UNIVERSITY OF CAPE COAST

CITRUS SEEDS AS A POTENTIAL SOURCE OF OIL FOR DOMESTIC, INDUSTRIAL AND MEDICINAL PURPOSES

VIDA ACKON

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CITRUS SINENSIS SEEDS AS A POTENTIAL SOURCE OF OIL FOR DOMESTIC, INDUSTRIAL AND MEDICINAL PURPOSES

BY

VIDA ACKON

Thesis submitted to the Department of Chemistry, School of Physical Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Chemistry

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DECLARATION

Candidate's Declaration

I hereby declare that this dissertation is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature:…… Date:……...

Name: Vida Ackon

Supervisors' Declaration

We hereby declare that the preparation and presentation of the dissertation were supervised in accordance with the guidelines on supervision of dissertation laid down by the University of Cape Coast.

Principal Supervisor's Signature:…… Date:……

Name: Prof. Victor Yao Atsu Barku

Co - Supervisor's Signature:…… Date:……

Name: Prof. Yaw Opoku Boahen

ABSTRACT

Citrus sinensis seeds have a lot of nutritional values as well as biological effective compounds responsible for the treatment of various diseases in the human body. However, greater percentage of the oranges gets rotten and this brings about reduction in the market value and an economic loss to the farmers who are into orange farming. The orange seeds are discarded as waste after the juice has been extracted. This has contributed greatly to environmental pollution. Also, there is an increase in demand of vegetable oils which has led to an increase in prices of vegetable oils. Seeds of sweet oranges *(Citrus sinensis*) were analyzed for oil and fatty acid composition. The crude oil was extracted by the use of soxhlet extraction. The results showed that oil content is as high as 71 %. The physico-chemical assessment gave the following results: refractive index (1.457), saponification value (165 mgKOH/g), peroxide value (4.12 meq/kg), iodine value (34.06 Wijs), moisture (5.803 ±0.1%) and ash (0.23 ± 0.15%). Crude fat (89.251 ± 0.2) with energy contents in the oil (803.261 Kcal/100g). The ATR-IR spectrum of oil showed prominent bands which was characteristics of unsaturated fatty acid. GC-FID analysis of fatty acid composition of *Citrus sinensis* seed oil indicated dominant fatty acids: Palmitoleic acid (C16:1), Linoleic acid and Palmitic acid (C16:0) with percentages 56.39%, 39.82% and 2.06% respectively. The fatty acid with the highest percentage composition (56.39%) is Palmitoleic acid (C16:1) a monounsaturated fatty acid. The *Citrus sinensis* seed oil showed greater degree of unsaturation forming 96.97% of the total fatty acids and 3.088% of saturated fatty acids. The oil was screened for antioxidant activities using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'- azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and phosphomolybdenum assays. These results indicated that the seed oil of sweet orange is possible suitable for both human consumption and industrial importances.

KEYWORDS

Antioxidant

Fatty Acids

Refractive index

Saponification

Spectroscopy

Transesterification

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DEDICATION

To my parents Mr. and Mrs. Ackon and brothers.

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LIST OF ABBREVIATIONS

ABTS	2, 2-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid)
ATR- IR	Attenuated Total Reflection Infra-Red
ATR	Attenuated Total Reflectance
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared
AV	Acid Value
B	Blank Titre Value
BHT	Butylated Hydroxytoluene
BHA	Butylated Hydroxyanisole
CO	Corn Oil,
CSO	Cotton Seed Oil,
DNA	Deoxyribonucleic Acid
DHA	Docosahexaenoic Acid
DMSO	Dimethyl Sulfoxide
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EPA	Eicosapentaenoic Acid
EAA	Equivalence of Ascorbic Acid
EFAs	Essential Fatty Acids
ET	Electron Transfer
FAME	Fatty Acid Methyl Esters
FFA	Free Fatty Acid
FID	Flame Ionization Detector
FT-IR	Fourier Transform Infrared Spectrometry
GC-FID	Gas Chromatography Flame Ionization Detector
IV	Iodine Value

IR	Infra-Red
M	Molar Mass
MUFA	Monounsaturated Fatty Acids
N	Normality
PUFA	Polyunsaturated Fatty Acids
P^H	Power of Hydrogen
PV	Peroxide Value
SFA	Saturated Fatty Acids
SV	Saponification Value
TAC	Total Antioxidant Capacity
TPP	Triphenylphosphine
TPPO	Triphenylphosphine Oxide
UV-VIS	Ultra violet visible infra-red spectroscopy
SOD	Superoxide Dismutase
GPX	Glutathione Peroxidase
ROS	Reactive Oxygen Species
ROS/RNS	Reactive Oxygen Species / Reactive Nitrogen Species
HAT	Hydrogen Atom Transfer
PM	Phosphomolybdenum Assay
SFA	Saturated Fatty Acids
MUFA	Monounsaturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
PV	Peroxide Value
SFA	Saturated Fatty Acids
UFAs	Unsaturated Fatty Acid

PO	Palm Oil
SBO	Soybean Oil
UV	Ultra Violet
LDL	Low Density Lipoprotein
PHSF	Partially Hydrogenated Soybean Fat
Oo	Olive Oil
GSO	Grape Seed Oil
CVD	Cardiovascular
TFA	Trans Fatty Acids
EVOo	Elaidic Acid Extra Virgin Olive Oil
HOSFO	High Oleic Sunflower Oil
TMUFAs	Trans Monounsaturated Fatty Acids
PHSF	Poly Hydrogenated Soya Fat
GLC	Gas Liquid Chromatography
FASFO	Fatty Acid Safflower Oil
SO1/ SO2	Sunflower Oil / Soya Bean Oil
RBO	Rice Bran Oil
PHMV	Partially Hydrogenated Mixture Vegetable
SMO	Silybum marianum oil
MOo	Moringa Oleifera Oil
UV-Vis	Ultra Violet Visible
NMR	Nuclear Magnetic Resonance
AOAC	Association of Official Analytical Chemist
MUFA	Monounsaturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids

LA	Linoleic Acid
IRS	Infrared Spectroscopy
GC	Gas Chromatography
TAC	Total Antioxidant Capacity
GC-FID	Gas Chromatography Flame Ionization Detector
SFA	Saturated Fatty Acids
UFA	Unsaturated fatty acids
MUFA	Monounsaturated Fatty Acid
TFAs	Trans Fatty Acids
PUFAs	Poly Unsaturated Fatty Acids
TAGs	Triacylglycerols
SB-ATR	Single Bounce Attenuated Total Reflection

LIST OF CHEMICAL SYMBOLS

CCl_4	Carbon Tetrachloride
C	Concentration
Ca	Calcium
Cu	Copper
HCL	Hydrochloric Acid
KI	Potassium Iodide
K	Potassium
KOH	Potassium Hydroxide
Mg	Magnesium
NaOH	Sodium Hydroxide
$Na_2S_2O_3$	Sodium Thiosulphate

CHAPTER ONE

INTRODUCTION

Background to the study

Fruits have a lot of nutritional values as well as biological effective compounds responsible for managing diseases in the body and they supply most essential vitamins and mineral nutrients that the body needs to perform its functions properly. Most fruits are eaten as food as well as playing an active role in reducing the risk of many diseases like cancers, neurological diseases, cardiovascular diseases (Jorge et al., 2016).Fruits are natural essential food for man containing important nutrients in suitable proportion. Fruits are good sources of vitamins, minerals, and enzymes. They are easily digested into the bloodstream and have the potency of cleaning the digestive tract and the blood. Most of the ailments that are usually caused by the intake of unusual foods can easily be remedied with fruits.

Fruits are also good source of medicine. *Citrus sinensis* is one of the most abundant fruit crops grown all over the world. It is very rich in folic acid, vitamin C (ascorbic acid) and fiber. In addition to the above they contain potassium (K), calcium (Ca), foliate, thiamin, niacin, magnesium (Mg), cupper (Cu) and vitamin B6 (pyridoxine). Citrus species are usually grown for the juice rather than the seed, the pulp, or the peels. The mostly grown citrus species belong to the Rutaceae family (Inglese et al., 2019).

The intake of fruits is one natural way of reducing the risk of oxidative stress through their interaction with free radicals in the body. Under stress condition, there is the production of reactive oxygen species also known as free radicals which have an adverse effect on the body. Oxidative activities of the

peroxide species are inhibited by some biological catalyst in the body such as the superoxide dismutase (SOD) and Glutathione peroxidase (GPX). An imbalance between the antioxidant defense and production of free radicals results in genetic and metabolic alterations. Antioxidants are necessary to prevent degenerative diseases including cancers, cardiovascular diseases, inflammatory diseases and builds the body's immune system (Sharma et al., 2019).

Citrus sinensis contains important phytochemicals such as synephrine, limonoids, polyphenols, hesperidin flavonoid, pectin, and adequate amount of calcium, folacin, potassium, niacin, thiamine and magnesium. Cancers, kidney stones, stomach ulcers, arteriosclerosis, and reduce cholesterol level and high blood pressure are inhibited by these biologically active compounds when used in diet (Etebu et al., 2014).

The orange fruit is an essential and popular fruits for consumers im the entire world due to its pleasant flavour and nutritional value (Sikdar et al., 2016b). The intake of orange fruit releases waste in the form of the seed, pulp and orange peel which are mostly discarded and contributes greatly to environmental pollution whiles these parts can be good sources of natural antioxidants such as ascorbic acid (vitamin), flavonoids, glutathione, vitamin E and carotenoids when used in diets (Goulas & Manganaris, 2012; Milind & Dev, 2012). The presence of phenolic compounds such as phenolic acids, flavonoids and tannins make them play an active role in reducing the risk of many diseases like cancers, cardiovascular diseases, neurological diseases and many types of cancer (Lin et al., 2016).

