE VOLUME (ml)	AVERAGE VOLUME (ml)	AVERAGE DIFFERENCE	SAPONIFICATION VALUE (mg $_{KOH}$ / g sample)	
Corn oil						
EC	2.000g	26.3ml	26.23ml	13.53	**189.79**	**190.35**
	2.001g	26.5ml	26.43ml	13.73	**192.50**	
	2.002g	26.3ml	26.17	13.47	**188.76**	

CC	2.000g	26.1ml	26.03ml	13.33	**186.99**	**184.56**
	2.002g	25.9ml	25.83ml	13.13	**183.99**	
	2.001g	25.8ml	25.733ml	13.03	**182.69**	
DC	2.000g	26.4ml	26.33	13.63	**191.19**	**187.10**
	2.001g	26.2ml	26.07	13.37	**187.45**	
	2.001g	25.7ml	25.73	13.03	**182.67**	

Appendix 14: Average Saponification Value for Soya bean oil samples

SAMPLES	**WEIGHT OF SAMPLE (g)**	**TITRE VOLUME (ml)**	**AVERAGE VOLUME (ml)**	**AVERAGE DIFFERENCE**	**SAPONIFICATION VALUE (mg $_{KOH}$ / g sample)**	
Soya bean oil						
SS	2.000g	26.3ml	26.23ml	13.53	**189.79**	**191.15**
	2.001g	26.5ml	26.43ml	13.73	**192.50**	
HS	2.000g	26.1ml	26.03ml	13.33	**186.99**	**185.49**
	2.002g	25.9ml	25.83ml	13.13	**183.99**	

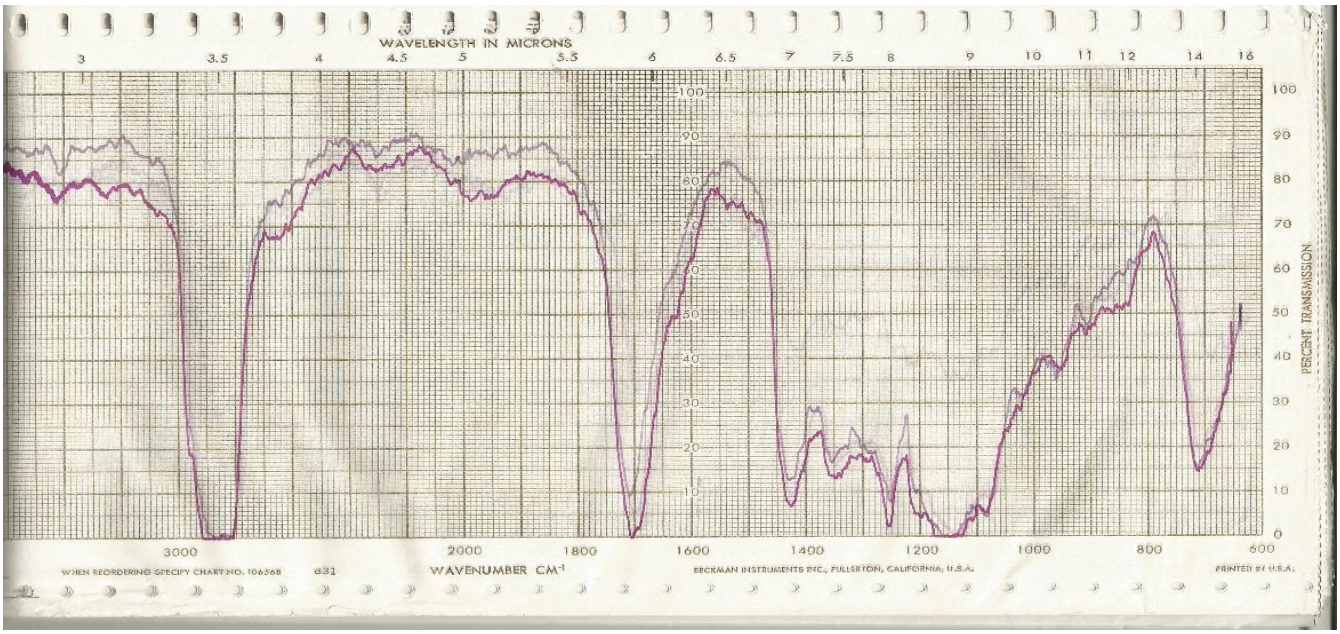
Appendix 15: Average Peroxide Value for Sunflower, Corn and Soya bean oil samples

SAMPLE	AVERAGE MASS OF $Na_2S_2O_3$ (g)	AVERAGE VOLUME OF $Na_2S_2O_3$ (ml)	AVARAGE PEROXIDE VALUE (mmol peroxide/ Kg sample)	AVERAGE PEROXIDE VALUE (mmol peroxide/ Kg sample)
SUNFLOWER				
RS	3.002	2.1	6.9968	6.665
	3.000	2.0	6.6665	
	3.000	1.9	6.3315	
SS	3.001	2.0ml	6.6643	6.553
	3.000	1.9ml	63315	
	3.001	2.0ml	6.6643	
ES	3.002	2.2ml	7.3316	7.111
	3.000	2.1ml	7.0015	
	3.001	2.1ml	6.999	
CORN OIL				
EC	3.000g	0.4ml	1.3065	1.083
	3.0001g	0.3ml	0.9715	
	3.001g	0.3ml	0.9715	
CC	3.000g	0.4ml	1.3065	1.418
	3.001g	0.5ml	1.6409	
	3.001g	0.4ml	1.3061	
DC	3.001g	0.5ml	1.9758	1.753
	3.000g	0.5ml	1.6415	
	3.000g	0.5ml	1.6415	
SOYA BEAN OIL				
SG	3.000g	3.6	12.0265	11.913
	3.000g	3.5	11.6915	
HS	3.000g	3.2	10.6865	10.463
	3.000g	3.1	10.3515	