Most fruits contain seeds with very important nutrients recommended in diets. They are also good sources of edible fats and oils. Oils from seeds were found to be of nutritional, industrial and pharmaceutical importance (Kumar et al., 2016). The calorific and index of nutritive of seeds from fruits qualify them better sources of vegetable oils and fats which are edible and can be used in diet (Ehi-eromosele, 2013). Since long time, edible oils obtained from seeds have been used in food as basic ingredients (Ehi-eromosele, 2013).

A number of plant seeds contain different percentage of an oily component, however, just a few of these plants are exploited industrially for food and other industrial applications: The most frequent used ones are soya, sunflower, peanut, rapeseed, cotton, colza, palm, coconut, olive, and grape seed. Vegetable oils have crucial function, especially in the industrial sector as in the wheat germ oil and avocado oil production and as well as man consumption. There are differences in chemical and physical characteristics of vegetable oils, so there is a need for assessing their composition on how possible they can be marketed. Fatty acid compositions of oil help to differentiate between the various types of vegetable oils. Most vegetable fats and oils contain mainly polyunsaturated and mono unsaturated fat and are low in saturated fats and oils. Climatic conditions and the type of soil affect the fatty acid composition but however, the trends in fatty acid composition is similar in different species. The seeds in fruit are considered as waste. Oils could be obtained from the seeds in the fruits because they contain the oily part of the fruit. This oil can be benefit to mankind. Although they make up a small frsction of the whole fruit (Sicari et al., 2017).

There is frequent increase in the manufacturing of food in the world market with consequently rise in waste production (Abdel-Shafy & Mansour, 2018). It is therefore important to develop researches for its uses, hence the concern in the uses of vegetable oils such as the oils extracted from fruit seeds because these oils contain active bioactive chemicals. The current study intends to explore the nutritional and industrial benefits humanity can get from *citrus sinensis* seed oil which is mostly discarded as waste by determining its nutritional parameters, fatty acid content, antioxidant capacity and the oil characteristics.

Vegetable oils are obtained from plant sources like melon, soya beans, groundnut, oil palm, corn, shea butter, coconut and other seeds from fruits. Plant seeds have been considered to be very good sources of fats and oils for nutritional, industrial and pharmaceutical applications. Some seed oils such as castor oils, groundnut oil, soybean oil etc are already in use for several purposes such as in pharmaceuticals, textiles, organic pesticides, oleo-chemicals, plastics, soaps, for cosmetic purposes and as ingredients in paint and varnish formulations. Two major constituents of Citrus seeds are seed meal, consisting of protein and seed oil.

Orange peels contain fragrant substances that can be processed into essential oils, which are used commercially for flavoring foods, beverages, perfumes and cosmetics (Nisha Pauline & Lakshmi, 2015). Most fruits in general, mango, banana, watermelon, pear, and oranges are not only eaten for their nutritional values but also for medicinal purposes as well. Oranges produced in Africa particularly in Ghana are eaten locally, with only a very small amount being used in the industrial sectors. The use of oranges releases

wastes in the form of peels, seeds and pulp which can be of use if properly handled instead of discarding. Currently, only the juice of the fruit is of commercial importance while the seed, the peels, and pulps which form about 50 % of the whole fruit are treated as waste (Reazai et al., 2014b).

The orange fruit has a delicious juicy taste and it's highly rich in vitamin C as well as other nutrients needed by the body to function. Oranges are popular and mostly account for about 70 % of the world's most important citrus all over the world (Akpan & Ozor, 2014).The orange fruit is part of human diet because of its nutrients quantity, health benefits and medicinal properties. Orange juice is widely used as a preservative and as flavour to various dishes. It is an important fruit because even its waste which is mostly discarded and contributes greatly to environmental pollution can have an important use. Several literatures reveal that the orange seed oil has an antioxidant properties and other important uses (Jorge et al., 2016).

Sweet orange (*C. sinensis*) accounts for more than two thirds of global coverage of the overall production of citrus in the world (FAO, 2004). Ten edible citrus species are currently known, eight of which are commercially cultivated and five of which are of great economic importance (Liu, Heying, & Tanumihardjo, 2012). Over 104 million tons of citrus are produced annually, and some 15 million tons are traded (FAO, 2004). In Africa the total area under citrus cultivation is 1.3 million hectares, 44 000 hectares of which are in South Africa and 4 500 hectares in Ethiopia. Citrus farming is scattered following its recent introduction to Ethiopia (Dagnew et al., 2014).

Statement of the Problem

A large amount of money, labor, time and resources is invested into citrus crop production every year in Ghana. This makes citrus fruit production a very tedious work. After the fruit is ripened, it is only harvested to the market for a very cheap price since consumer's requirement is only the fruit juice. The peel, the seed, the pulp and the rotten fruits which form about 50 % of the whole fruit is discarded as waste contributing greatly to environmental pollution. This finally brings about an economic loss to the farmers and the nation. This is a disincentive to farmers who are engaged in citrus fruit production in Ghana. The need to reduce environmental pollution, as well as, finding alternative means of adding value to citrus fruit production in Ghana call for this research. More also, there has been a concern to seek for renewable energy sources as alternative fuel sources to substitute the fossil fuel used in Ghana. Additionally, the need for other industrial raw materials like oils for paint and soap industries calls for research into this waste to possibly identify orange seed oil as a potential source of raw materials for our industrial sectors. Moreover, an increase in demand for vegetable oils has led to an increase in prices of vegetable oils, supporting the need to search for substitution of more quality and cheaper oil.

Purpose of The Study

This study, aims to evaluate the oil content, fatty acid composition and other potential benefits of *Citrus sinensis* seed oil cultivated in Ghana for its benefits for human and industrial purposes.

Research Objectives

This research seeks to:

1. Assess the physicochemical properties of the seed oil.

2. Examine the nutritional value of *Citrus sinensis* seed oil.
3. Study the antioxidant properties of *Citrus sinensis* seed oil.
4. Identify the fatty acids composition of *Citrus sinensis* seed oil.
5. Investigate the potential uses of the seed oil in soap production.
6. Determine if repeated use of orange seed oil for frying will affect the oil quality.

Research Questions

The following research questions were posed to guide us to achieve our stated objectives.

1. Does orange seed contain a sizeable amount of oil?
2. Does the oil obtained from an orange seed have biodiesel properties?
3. Does the oil obtained from an orange seed contain some useful fatty acids?
4. Does the oil possess antioxidant properties?
5. Is the orange seed oil useful for soap production?
6. Does repeated use of the oil in frying have any effect on the oil quality?

Significance of the Study

The study will help students and other researchers to realise the usefulness of the by-product of orange fruit which is greatly discarded as waste contributing greatly to environmental pollution.

This knowledge will lead to waste reduction, waste minimization and pollution control. This work will add more value to citrus crop production and will remove the limitations that only the juice is of commercial use. The

exploration for other uses of citrus fruits will increase the demand for orange by the world market for other useful products.

Delimitations of the study

Soxhlet extraction was method chosen to extract the oil from the sweet orange seed hence, long extraction time and increased temperature in the Soxhlet extraction process which will enhanced the thermal degradation of volatile compounds was not a major factor unlike in flavor compounds extraction, environmentally friendly technique such as microwave pretreatment-improved steam distillation can be used to prevent flavor compounds deterioration due to the adjustment in the extraction process (Shaghaleh et al., 2018).

Limitations of the study

In Africa, sweet orange seed oil dissimilar in chemical composition especially those of various geographical areas (Nwobi & Adesina., 2006). This results to a distinguish factor in a little variation in the results obtained in this experiment.

Definition of Terms

Antioxidant are compounds that inhibits reactions or prevent oxidation promoted by peroxides or oxygen.

Fatty Acids are substance containing a long chain hydrocarbon and a carboxylic acid as a terminal group.

Transesterification is defined as the chemical reaction of triglycerides and alcohol which results to alkyl esters in the presence of a catalyst.

Saponification when oil, lipids, or fat are converted into soap and alcohol in the presence of aqueous alkali is referred to as saponification.

Refractive index the ratio of speed of light in space to the speed of light in the substance is referred to as index of refraction.

Spectroscopy is the study of emission and the absorption of light and other ration by matter.

Organisation of the Study

Chapter one gives an introduction to the study which explains the main aims and objectives of the research, the significance of the study, which explains the importance of the research. The organized thesis structure was also given in this chapter.

Chapter two provides some technical information and some main report of literature review to the present research, with background and chemical composition of *Citrus cinensis* seed oil and some examples. Some advanced implemented technology and research was also provided.

Chapter three gives a technical detail of the analytical equipment used, setup reactions in the laboratory, reaction conditions, and the materials used, during the course of the research a well analytical methods and experimental methods was considered.

Chapter four give the results and discusses the outcome of the research, such as: physicochemical analysis, proximate composition, GC-FID analysis of fatty acid composition, the IR spectrum and antioxidant activity of the *Citrus cinensis* seed oil.

Chapter five present a brief main finding of the work and conclusions obtain from the findings. Some recommendations were suggested for future studies.

Chapter Summary

In this chapter, a general overview of the research has been described. This chapter elaborated the general background to the issues concerning the discarding of sweet orange seeds as waste. The motive behind this study and the importance of the study.

CHAPTER TWO

LITERATURE REVIEW

Introduction

This chapter present the literature of the study of this work. It includes the following: morphology of *Citrus sinensis*, scientific classification of *Citrus sinensis*, phytochemistry of citrus fruits, uses of orange seed oil, nutritional composition of orange/orange seed oil, Antioxidants properties of *the Citrus sinensis.* Fatty acid compositions and properties of the orange seed oil, effects on the physiochemical properties of the oil after continuous use for frying, chemical indices of the orange seed oil, Infrared spectroscopy, the approach in fatty acid research, isolation of fatty acid compounds and detecting of fatty acids.

Morphology of *Citrus sinensis*

Citrus sinensis (sweet orange) belongs to the family Rutaceae. *Citrus sinensis* is among the most widely used fruit of the citrus family and it is originated from South-East Asia (Ibrahim & Yusuf, 2015; Rachel et al., 2013). The fruit has been introduced to the new world by the Arab traders through the great trade routes of Africa to the eastern mediterranean while in the year 1000 AD the crusaders brought the fruit to Portugal, Italy and Spain (Aubaile, 2012). *Citrus sinensis* is often grown in the rainforest and guinea savannah area where most of their farmlands are in the remote part of the country where the road is very poor. Due to poor means of transportation of the fruit, about 30-50 % of these citrus fruits get spoiled on the way before reaching the final consumers in the urban centres. Most citrus fruit cultivated are oranges, but large quantities

of grape fruits, limes and lemons are cultivated as well. Present annual worldwide production of citrus is estimated at over 105 million tons. Botanically, citrus is a large family whose dominating members include *citrus sinensis* (sweet orange), *citrus paradisi* (grape fruit) and tangerine orange (Inglese et al., 2019).

Oranges are citrus fruits consisting of an outer coating called pericarp or the peel, a thin white membrane covering the pulp. Sweet orange tree is a medium size plant often grown to a height of 6.0 m–l00 m. The broad, green leaves are medium sized and ovate with crenulated margins and about 4-12 cm in diameter. The petioles (leaf stalks) have narrow rings (Ibrahim & Yusuf, 2015; Milind & Dev, 2012). The peel is 0.5 cm in thickness and attached tightly to the segments. It changes into an orange color but often remains green or pale yellow in tropics. The pulp is very juicy and slightly acidic. The central line is solid and it may contain no seeds or many seeds (Das, Sachan, Shuaib, & Imtiyaz, 2014). The seeds of Sweet orange (*Citrus sinesis*) occur within the berry and are embedded in juice sacs of the loculus which is very close to the central axis.

The sweet orange (*Citrus sinesis*) is a fruit-bearing plant that provides edible fruits through the whole tropical and subtropical lands (Ibrahim & Yusuf, 2015). The fruiting stage starts from flowering. Flowers of the plant are axillary and having both functional stamens and pistils and has a diameter of 2–4 cm (0.8–1.6 in). There are generally five petals and contain some oil glands whiles the calyx is 4–5 lobed (Das et al., 2014; Milind & Dev, 2012). Orange yield very little pollen grains when they blossom hence, orange growers need not to practice artificial pollination. Self-pollination is enhanced for citrus having both

sex on the same blossom and nectar for pollination by insects are also available. Cross-pollination is used only by some farmers and the flowers matures into a bud which grows to yellow orange with many or few seeds when ripened. The fruit, ripen to orange to yellow and are in oval shape for about 6.5 to 9.5 cm wide. The orange fruit consists of two different regions in structure, the endocarp, or pulp or juice sacs and the pericarp or the peel, skin. The skin which gives the orange a characteristic smell is made up of pericarp of epicuticular wax with small oil glands. The quantity of wax is dependent on the variety of the orange, growth rate and climatic conditions (Etebu et al., 2014).

No signs of acute oral toxicity were recorded when Protus and co-workers (2012), studied acute oral toxicity of *Citrus sinensis* (sweet orange) where the extracts from the leaf was administered to mice at a dose of 5000 mg/Kg body weight (Tarkang et al.,2012).

Figure 1: Images of orange

Reference: Dharmawan et al. (2007)

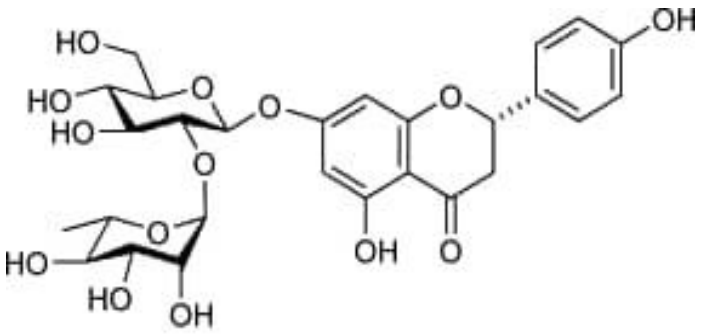
Scientific Classification

Kingdom	Plantae
Division	Magnliophyta
Class	Dicotyledons
Order	Sapindales
Family	Rutaceae
Genus	*Citrus*
Species	*sinensis*

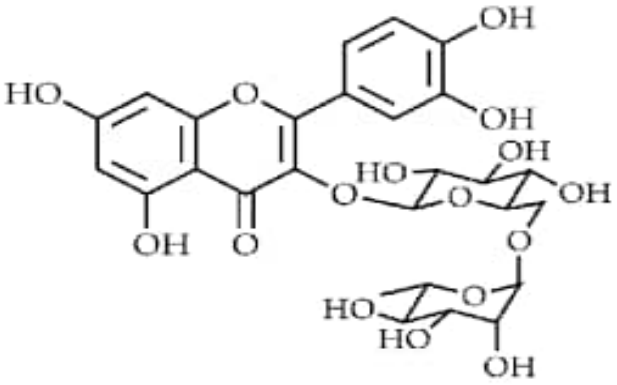
Phytochemistry of Citrus Fruits

Orange fruit contains 1.5 % essential oil. Several literatures review that the key phyto-compound present in orange fruit is D-limonene constituting about 90 % of all the oils extracted (Milind & Dev, 2012; Pandharipande & Makode, 2012). A key phyto compound in various citrus oils is D-limonene (1-methyl-4-(1-methylethanyl cyclohexane) D-limonene is a monoterpene with a smell like lemon (Shekhar Pandharipande, 2012). Limonene is a naturally occurring chemical used in many food products, soaps and perfumes due to it lemon-like odor and flavor (Sikdar et al., 2016b). Limonene is also registered active ingredient in most pesticide, insecticides, insect repellents, and cat repellant. Furanocumarines and lipophilic flavonoids are have been stated to be present in orange oils (Milind & Dev, 2012).

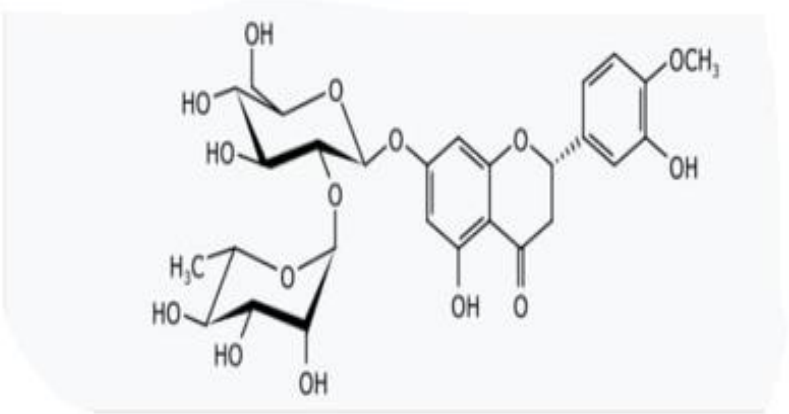
Orange fruit also contains many bitter flavone glycosides like naringin and neohesperidin, whose sugar component is rutin and neohesperidose (Milind & Dev, 2012).



Naringin



Rutin



Neohesperidin

Figure 2: Flavone glycosides

Reference: Milind & Dev (2012).

The following minerals: potassium, magnesium, calcium, zinc, iron and phosphorus and the following vitamins (B1, B2, B3, B5, B6) and as well as vitamin C were obtained from the fruits (Milind & Dev, 2012). They are also good sources of edible fats and oils. Oils from seeds were found to be of nutritional, industrial and pharmaceutical importance (Kumar et al., 2016). They also contain phenolic bioactive chemicals such as phenolic acids, flavonoids and tannins which make them inhibit many diseases like cancers, cardiovascular disease and neurological diseases. Oils obtained from the seeds of orange fruits are stated to be good sources of tocopherols, phenolic compounds, phytosterols and carotenoids (Jorge et al., 2016; Kaur et al., 2021).

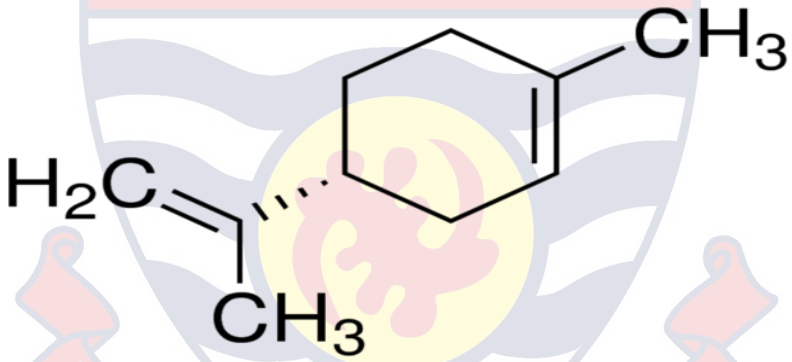


Figure 3: D-Limonene

Reference: Jorge et al. (2016)

Uses of Orange Seed Oil

Medicinal Uses of Orange Seed Oil

There are some reports that active component of orange enhances the secretion of gastric juice. Many pharmacological studies stated that citrus fruits possesses anthelmintic, antioxidant, antimicrobial, antidiabetic, anticancer, anti-inflammatory, insect repellent, cardiovascular, respiratory, central nervous, analgesic, reproductive, gastrointestinal, immunological and many

pharmacological effects (Al-snafi, 2016). The Orange fruit is part of human diet because of its nutritional value and other medicinal properties (Nwobi et al., 2006)(Sikdar et al., 2016a). It also has several health benefits and the juice is widely used as flavor and preservative to many dishes.