Appendix 16: Average % Free Fatty Acid for Sunflower, Corn and Soya bean oil samples

SAMPLES	AVERAGE MASS(g)	AVERAGE TITRE (ml)	AVERAGE ACID VALUE	AVERAGE % FREE FATTY ACID	AVERAGE %FFA
SUNFLOWER					
RS	**5.000**	**0.2**	**0.224**	**0.113**	**0.094**
	5.000	**0.2**	**0.224**	**0.113**	
	5.000	**0.1**	**0.112**	**0.056**	
SS	**5.000**	**0.3**	**0.337**	**0.169**	**0.150**
	5.000	**0.3**	**0.337**	**0.169**	
	5.000	**0.2**	**0.224**	**0.113**	
ES	**5.001**	**0.2**	**0.224**	**0.113**	**0.075**
	5.001	**0.1**	**0.112**	**0.056**	
	5.000	**0.1**	**0.112**	**0.056**	
CORN OIL					
EC	**5.000**	**0.3**	**0.449**	**0.226**	**0.266**
	5.000	**0.3**	**0.449**	**0.226**	
	5.000	**0.3**	**0.449**	**0.226**	
CC	**5.000**	**0.2**	**0.224**	**0.113**	**0.094**
	5.000	**0.2**	**0.224**	**0.113**	
	5.000	**0.1**	**0.112**	**0.056**	
DC	**5.000**	**0.2**	**0.224**	**0.113**	**0.075**
	5.000	**0.1**	**0.112**	**0.056**	
	5.000	**0.1**	**0.112**	**0.056**	
SOYA BEAN OIL					
SG	**5.000**	**0.2**	**0.224**	**0.113**	**0.113**
	5.000	**0.2**	**0.224**	**0.113**	
HS	**5.000**	**0.3**	**0.449**	**0.226**	**0.188**
	5.000	**0.3**	**0.449**	**0.226**	

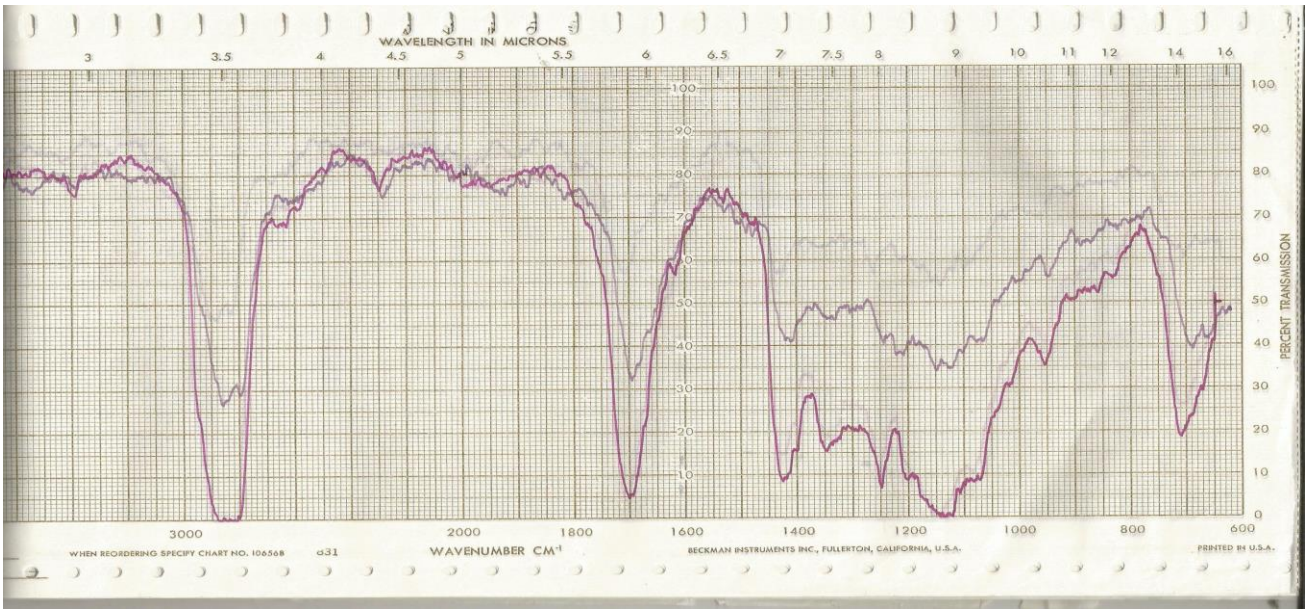
Appendix 17: Infrared Spectra for Sunflower oil RS and ES samples



Key:

———	Sunflower oil sample RS
———	Sunflower oil sample ES

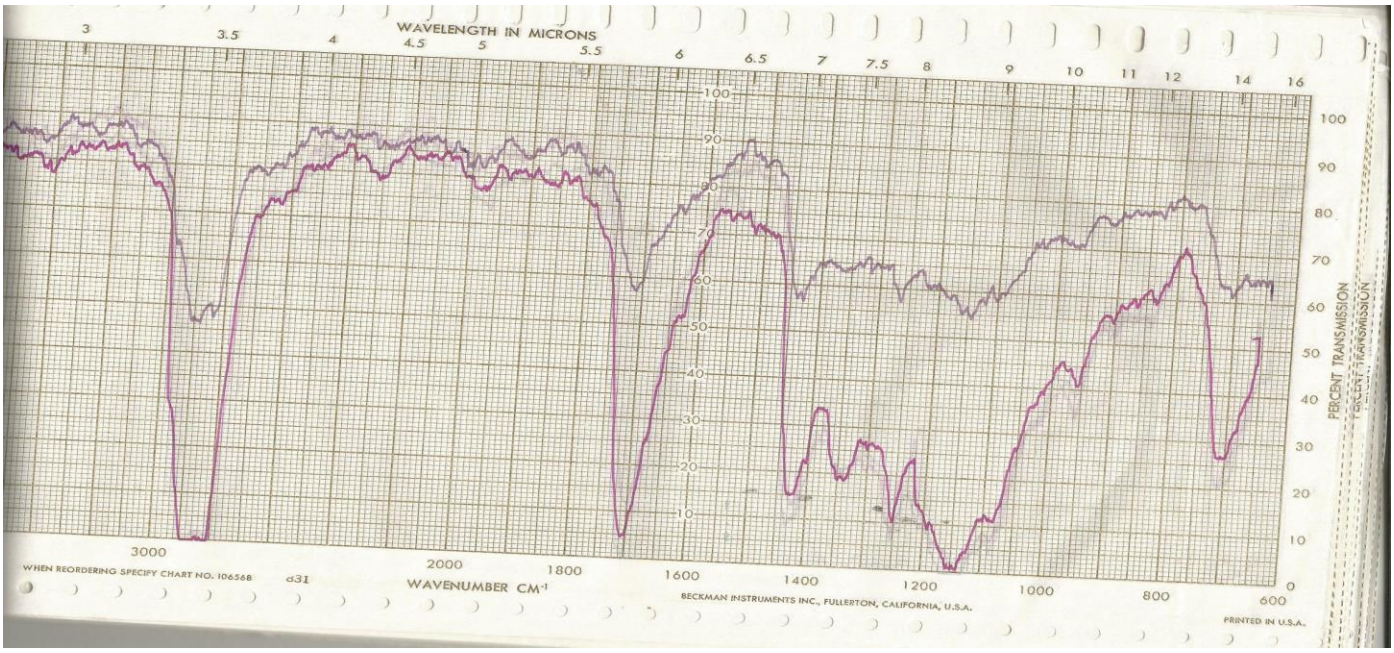
Appendix 18: Infrared Spectra for Sunflower oil RS and Corn oil DC samples



Key:

———	Sunflower oil Sample RS
———	Corn oil Sample DC

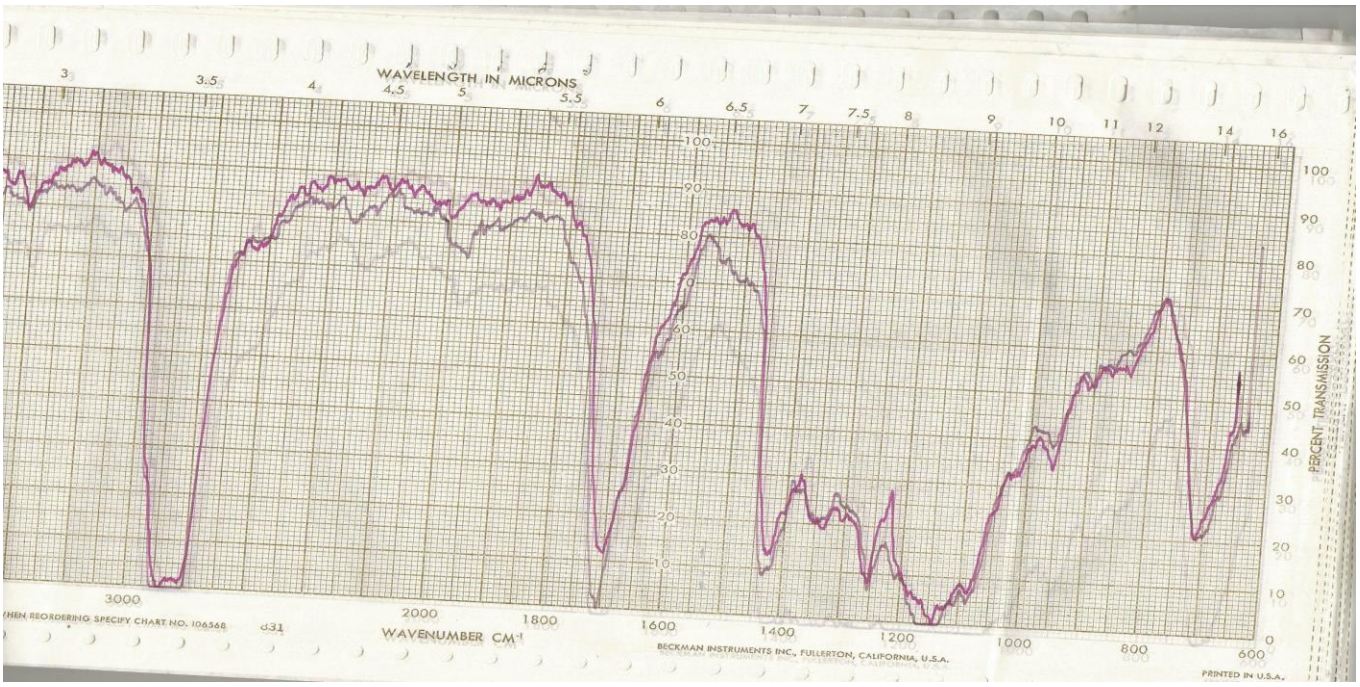
Appendix 19: Infrared Spectra for Sunflower oil RS and Corn EC samples



Key:

▬	Sunflower oil sample RS
▬	Sunflower oil sample EC

Appendix 20: Infrared Spectra for Sunflower oil SS and Corn oil EC samples

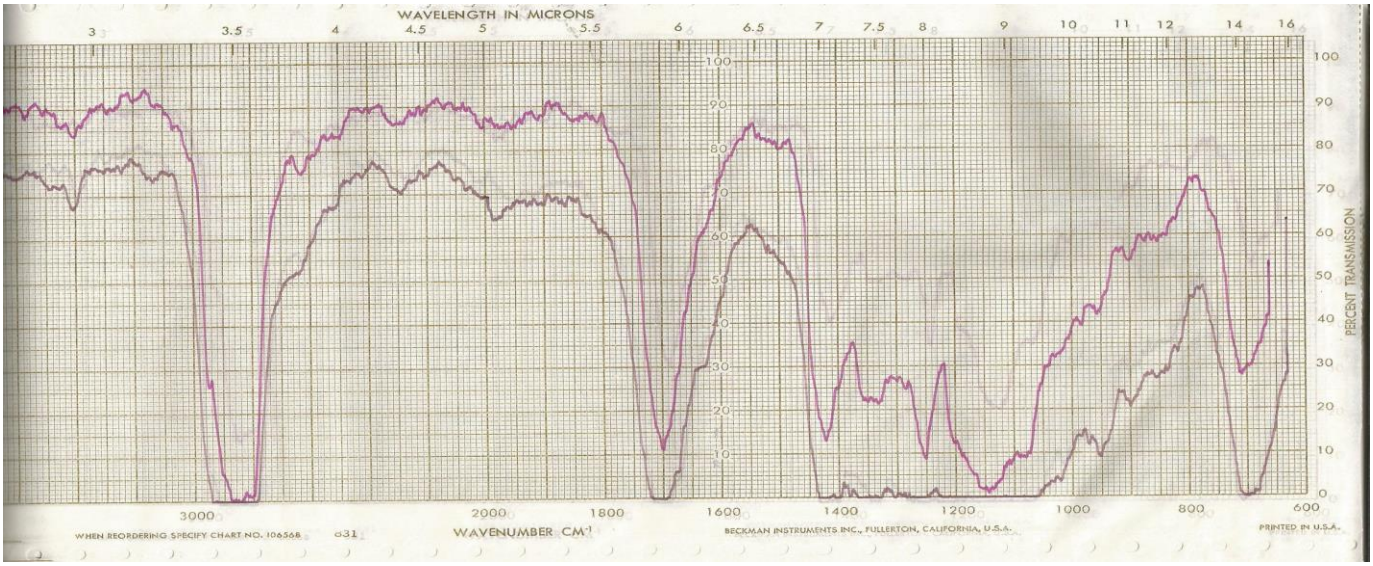


Key:

Sunflower oil Sample SS

Corn oil Sample EC

Appendix 21: Infrared spectra for Sunflower oil SS and Corn oil CC samples

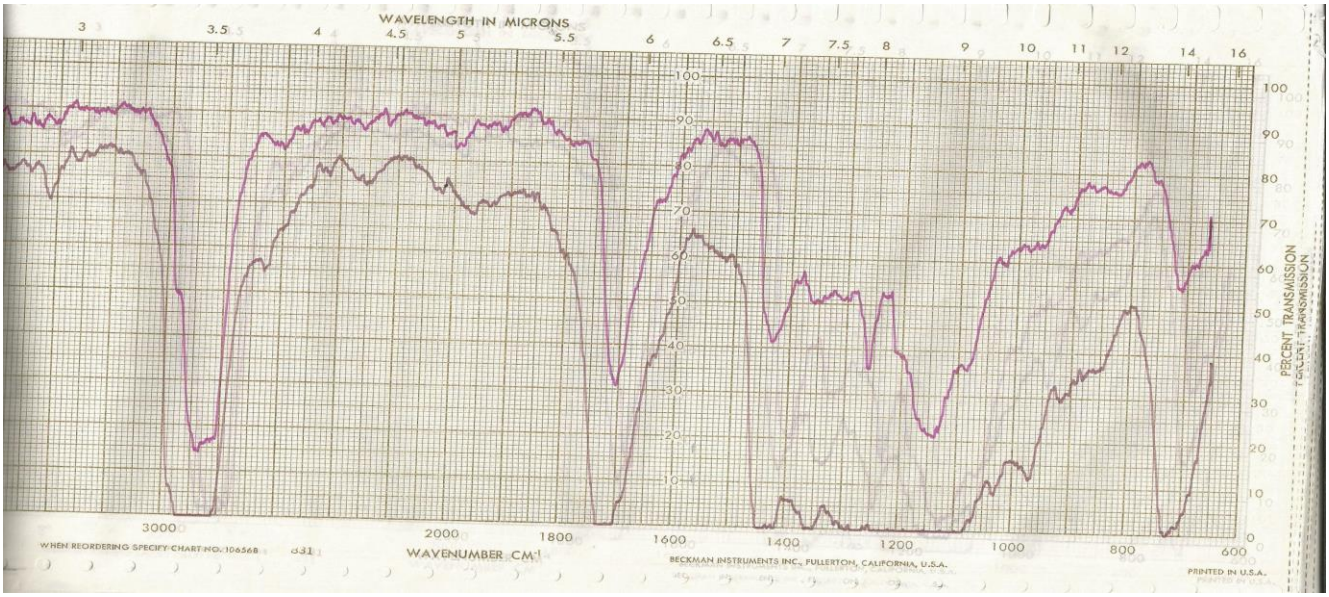


Key:

Sunflower oil Sample SS

Corn oil Sample CC

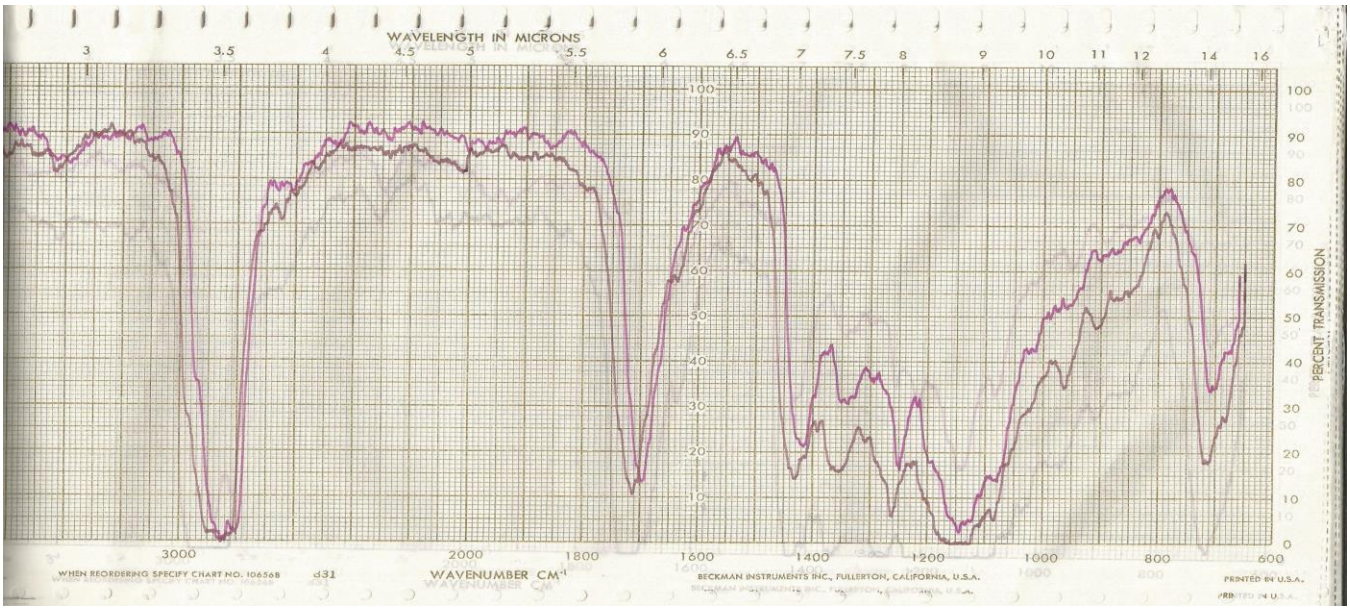
Appendix 22: Infrared Spectra for Sunflower oil SS and Corn oil CC samples



Key:

▬	Sunflower oil Sample SS
▬	Corn oil Sample CC

Appendix 23: Infrared Spectra for Sunflower oil ES and Corn oil CC samples

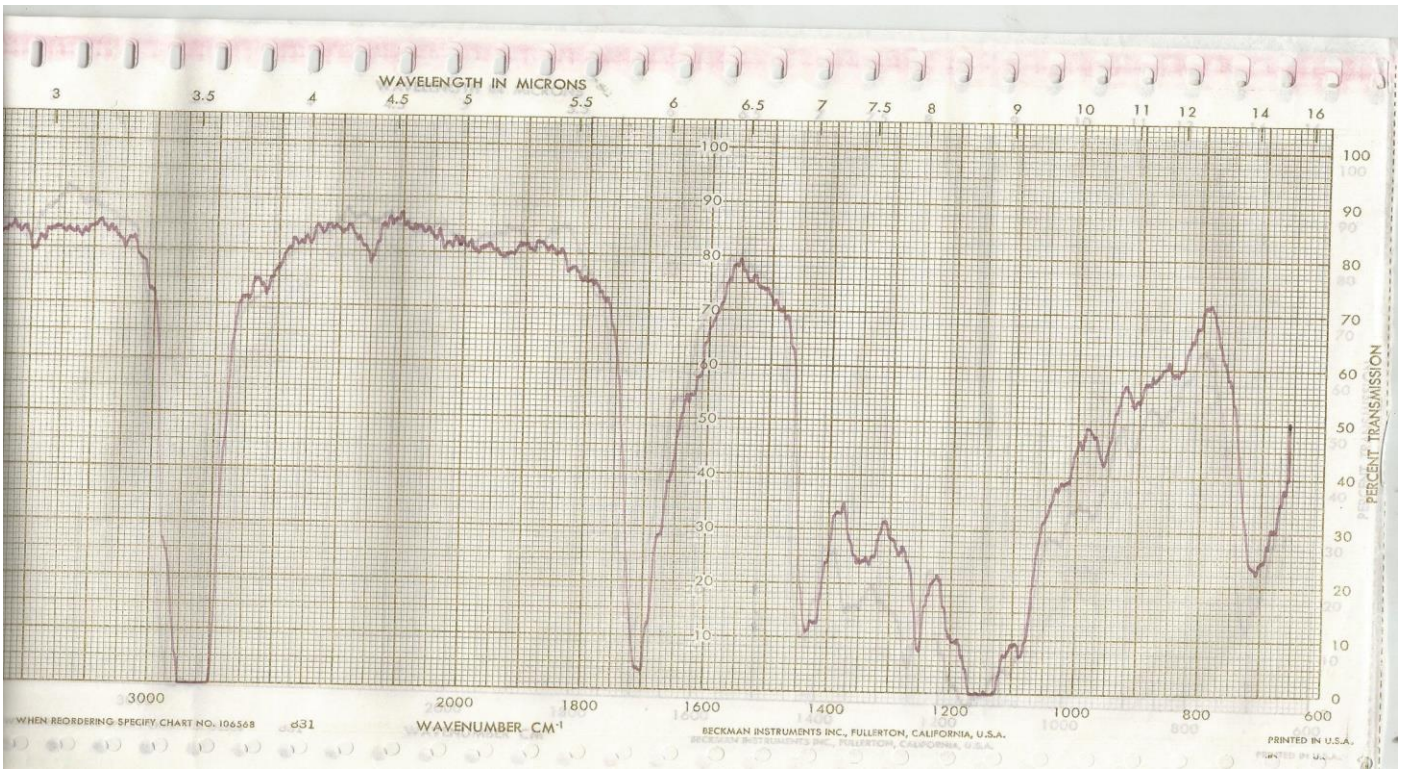


Key:

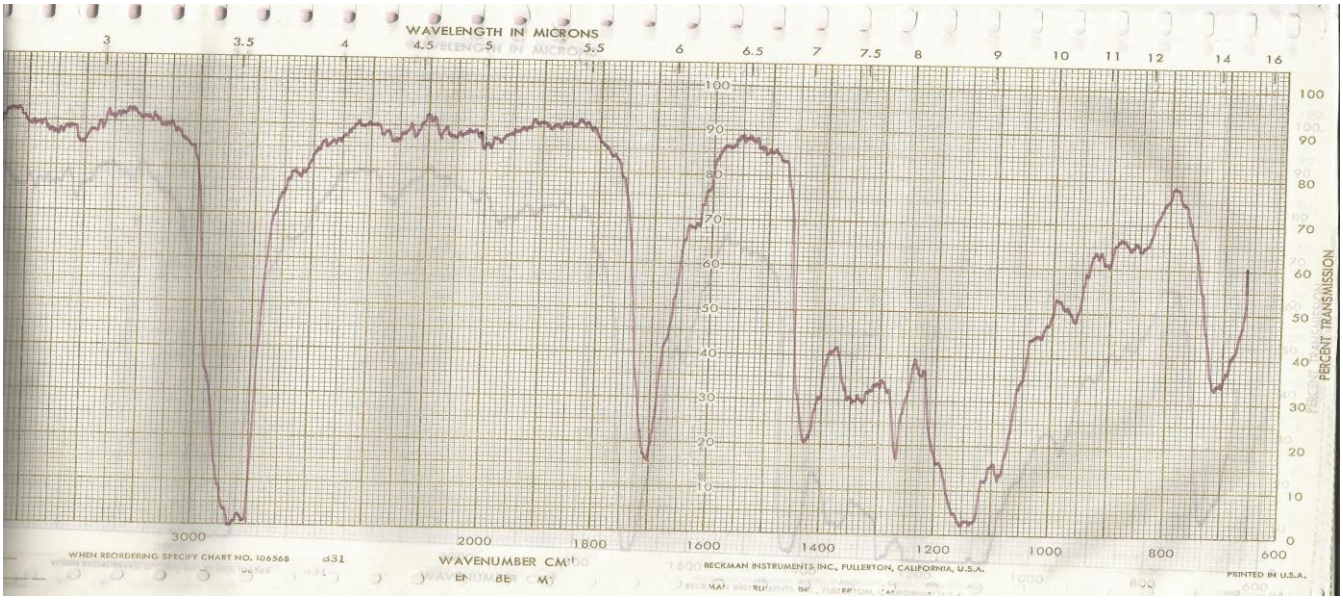
Sunflower oil Sample ES

Corn oil Sample CC

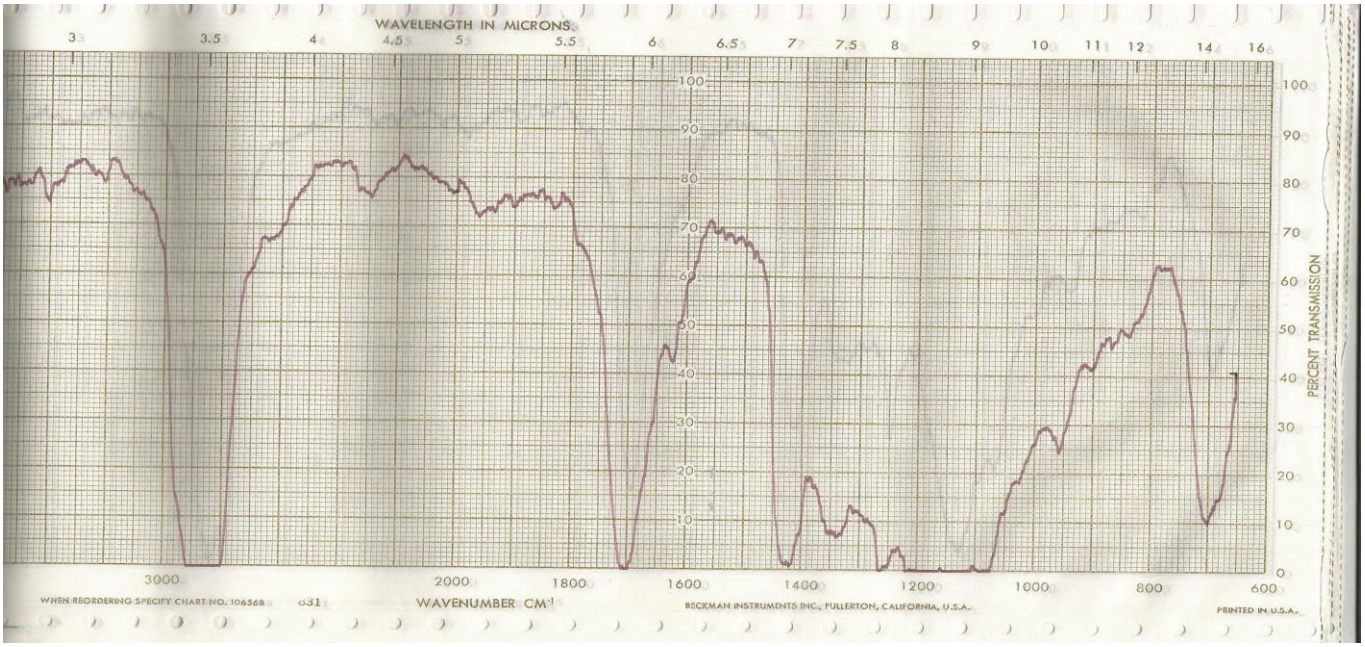
Appendix 24: Infrared Spectrum for Corn oil DC sample