Citrus sinensis (oranges) were eaten to relieve catarrh and fever. A poultice prepared from the roasted pulp was used for skin diseases. The immature fruit was eaten to stop stomach and intestinal problems. Orange flower was used as sedative and antispasmodic in Italy and France. Also, in Italy, the decoction of the flowers and the dried leaves was given as a remedy for flatulence, indigestive and antiemetic. In China, decoction of the husked orange seeds was recommended for urinary diseases, the bark is also infused and macerated in wine and then taken as a tonic wine. In Ecuador, An orange seed extract was given as malaria treatment (Al-snafi, 2016).

Citrus limonum (lemon juice) is widely used as antiscorbutic, diuretic, astringent and febrifuge. In Italy, the juice is given to relieve gingivitis stomatitis and tongue inflammation (Al-snafi, 2016). *Citrus limonum* juice in hot water, honey or ginger has been accepted and used as a daily laxative and to prevent common cold (Al-snafi, 2016).

Citrus medica has also been accepted as traditional medicine used for cough, vomiting, sore throat, asthma, hiccough, nausea, anti-scorbutic, tonic, expellant of poison and stimulant. Flowers, seeds, leaves, peels and roots were used to trreat many ailments (Al-snafi, 2016).

Industrial Uses of Orange Seed Oil

Orange Seed Oil as Potential Source of Energy

Fossil fuels are the main source of energy for industrial and domestic uses but economically oils from orange seeds could be a potential alternative source to reduce over dependance on this main source. Some of these renewable and alternative sources of energy include solar energy, fuel cells, bio-fuels hydroelectric power and wind mill (Bull & Obunwo, 2014). Recent studies have shown that the orange seed oil as well as orange peel oil-extracted can be combined with diethyl ether to provide another fuel source diesel engine such as compression-ignition engines that could be used with/without Di-ethyl ether for diesel engines. Several studies have been carried out to transform orange waste into wealth by enhancing the production of biodiesel with orange seed oil (Agarry et al., 2013).

Orange Seed Oil as a Potential Oil for Soap

Apart from medicinal and nutritional properties, orange seed oil is proven to be useful in soap making and other industrial applications such as in the paint industries (Ibrahim & Yusuf, 2015; Nwobi et al., 2011). Atasie and Akinhanmi (2009) reported that oil with high saponification number is useful in soap industries (Atasie & Akinhanmi, 2009). The saponification values of most oil ranges withing 188-196 mg KOH/g as a standard value required for soap making. Reduced saponification number indicates that the oil may not qualify for an industrially important whiles higher saponification number sugest that the industry can make use of the oil (Amoo et al., 2004). Lager saponification number, enhances the soap-making quality (Odoom & Edusei, 2015).

Nutritional Composition of Orange/ Orange Seed Oil

Citrus fruits contain many phytochemicals together with essential oils, flavonoids, alkaloids, coumarins, carotenoids, psoralens including vitamins, minerals and trace elements with nutritional value (Al-snafi, 2016). The citrus juices contain minerals, vitamins, carotenoids, organic acids, amino acids, sugars, phenolics, nucleotides, limonoids, enzymes, lipids, pectins, proteins, and other both insoluble and soluble solids (Dharmawan, 2008). Citrus fruits contain phytochemical compounds which are responsible for the antioxidants and nutritious property of the fruit. *Citrus sinensis* (orange) has been proven Scientifically to contain vitamins and minerals with many health benefits. Other non-nutrient biologically active compounds which are found in *citrus sinensis* fruits are phytochemical antioxidants, soluble and insoluble dietary fibres. They are known to be helpful in reducing the risk of cancers and many chronic diseases when used in diet (Dharmawan, 2008). Citrus seed is a good source of oil rich in essential fatty acids, protein, minerals, vitamins, fibre (Adeyeye et al., 2015). Fatty acids, both free and as part of complex lipids, play a number of important roles in metabolism as essential components of all gene regulators and membranes. Dietary lipids provide polyunsaturated fatty acids that are natural metabolites (Rustan, 2005). Citrus are rich in ascorbic acid (vitamin C) and folic acid, as well as an important source of fiber. In addition, they contain K, Ca, foliate, thiamin, niacin, vitamin B6 (pyridoxine), P, Mg and Cu.

Antioxidants

Antioxidants are substance which inhibit rate of lipid oxidation reaction by opposing oxidation reactions promoters such as oxygen or peroxides especially in foods. Many food products utilized antioxidant as food

preservatives. Biologically, antioxidant is “natural or synthetic substances added to products to inhibits or reduce their decomposition by action of atmospheric oxygen. In medicine and biochemistry, organic substances and enzymes, for instance, β-carotene or vitamin E which enables counteracting the detoriating effects of oxidation in the body are antioxidants.

Antioxidant can be applied in the chemical industries to prevent autoxidation. The primary cause of autoxidation is a radical chain reaction which transpire between oxygen and the substrate. Sterically hindered amines and phenols are antioxidants the breaks down radical chain reaction and also reduces radical scavengers. Antioxidants are components that act against rancidity in oils and fat according to chemistry of food. It is a chemical compound that effectively reduces the negative effects of reactive oxygen species (ROS) on the physiological functions of human. Antioxidant serves as a dietary that reduces reactive nitrogen species /reactive oxygen species (RNS/ROS) to prevent chain reactions caused by radicals. The oxidation brought by Reactive oxygen species (ROS) may cause disintegration of cell membrane, mutations of the DNA and protein damage which have an important function in aging and also have the ability to promote the development of various diseases, examples includes as arteriosclerosis, arthritis, skin damages diabetes mellitus, cancer, inflammation, liver injury, coronary heart diseases (Kaplan, 2010). Antioxidants can be Lipid-Soluble (Lipophilic) Antioxidants or water-soluble (Hydrophilic). The lipid-soluble antioxidants like that of lipoic acid, vitamins E, vitamin A, and carotenoids and are located in the cell membranes, whiles the water- soluble antioxidants like that of Polyphenols, vitamin C, glutathione are located in the aqueous body fluids, as in fluids around

the cells (cytoplasmic matrix) and blood fluids within the cells. Free radicals may attack the fatty cellular membrane and watery cell contents hence, the body needs protection for both. Cell membranes lipid peroxidation is prevented by the lipid- soluble antioxidants. Many existing natural antioxidant substance that can be found in vegetables, dietary supplements and fruits are α-tocopherol, trans-cinnamic acid and hydroxycinnamic acid, phenolic polymers, ascorbic acid, phenolic acids, Benzoic acid, stilbenes (in glycosylated form), lignans, coumarins, isoflavonoids and flavonoids.

Role of Antioxidants

An antioxidant inhibits the oxidation of another compound by releasing their electrons to free radicals to terminate a free radical chain reaction without free radicals themselves. Antioxidants are natural way of preventing living cells from being attacked by reactive nitrogen and oxygen which are reactive species (RNS/ROS). The body naturally produces a various nutrient for their antioxidant activities and also produces enzymatic antioxidants to enable reduce these damaging chain reactions. Vitamin C, carotenes, vitamin E and lipoic acid are examples of such antioxidant nutrients (Kaplan, 2010).

Classification of Antioxidants

There are two categories of antioxidants, enzymatic and Non-enzymatic antioxidants; those antioxidants that operate by the inhibiting of free radical chain reactions is known as Non-enzymatic. Vitamin E for example may prevent free radical chain activity through five (5) reactions pathway (Kaplan, 2010). While, those that operate by breaking and removing free radicals are known as enzymatic antioxidants. Consequently, these antioxidant enzymes blush out destructive oxidative products by transformng them to hydrogen

peroxide which is further converted to water, in a many steps process. Cofactors trace metal (zin, manganese, copper and iron) are also required. Enzymatic antioxidants cannot be used orally as drug supplement but are manufactured in the body. Non enzymatic antioxidants are from food source and can be supplemented to the body through oral means.

Antioxidant Activity of Orange Seed Oil

Orange fruit is believed to have compounds such as tannins, flavonoids and phenolic acids that enables them able to attack free radicals and act against oxidative stress. Risk of many diseases like cancers, cardiovascular, and neurological diseases can be reduce by the presence of many phytochemicals (Al-Hadeethi, 2015).

Constantly, the body produces free radicals due to oxidation and other activities in the body. Free radicals can trigger cell damage and this caused by oxidative stress. Oxidative stress has an important role in many diseases including cancer, eyes diseases, cardiovascular diseases and Age-Related Macular Degeneration (Milind & Dev, 2012).

Antioxidants are natural or man-made compounds that have been proven experimentally to inhibit oxidation that is the production of free radicals they have the ability to prevent or slow damage to cells as a result of free radicals. These have the ability to suppress, reduce, or delay the oxidative stress. Antioxidants such as thiols and ascorbic acid breaks chain reactions caused by free radicals. Natural by-products of chemical processes that occur in the human body such as the body's reaction to environmental changes and metabolism are free radicals (Nimse & Pal, 2015). Antioxidants act as free-radical scavengers. Antioxidant defensive action is unique and it relies on the

species of antioxidant used even though an existence of the antioxidant defense is general (Birben et al., 2012). Antioxidants can exist both in enzymatic and non-enzymatic ways within the cell and outside the cellular environment. The occurrence of inflammation, exposure to environmental pollution, UV exposure, cigarette smoke, high levels of food additives and pesticides facilitates the production of reactive nitrogen and oxygen which are reactive species (RNS)/ (ROS). These RNS and ROS often results in oxidative stress in causing disordered physiological process as the cellular constituents are altered resulting in various disease conditions such as heart related disease, respiratory related diseases, cancer, stroke, immune deficiency, arthritis (Nimse & Pal, 2015). Antioxidants in their operation turn to amend cellular oxidative status and avert biologically key molecules like membrane, lipids, DNA and proteins from damage due to oxidative stress (Kurutas, 2016).

There are natural antioxidants and synthetic antioxidants or antioxidant drugs. Natural antioxidants are obtained from fruits and vegetables. Some plant-based foods have high content of phytoestrogens, flavonoids, flavonoids, polyphenols and catechins which are antioxidants and the major antioxidants found in natural products are vitamin c, carotenoids (vitamin A), tocopherols (vitamin), Polyphenols, and flavonoids (Yadav et al., 2016). Synthetic antioxidants do not exist in nature but are chemically produced substances added to food as preservatives to inhibit lipid oxidation. Many synthetic antioxidants have been utilized to stabilize fats and oils because most natural antioxidant are unstable. Since 1954, Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are common synthetic antioxidant that have been utilized as antioxidants in human foods (Venkatesh & Sood, 2011).

Antioxidant Assays

There are different types of assay used to measure antioxidant activities. Assays work by hydrogen atom transfer (HAT) or electron transfer (ET) (Gupta, 2015). The electron transfers-based assays are redox reaction with the oxidant as an indicator at the endpoint of the reaction. Hydrogen atom transfer assay technique consisting of an oxidizable molecular probe, a synthetic free radical generator and an antioxidant. Hydrogen atom transfer and electron transfer based assays measures the oxidant (or radical) inhibiting ability, instead of the inhibiting antioxidant ability of a sample. Examples of electron transfer based assays are 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS). However, in biological systems, these radicals are foreign material (Shalaby & Shanab, 2013). Hydrogen releasing antioxidant such as lyophilic compounds, hydrophilic compounds and food extracts such carotenoids, hydroxycinnamates and flavonoids inhibits radical cation (Stämpfli et al., 2007). Maximum absorption at 517 nm is an absorption frequency of a stable DPPH radicals. DPPH radical which is in powdered form changes color from red to yellow when it has been scavenged. The DPPH assay utilizes this color changes to show free radical inhibiting ability. DPPH react with the antioxidants and it is reduced to DPPH-H which results in the decrease in absorbance. 2, 2-diphenyl-1-picrylhydrazyl, which is a stable free radical, becomes decolorized when it reacts with antioxidants. The extent of decolorization asses the inhibiting ability of the antioxidant substance or in terms of the extract ability to donate hydrogen.

Another assay method is the Total antioxidant capacity using phosphomolybdenum assay (PM) the principle is the reduction of molybdenum

(VI) to molybdenum (V) by the antioxidant sample. Colored green phosphate/molybdenum (V) complex at acidic pH is form at end of reaction process (Beghdad et al., 2014). This assay enables the determination of amount of fat soluble antioxidant capacity (total antioxidant capacity) and water-soluble (Beghdad et al., 2014).

Fatty Acids

Fatty acids are chemical compounds containing a terminal carboxylic acid group and long hydrocarbon chain. The nature of the bond in the chain determines whether fatty acid can be saturated or unsaturated. Both lipids fatty acids or free fatty acids play significant roles as an essential components of all membranes and in metabolism as gene regulators (Glick & Fischer, 2013; Palmquist, 1964). Natural metabolites which are also polyunsaturated fatty acids are being provided by Dietary lipids (Rustan, 2005). However, free fatty acids have the ability to form micelles and amphipathic properties this enables the acids and their salts to be use as soaps and detergents. The major components reported in most studied lipids includes sterols, hydrocarbons, , sterol esters, triacylglycerol, wax esters, free fatty acids, 1,3-diacylglycerols, alcohols, 1,2-diacylglycerols, Palmitic, oleic and linoleic acids (Reazai et al., 2014a).

Different group of fatty acids are unique with specific biological properties and health effects. Fatty acids have carboxylic functional group with aliphatic straight chains, however, C4 to C18 are the most commonly found fatty acids but naturally fatty acids C4 to C22 exist naturally. Fatty acids can be categorized according to the number of double bonds it contains: Saturated fatty acids (SFA) is a type of fatty acids which contains single bonds that dominate in the fatty acid chain examples includes myristic acid, stearic acid, butyric

acid, lauric acid and palmitic acid (Briggs et al., 2017). These can be found in foods such as butter, coconut oil, palm kernel oil, cow's milk, breast milk, dairy products, palm oil and meat. Monounsaturated fatty acids (MUFA) contain one double bond that dominate in the fatty acid chain. Both type of fatty acids helps in maintaining good health of cells. And also, they reduce stroke in the long run and the risk for heart disease by reducing bad cholesterol. Examples of includes oleic acid (18:1 n−9) palmitoleic acid (16:1 n−7), and cis-vaccenic acid (18:1 n−7) (Schwingshackl & Hoffmann, 2012). MUFA can be found mostly peanut butter nuts including avocados, peanuts, sesame seeds and cashews and in cooking oils, including canola oil, olive oil and sesame oil. Polyunsaturated fatty acids (PUFA) are fatty acids contain more than one double bond that dominate in the fatty acid chain. Examples includes omega-6 fatty acids and Omega-3 fatty acids (Ander et al., 2003). They are fat and can be gotten from mainly in fish, seeds, nuts, seed oils, and oysters.

Fatty Acid Composition of Orange Seed Oil

Mieko and Neuza. (2012). Stated fatty acids profiles of Brazil orange seed oil they are Palmitic (C16:0), linoleic (C18:2 n-6), stearic (C18:0), oleic (C18:1 n-9), and Linolenic acid (C18:3 n-3). Palmitoleic (C16:1), Margaric (C17:0), Lignoceric (C24:0), Arachidic (C20:0), and Behenic (C22:0) had also been reported (Mieko et al., 2012). Traces of Linoleic acid was found with no traces of Linolenic acid (B. Engineering, 2013; B. Nwobi et al., 2011). Another literature revealed that orange seed oil from Kerman, Iran contains (33.2 % to 36.3 %) Linoleic acid as the main fatty acid found in citrus seeds oil whiles palmitic acid and oleic acid was found within the ranges 23.5 % to 29.4 %. There are other acids found at small rates such as stearic, linolenic acid and

palmitoleic (Reazai et al., 2014a). Citrus seeds contains mostly saturated palmitic acid component (Reazai et al., 2014a). The content of Palmitic acid depends on the types of seeds. Reazai et al. (2014) reported the content of oleic acid of orange seed oil 29.3 % in samples from Jiroft and 27.1 % in samples from Qaleh Ganj in Iran. The average unsaturated fatty acids found in sour orange and lemon varieties are and 68.83 % and 66.46 % respectively (Reazai et al., 2014a).

Essential Fatty Acids from Seed Oils

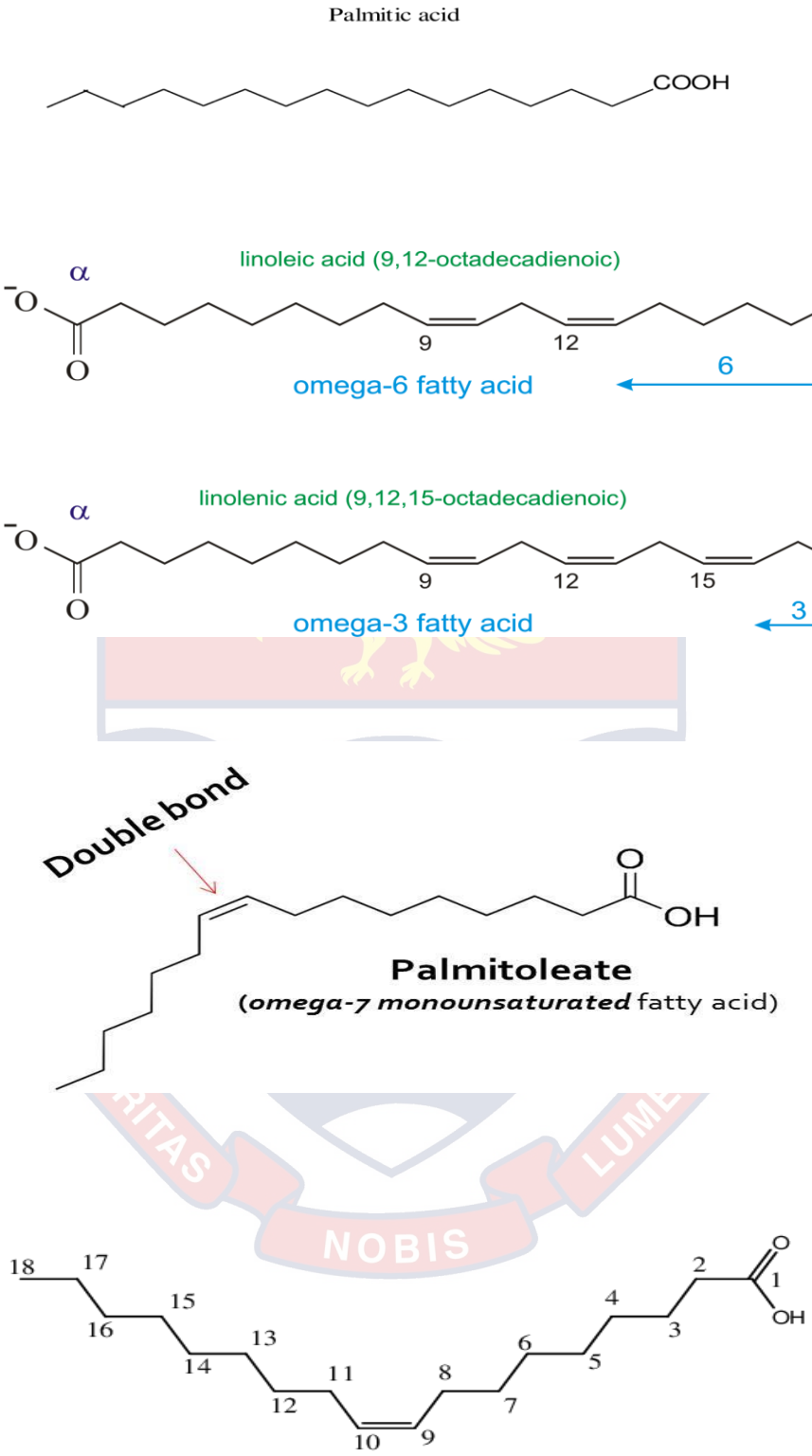
The essential fatty acids (EFAs) are fatty acids obtained from dietary plant sources responsible for biological activities. The Humans system requires two types of fatty acids, a-Linolenic acid (C18:3n-3), n-3 fatty acid with the first double bond at the third carbons from the methyl end and the linoleic acid (C18:2n-6), the first double bond 6 carbons from the methyl end (Glick & Fischer, 2013).