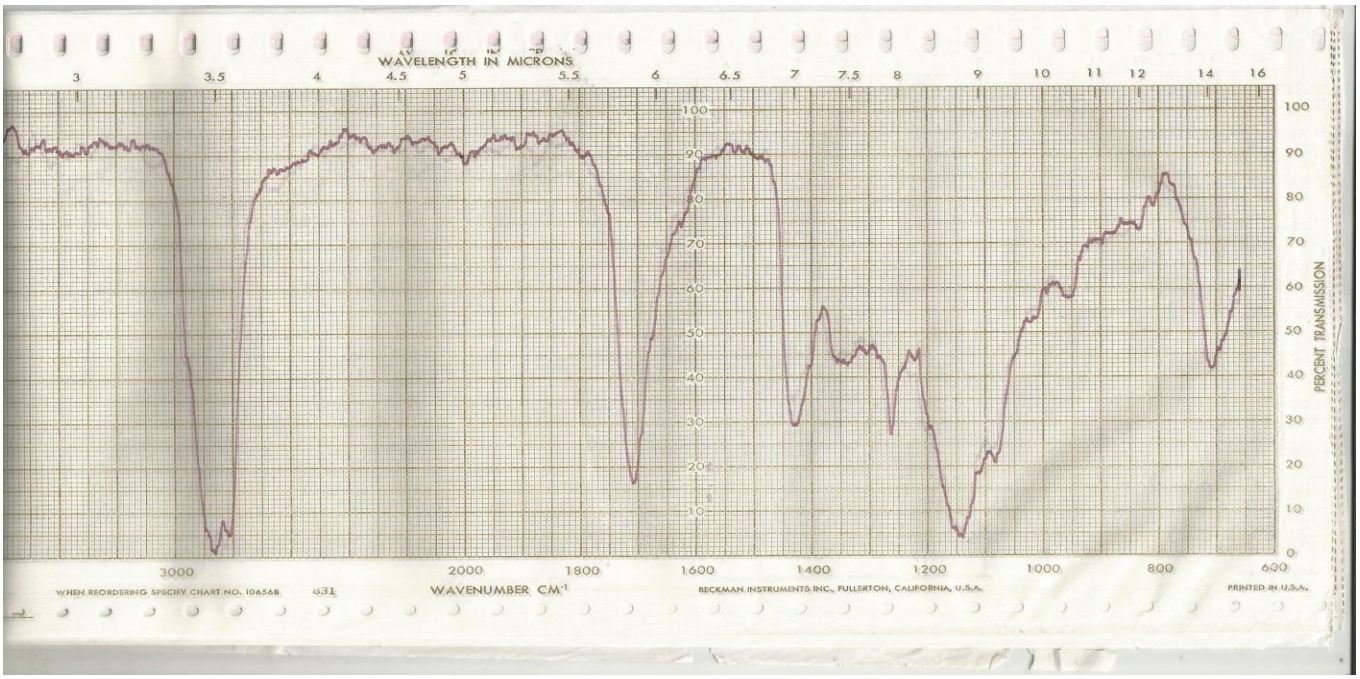
Appendix 25: Infrared Spectrum for Soya bean oil SG sample



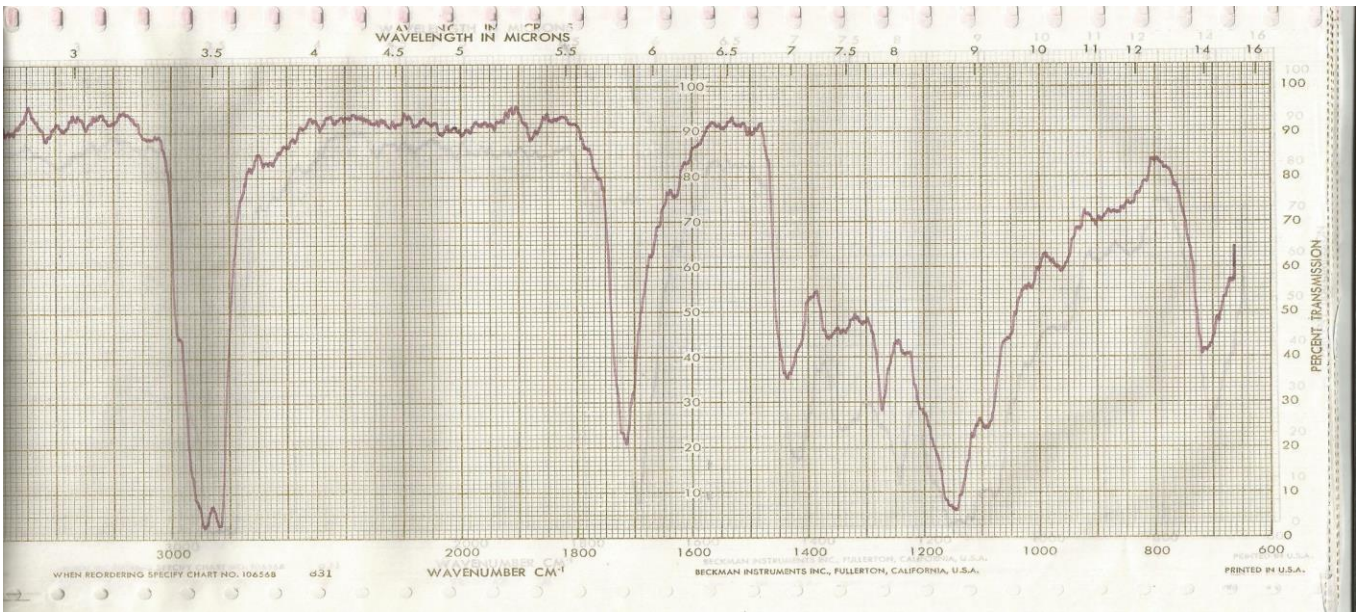
Appendix 26:Infrared Spectrum for Soya bean oil SG sample