The essential fatty acids (EFAs) are the most valuable fats as the body requires them to survive. Most fats, such as saturated fatty acids, cholesterol and unsaturated fatty acids in the human body can be produce from other fats. Two groups of fatty acids exist, known as essential fatty acids, based on alpha-Linolenic acid (omega-3 group including EPA and DHA) and linoleic acid (omega-6 group including GLA) which cannot be produced in the human body (Pasquale, 2017; Sampath, 2009). An example of long chain polyunsaturated fatty acids is α-Linolenic acid which includes docosahexenoic (DHA) and eicosapentenoic (EPA) are elongated and desaturated by enzymes (Sampath, 2009). These derivatives have anti-hypertensive, anti-inflammatory, anti-thrombotic, and anti-arrhythmic (Sampath, 2009). N-6 fatty acids are

accountable for thrombotic metabolites and high-level inflammatory and therefore require a correct equilibrium of the fatty acid proportion n-6 to n-3 (4:1 or 2:1) in the diet (Reazai et al., 2014a).

Importance Fatty Acids in Diet

Fatty acids have great influence in the body composition of man. They improve metabolism, increase weight, fat loss, muscle mass retention, hormonal (including testosterone and growth hormone), decreased cardiovascular, neural, musculoskeletal and inflammation in the body thus offering health advantages. They also enhance lipid serum profile (cholesterol, triglycerides) including concentrations of cholesterol, improve working of the immune system, Mental health improvement and have anti-aging effect (Pasquale, 2017).



cis-9 oleic acid

Figure 4: Structures of some fatty acids

Reference: Reazai et al. (2014a)

Transesterification of Fatty Acids

Fatty acid methyl esters (FAMEs) are prepared for the assessment of fatty acid composition using Gas chromatography. This may be achieved by alkali or base methanolysis which starts with saponification of the fatty acid followed by esterification (Lago, 2014).

Fatty acid methyl esters can be gotten from animal fats or vegetable by means of trans esterification. In the process of Tran's esterification, the triglycerides react with a short chain alcohol in the presence of a catalyst. Glycerol and fatty acid methyl esters are the products of transesterification process (Hamid, 2011). In the presence of a catalyst, condensation of a carboxyl group of the acid and the hydroxyl group begins to during the reaction process. The acid group becomes more reactive because there a catalytic protonation of the oxygen atom of the carboxyl group. The alcohol (methanol) then reacts with the protonated acid to produce an ester which the produces water as a by-product. The alkyl chain length of the resulting esters produced depends on the alcohol utilized. The use of ethanol will result in ethyl ester whiles for methanol will result in the formation of methyl esters (Apita, 2014; Knothe, 2005). The free form of fatty acids may be difficult to assessed because the hydrogen bond exists between the compounds and they are highly polar and tends to adsorption to the surface of the column of the chromatography used, this account for reason to assess fatty acids in fatty acid methyl esters form. Converting the fatty acids to methyl esters reduces their polarity and thus makes way for easy analysis. Because of the differences in the unsaturated fatty acid, the polar carboxyl functional group of the fatty acids has to be first neutralized for easy

identification. This then allows the column to perform separations based on boiling points and also by degree of unsaturation.

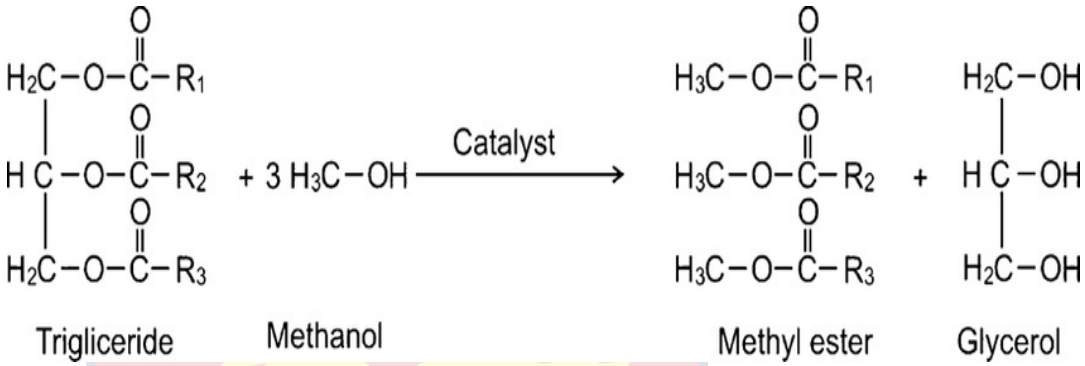


Figure 5: Trans esterification of fatty acids

Reference: Hamid (2011)

Composition of Fats and Oils

Since long time, edible oil and fats have been used in food. Proteins and carbohydrates more complex than fats and oil because these fats and oil contains molecules of triglyceride. In the classical definition of oils and fats is that, oils are liquids at room temperature (20 ℃) whiles fats are solids. Fats and oil composition may vary based upon the sources. Composition of fatty acids is an important criterion that decides the use of fats and oils. Fats and oils contain triglyceride. Free fatty acids, sterols fatty alcohols, phosphatides, fat-soluble vitamins, mono- and diglycerides, tocopherols, pigments, and waxes are the minor component of triglycerides. The source of the fatty acids account for its content of free fatty acids especially in crude oil. In addition to free fatty acids, animal fats contain smaller amounts of the minor component whiles crude vegetable oils contain about 2 %. Figure 6 shows the hydrolysis of fat (triglyceride) to produce the various components.

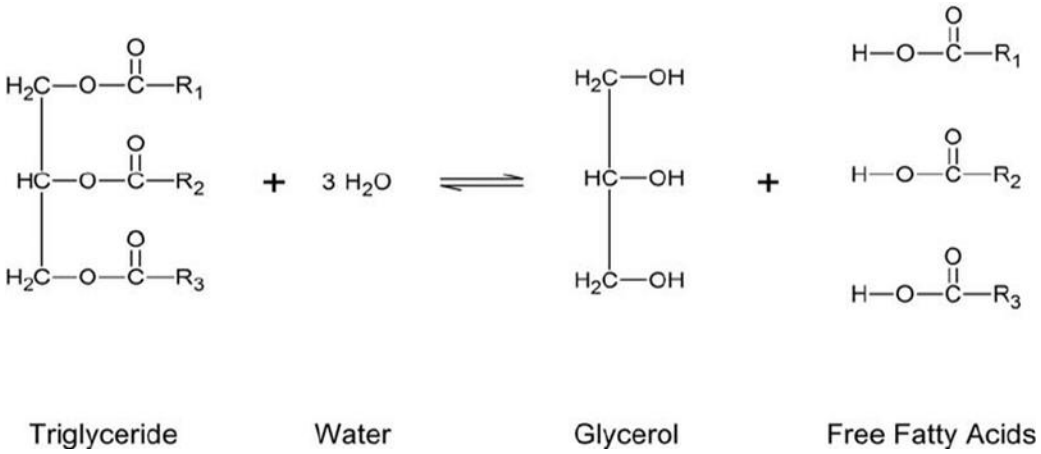


Figure 6: Hydrolysis of fatty acids

Reference: Hamid (2011)

Oxidation of Fats and Oils

Oxidation results in deterioration in quality of food especially in triglycerides and phospholipids. Lipids or phospholipids and neutral lipids or triglycerides are the two major classes of lipids. Oxidation of lipids is one of the major problems faced by the industrial sectors and even in the home. Oxidation reaction is one of the main sources of degradation that occurs during manufacturing and storage of lipid food. Oxidation of fatty acids and oils is a chemical reactions which takes place in the presence of moisture, temperature, light, availability of oxygen, the presence of iron and the type of oil (Wasowicz et al., 2004).

Oils with saturated fatty acids are less susceptible to oxidation than polyunsaturated fatty acids (PUFA) oils. Double bonds presence between the carbon atoms enhances unsaturated oils to oxidation. More single bonds presence in saturated fatty acids retards oxidation in oils (Wasowicz et al., 2004). Oxidation eventually produces rancidity in oil, which is noticed by its off flavor and smell. Oxidation of oils produces various breakdown products which includes peroxides, primary oxidation products, dienes, , free fatty acids,

and secondary products of carbonyls, trienes and tertiary products (Wasowicz et al., 2004). Oxidation occurs by mechanisms such as free radicals' formation reactive oxygen precursors leads to oxidation of oil. The oxidized oil leads to the formation of both desirable and undesirable products as a results of interactions among food constituents (Himaja et al., 2010; Pickova et al., 2016; Pignitter & Somoza, 2012). The production of peroxides which is responsible for undesirable smell of lipids in food reduce the shelf-life of products. Oxidative reactions in fatty acids result in rancidity of the oil. Rancidity ends the oil in a bad smell, discoloration, altered nutrient value, and also produce toxic compounds, which can endanger the health of humans.

Antioxidants and chelating agents are the mostly remedies for lipid oxidation. Assessing oxidation involves testing for the presence of peroxides and other secondary breakdown products in the oil. The simplest tests are iodine value (IV), peroxide value (PV), free fatty acid FFA and acid value (AV).

Unsaponifiable Extract

The unsaponifiable matter of fats and oils consists of nonglyceridic bioactive substances such as pigments, aliphatic alcohols of high molecular mass, hydrocarbons, sterols and, ketones, and fat soluble vitamins which are formed naturally or formed during processing or oil degradation (Freitas, 2014). These components are not saponifiable by alkali hydroxides but dissolves in ordinary fat solvents (Freitas, 2014; Yildiz & Dasgupta, 2016). Unsaponifiable matter has the strongest antioxidative proper this account for their popularity. Therefore, unsaponifiable matter can be introduced as a natural antioxidant which has larger antioxidative property and they can also introduce as an alternative to thermal synthetic antioxidants because of their stability an

example is Tert-Butylhydroquinone (TBHQ) whose antioxidant property has been reported (Tavakoli et al., 2017).

Frying Process

Food are heated in hot fats and oils during frying procedure. Some foods are made attractive and delicious by frying them especially practiced in food industries, households and eating places. 150-190 ℃ temperatures range is applied during the frying procedure this range transfers mass heat which result in interaction between the material and the medium. (Ahromrit & Nema, 2010).

Deterioration of Oils During Frying Process

As the frying process occurs, several chemical reactions which includes hydrolysis and oxidation reactions occur leading to deterioration of the oils (Song et al., 2015). Other factors considered in the frying process are reduce temperature and an increased time. Oil deterioration takes place at higher frying temperatures (Abdulkarim et al., 2008). At higher temperatures, hydrolysis of the oil occurs speedily and is as a result of the presence of moisture associated with the food being fried in the oil. The consequence is polymerization and oxidation reaction which results to the degradation in the quality of the oil (Abdulkarim et al., 2008; Song et al., 2015). Therefore, the autoxidation result is the off-flavor of a frying oils (Falade & Oboh, 2015).

The hydroperoxides are the main products whiles the secondary oxidation products are alcohol, aldehydes, hydrocarbons, acids, ketones, etc. (Domínguez et al., 2019). key chemical values utilized to evaluate the extent of oxidation of frying oils is by assessing the oil primary or secondary oxidation products. Lipids oxidation is mainly assessed by a free-radical chain mechanism in the following procedure below;

(1) Radical formation in the initial step

$RH \rightarrow R\bullet + H\bullet$

(2) Free radicals are propagated and formation of hydroperoxide as the primary oxidation product.

$R\bullet + O_2 \rightarrow ROO\bullet$,

$ROO\bullet + RH \rightarrow ROOH + R\bullet$

(3) Secondary oxidation products are produced as a result of termination steps

$R\bullet + R\bullet \rightarrow R–R$,

$R\bullet + ROO\bullet \rightarrow ROOR$,

$ROO\bullet\ ROO\bullet \rightarrow O_2$ +ROOR or alcohol compounds or carbonyl (Domínguez et al., 2019).

The increased level of rancidity in frying oils is caused by repeated use of oil and this also affects the shelf life of fried foods. (Che Man & Jaswir, 2000). When oil is used at increased temperatures and in the presence of air, thermal deteriorations occurs. The physical, chemical, dietary sensory property of the oil is changed as a result of deterioration (Kaur et al., 2008; Nayak et al., 2016). The deteriorated products presence in the oil are dangerous to human health because they inhibit enzymes (Abrahamse et al., 2012) as vitamins are destroyed which results to gastrointestinal irritations especially cancer, heart disease, diabetes, stroke or mutations, have been reported (Craig-Schmidt, 2006).

The Fatty acid composition is key parameter in assessing the stability and flavor of oil in fried foods. Normally, frying procedure changes the Fatty acid composition of the oil which results to the formation of Trans fatty acids (Abrahamse et al., 2012) also there is reduction in the level of UFAs and

increase in saturated fatty content. Consequently, chemical parameters like iodine value, free fatty acids and peroxide value are also altered (Abrahamse et al., 2012). Trans fatty acids formation depends on the nature of frying oil used, frying time and temperature (Kaur et al., 2008). Trans fatty acids was used as safe alternative for butter fat as the harmful effect of saturated fatty acids has been reported. However, total and low-density lipoprotein cholesterol increases and the "good" high-density lipoprotein cholesterol decreases in foods because of Trans fatty acids (Iqbal, 2014). Mozaffarian et al. (2004) at Harvard University reported that the ratio of low-density lipoprotein cholesterol to high-density lipoprotein cholesterol increase because the presence trans fatty acids which may result in the risk of coronary heart disease.

Trans fatty acids are associated with systemic inflammation markers in women, especially protein (C – reactive) (Mozaffarian et al., 2004). Lopez-Garcia et al. (2005) enunciated that consuming more trans-fat may result in endothelial dysfunction. The heat applied type of oil used during frying procedure produces many trans fatty acids (Romero et al., 2000; Bansal et al., 2009). Wakako et al. (2008) also reported that most of these trans fatty in foods are produced from the oil used but not from the frying process. In countries, especially where technology is advance, food sources are either free from trans fatty acids or to a reduce level due to the strict rules and regulations associated with the ingestion of fatty acids also, modern methodological industrial process are applied in these countries to control trans fatty acid formation (Food and Agriculture Organization, 2003). however, other developed countries like Pakistan and other developing countries, commercial fryers do not use better oil for frying food because there no standard to levels of oxidation indices

especially to the trans fats. Tynek et al. (2001) have reported that after heating oil there was a reduction in the iodine value and an average loss of the C18:2 fatty acids when compared to heated oil containing food, this resulted from more intense thermo-oxidative transformations in the oil.

Reduction in the iodine value resulted from the degradation of double bonds by polymerization, scission and oxidation hence, heated and unheated oils and fats should be monitored by assessing parameters like fatty acid composition and iodine value, which can indicate destruction of the FAs. Typically, oils utilized for frying can be palm oil (PO), canola oil, Sunflower oil cottonseed oil (CSO), soybean oil, sesame oil (SO), and corn oil (CO) (Hashempour-Baltork *et a*l., 2016). A mixture of different oils is formulated to obtain to healthy oil. Hence, the formulated oil should contain a contain increased level of natural antioxidants and reduced level of linoleic and linolenic acids which can withstand the heat during the heating procedure. Assessing free fatty acids measures the fatty acids that hydrolyzed on the triacylglycerol backbone.

Free fatty acids contents in oil is a means of determining the importance of the frying procedure. The determination of free fatty acids assesses the safety of the frying oils for human, and the oil is rejected if a value of 2 percent is obtained (Matthaus, 2006). Peroxide value is a technique that is used to evaluate hydroperoxides which is a first lipid oxidation products and it is expressed as meqO_2/Kg of oil (Halvorsen & Blomhoff, 2011). During frying, hydroperoxides becomes unstable and accumulates because of the frying temperatures. It is also stated that the peroxide value is insufficient for estimating frying oils.

According to Xia and Budge (2017), secondary oxidation products contains carbonyl groups this results from the primary oxidation of the oil which initially contains the peroxides (Xia & Budge, 2017). In terms of stability, the primary compounds are less than the secondary compounds and the carbonyl value is a good index for assessing frying oil oxidation levels. Rancidity and unpleasant flavors of the oil is as result of the secondary products being oxidized and this results in the loss of fried food nutritional values (Endo et al., 2001).

During the frying procedure, evaluating the number of polar compounds present in the oil is directly link with the lager molecular weight compounds. To assess the degradation of oil used to fry, total polar compounds present in the oil is an accepted method to decide either to reuse or discard the oil (Gertz, 2001). There is a change in the double bond of lipids especially through oxidation as in methylene-intervallic polyenes or dienes. Farhoosh et al. (2008) assessed that some products such as carbonyl, conjugated triene and diene in fat and oils undergoes oxidation when the heated at high temperatures. These are better indication for change in oxidation. Which is leads by increased UV absorption at wavelengths of 420, 234 and 273 nm.

Mono- di-acylglycerols, glycerols and free fatty acids are also formed giving rise to off-flavour of frying oils. The shelf life of oils used repeatedly in frying food becomes shorter due to high rancidity rate attached to frying oils (Esfarjani et al., 2019).

Effect of Frying Process on Fatty Acid Composition

The fatty acid composition of frying oils is a key factor affecting the flavor and stability of fried food. The fried food is immersed in the frying oil during frying and hence the quality of the oil is very critical as it contains an

important part of food constituent. Researchers have proposed that fatty acid composition changes as a result of changes in the oil during frying.

Slama et al. (2019) discovered an increase of saturated fatty acids (C18:2) and decrease of Polyunsaturated fatty acids (C18:3) at frying temperature about 180 ℃ from 11.5 % and 27- 46 % for 40 h respectively. When both fatty acids were compared, C18:2 Fatty acids content was more than that of C18:3 fatty acids. Rossell (1989) stated that the C18:3 rates of oxidizes 100 times larger than stearic acid (C18:0) whiles C18:2 is 10 more higher than oleic acid (C18:1). Also, Rossell (1989) stated that rate of oxidation C18:3 fatty acid is 15 more larger than C18:1 fatty acid as stated. The quality of a frying oil is determined by the number of C18:3 fatty acids it contains frying. Frying oil with a reduced C18:3 fatty acids level is good or quality oil to meet consumer demands. Vever-Bizet and coworkers (2011) have assessed the reaction rates C18:0, C18:1, C18:2 and C18:3 fatty acids which are 1.2 × 104, 5.3 × 104, 7.3 × 104, and 10.0 × 104 $M^{-1}s\text{-}^{1}$, respectively when these rates was measured with oxygen. Relative reaction rate of C18:3 fatty acids was very fast than that of C18:2 and C18:1 fatty acid. Hence, C18:1 fatty acid was more than C18:3 fatty acid. Kaur et al. (2014) have stated that the building blocks of fats and oils are fatty acids (FAs) and very essential. The human body cannot produce these fatty acids but can be gotten via nutrition means.