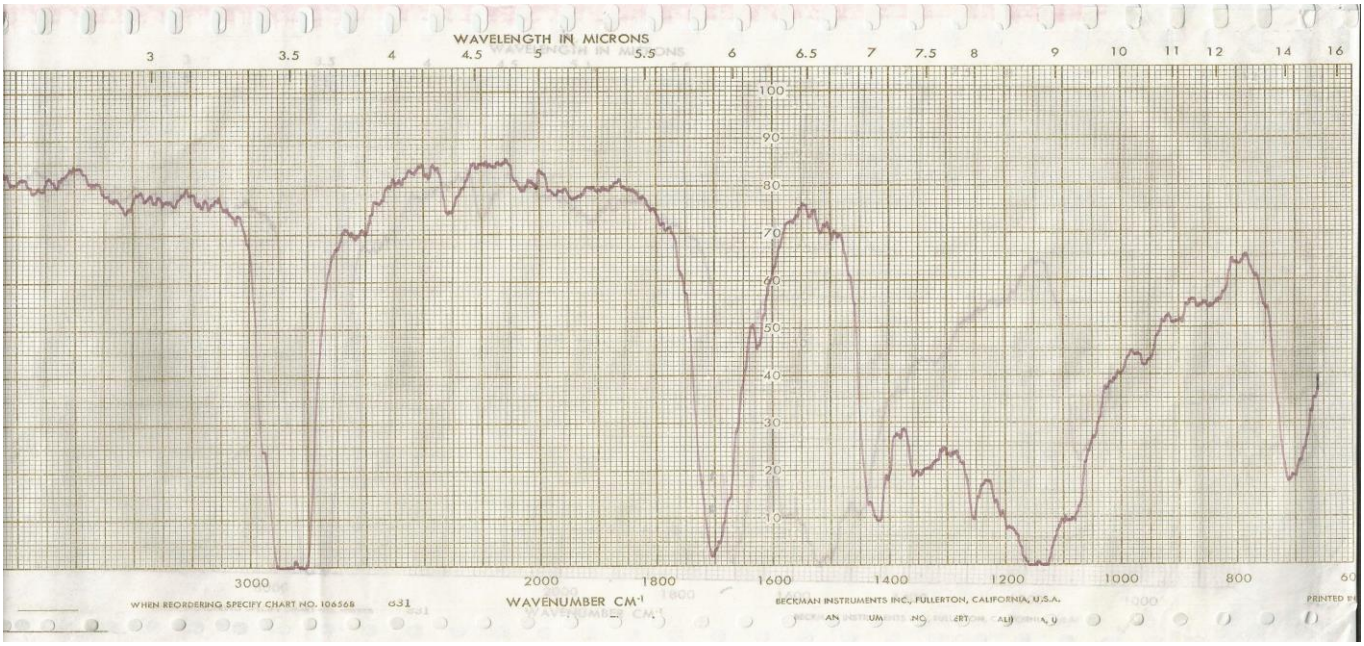
Appendix 27: Infrared Spectrum for Soya bean oil HS sample



Appendix 28: Infrared Spectrum for Soya bean oil HS sample



Appendix 29: Infrared Spectrum for Corn oil DC sample



Appendix 30: Sunflower oil samples IR spectra

SUNFLOWER SAMPLES FREQUENCY (cm^{-1})								
RS			**SS**			**ES**		
3420	3400	3400		3400	3420	3420	3420	3420
2925-2800	2860	2900-2800	2900-2800	2950-2800	2950-2800	2910-2800	2900-2800	2900-2800
1710	2800	1710	2300	2250	2300	1990	2640	2300
1430	2300	1430	1960	1970	1970	1710	2000	2100
1350	1700	1260	1700	1700	1700-1740	1430	1720	2050
1250	1420	1150	1430	1420	1440-1420	1350	1430	1700
1130-1160	1330	1100	1350	1250-1050	1350	1250	1350	1630
1080	1240	1030	1260	950	1040	1150	1260	1430
960	1220	960	1150	900	970	1080	1080	1250
910	1150	910	1080	850	920	960	960	1240-1080
710	1080	700	960	820	850	910	910	1050
	950		900	700	730	710	850	1030
	700		860				720	950
			840					900
			710					

Appendix 31: Corn oil samples IR spectra

CORN OIL SAMPLES FREQUENCY (cm^{-1})								
EC			**CC**			**DC**		
3400	3420	3400	3400			3400		3400
2900-2800	2900-2800	2950-2800	2900-2800	2900-2800	2860	2900	2900	2900-2800
2300	1700	2300	1700	2600	1700	2800	2800	2300
1980	1640	2100	1430	2000	1420	2300	2300	1880
1700	1430	2050	1350	1700	1350	1700	2100	1700
1430	1350	1700-1730	1250	1430	1300	1430	2050	1630
1350	1260	1630	1150	1350	1250	1350	1700	1430
1250	1150	1430	1090	1260	1150	1250	1430	1350
1200	1040	1350	990	1140	1080	1200	1330	1250
1150	960	1250	960	1080	1020	1150	1250	1150
1070	900	1240-1080	860	1020	950	1070	1210	1080
1020	860	1050	840	920	900	1020	1140	1010
950	840	1030	700	830	830	950	1080	960
900	710	950		710	710	900	950	900
860		900				860	850	850
830						830	700	700
710						710		
1700								

Appendix 32: Soya bean oil samples IR spectra

SOYA BEAN OIL SAMPLE FREQUENCY (cm^{-1})			
SG		**HS**	
2880-2800	2900-2800	2880	2900-2820
2300	2300	2000	1880
2000	1960	1430	1710
1700	1700	1350	1440
1430	1620	1260	1360
1350	1430	1150	1270
1270	1350	1080	1270
1150	1260	1020	1150
1090	1220-1080	960	1090
960	960	900	960
900	900	810	900
850	860	710	720
710	840		
	710		