Also, during frying process, especially at larger temperatures oxidative change occurs due to thermal means as a result oxygen enters the oil whiles moisture is released from the food (Choe & Min, 2007). It is found that the type of food being fried (Stevenson et al., 1984), operating conditions and the design of the fryer contributes to the oxidized product formed. Liu et al. (1992)

explored number of fatty acids produced during frying as a result of oxidation process, that further results in a decreased in total unsaturated fatty acids or a rise in saturated fatty acids levels. The rise in levels of cholesterol, as in low-density lipoprotein (LDL), occurred as a because of the increased levels of saturated fatty acids during the frying procedure, and may result in health problems. A repetitive use of oils at high temperature leads to an exchange of fatty acids between the oil and the fried food, these phenomenon effect in the change in the level of fatty acids in the oil (Sanibal et al., 2004). Min et al. (2000) compared the average oxidation rate of polyunsaturated fatty acids similar to C18:2 and C18:3 fatty acids as 1:40 to 50:100 on the basis of the oxygen absorption to Monounsaturated acids like C18.

Oxidative deterioration of oil during frying depends on the quantity of unsaturated fatty acids present in oil. At frying temperature of 180°C, tend to oxidation and degradation of unsaturated fatty acids into secondary products occurs which leads to the loss oil flavor (Park & Kim, 2016). Tyagi et al. (2001) assessed the thermal oxidation in frying soybean oil which is an edible oil, from the results the amount of linoleic (C18:2) and linolenic (C18:3) fatty acids reduce through 79 % to 60 % which indicates the loss of losses of unsaturated fatty acid.

Sanibal et al. (2004) investigated the changes in the reduction of polyunsaturated fatty acid in the fatty acids profile of soybean oil and partially hydrogenated soybean fat (PHSF) at the time of frying procedure. The reduction was 12.8-7.3 % and 59.9- 32.6 % at 50 h of frying method respectively. Sulieman and coworkers (2006) have analyzed the characteristics of fatty acid composition cottonseed oil (CSO), Sunflower oil and palm oil which are

vegetable oil during the frying process for 16 h. The assessment on the alteration in fatty acid composition revealed that the polyunsaturated fatty acids (C18:2 and C18:3) reduced in value of 52.87 to 44.26 %, 65.68 to 63.61 % and 14.26 to 12.73 % whiles the saturated fatty acids rises from the value of 24.99 to 26.05, 9.77 to 10.50 and 41.97 to 44.18 % in cottonseed oil, Sunflower oil and palm olein respectively. Similarly, Machado et al. (2008) have stated a crucial reduction of polyunsaturated fatty acids most oil. Aladedunye et al. (2009) have described a fall in level of polyunsaturated fatty acids like C18:2 at 185℃ and C18:3 at 215℃ in canola oil during frying method. Respectively, reduction in both values were 8.5 % and 13.3 % at 185 ℃ and 215 ℃. Decreased fatty acid content of C18:3 was very noticeable during frying method from the range of values of 24.0 % and 47.1 % at 185 ℃ and 215 ℃. Alireza and coworkers (2010) reported the characteristics palm oil (PO), canola oil and sesame oil (SO) for used for frying potato chips at 180 ℃ for five days period. The fatty acid composition shown that saturated fatty acids such as the palmitic acid (C16:0) rises in level than with a continued frying time in all three oil samples whiles both the polyunsaturated fatty acids namely C18:2 and C18:3 fatty acid contents decreases in level. Juarez et al. (2011) and Marinova et al. (2012) observed similar changing trends occurring in both saturated and polyunsaturated fatty acids for different frying vegetable oils.

Effect of Frying Process on Trans Fatty Acids

The ingestion of trans fatty has increased the risk of certain diseases such as cardiovascular (CVD), degenerative diseases, certain cancers, chronic respiratory and neural hence more attention has been drawn on trans fatty acid (Sun et al., 2015). C – reactive protein, which is an inflammation marker in the

body increases as result of the intake of trans fatty acid according to research. (Mozaffarian et al., 2004). Lopez-Garci and coworkers (2005) explain that, higher intake of the trans fatty acid may also cause some disease complications such endothelial dysfunction. But, the quantity of trans fatty acid present in an oil depends on the heat applied to a particular type oil during the frying procedure (Romero et al., 2000; Liu et al., 2007; Bansal et al., 2009). Many trans fatty acids (TFA) in fried foods are produce from a particular type of oil used for frying it buy not the frying procedure (Wakako et al., 2008).

Cuesta et al. (2000) have stated that, during frying procedure of sunflower oil, oxidation of the oil occurs due to the heat applied to the oil, this result in the rise in level of Trans linoleic acid or linoelaidic acid (C18:2t) and Trans oleic acid or elaidic acid (C18:1t) in the oil.

Moreno et al. (2009) revealed the quantity of Trans isomers found in sunflower oil increases 1.10 % to 11.45 % for the same time duration of 40 min at different temperature of 200 ℃ and 300 ℃ respectively. Also, Romero and coworkers (2000) assessed extra virgin olive oil (EVOo), high oleic sunflower oil (HOSFO) and Sunflower oil, in which the minimum quantity of the elaidic acid was recorded in these oils.

Degradation of a frying oil is enhanced by the presence of water, as the water from the fried food undergoes hydrolysis reaction (Hara et al., 2006). A reference to trans fatty acids in frying oil, hydrolysis results to the isomerization of the double bonds in unsaturated fatty acid (Bansal et al., 2009). Because of this reason its advice to fry an oil at lower temperature of about to 160 °C to prevent the deteriorating the oil and to prevent the intake of less amount of trans fatty acids by human consumers.

Bansal et al. (2009) and Aladedunye et al. (2009) have reported that, the foods which were initially frozen releases trans fatty acids into the frying oil during the frying procedure and this causes the number of C18:1t fatty acid to increase in the heated oil (HO). In the frying procedure using poly hydrogenated soya fat (PHSF), elaidic acid was the key fatty acid among trans isomers released into the oil and this is the characteristic changes that occurred after the frying process. After 10 and 50 h of frying procedure, the trans monounsaturated fatty acids increased to 2.1 % and 14.3 %. The Polyunsaturated fatty acids reduced from the range of 59.9 % to 32.6 % in frying oil after 50 h of frying procedure. These reports depict the formation of trans monounsaturated acids in heated oil and frying oil.

The small quantity of unsaturated fatty acids which were present in the oil was as a result of the lower percentage formation of trans isomers in the frying oil. Farag an co-worker (2010) reported the formation of trans fatty acids in distinct blending of oil samples (sunflower oil + canola oil + palm olein) using gas liquid chromatography (GLC). Formation of trans fatty acids increased during the frying process during frying procedure at 180 ℃ for the period of 20 h. The mixture of Sunflower oil with palm olein or canola oil yielded a reduced trans fatty acid during frying procedure.

The amount of trans fatty acids increased with prolong frying at high temperature was indicated by Wakako and co-workers (2010) as they investigated the effect of heated oils (HOs) on utilizing of trans fatty acids, by frying and heating process.

It is important to raise scientific awareness on the risk in the intake of trans fatty acids as a nutritional quality. This is because many countries,

including Pakistan, large population continue to consume fried foods as part of their diet due to their traditional eating habits and lifestyle.

Effect of Frying Process on Free Fatty Acids

Frying oil hydrolysis to form free fatty acids, glycerol, monoacylglycerols and diacylglycerols as it being used to fry food materials (Bordin et al., 2013). Another significant parameter for the determination of frying oil deterioration as a result of the oxidation of the oil is the presence of free fatty acids.

Nasirullah (2001) revealed that the unsaturated fatty acids in the oil oxidize by thermal degradation which leads to the formation of free fatty during the frying process. The determination of free fatty acids assesses the safety of the frying oils for human, and the oil is rejected if a value of 2 percent is obtained (Matthaus, 2006).

Abdulkarim and coworkers (2007) stated that the free fatty acids in soybean oil, canola oil, palm olein and *Moringa oleifera* seed oil during frying process. The percentage amounts of free fatty acids determined were 0.22, 0.10, 0.19 and 0.17 %, for soybean oil, canola oil, palm olein and *Moringa oleifera* seed oil respectively. Soyabean oil recorded the lowest amount of free fatty acid value (60 %). Palm olein was the next (65.0 %) followed by MoO (66.6 %) and canola oil recorded the highest free fatty acid value (71.4 %) after the 5 days frying process. Manral and coworkers (2008) determined the free fatty acids in Sunflower oil during fish frying at 180 °C for 14 h. Index of free fatty acids increased from 0.05 % to 0.585 % in the oil for the 14 h in the process of frying.

Similar study conducted by Juarez et al. (2011) on soyabean oil, sunflower oil and partially hydrogenated mixture of these vegetable oils

recorded significant increases in the free fatty acids. However, the greater change in the free fatty acids occurred in the partially hydrogenated oils.

Moros et al. (2009) investigated the deterioration of Sunflower oil and the seed oil during heating for the evaluation of cis-unsaturation and trans fatty acids correlation with free fatty acids when a temperature of 147, 171 and 189 ℃. After frying period of 32 h, it was found that the cis-unsaturation decreased while both free fatty acids and trans fatty acids content increased.

Effect of Frying Process on Peroxide Value

Peroxides are primary oxidation products of the frying oil and they quantified by measuring the peroxide value of the oil (Goburdhun et al., 2000). Many researchers agree that the current studies show an increasing trend of peroxide value with frying time. Tan and co-workers (1999) stated a rise of peroxide index in in the oil during frying and heating process.

There was an increase of peroxide value from 0.91 to11.70 meq/Kg when Jaswir and coworkers (2000) assessed frying used to fry potato chips. It was stated by Goburdhun and coworkers (2000) that the rise in level of 6.6-12.6 meq/Kg peroxide level of the oil was obtained when it is used to deep fry potato chips for 300 min. An increased in peroxide value from 3.6 to 6.0 and 5.7 to 11.2 meqO2/Kg was recorded for rice bran oil and refined soybean oil respectively throughout frying procedure (Nasirullah, 2001).

Bangash and coworkers (2006) assessed the peroxide value and other chemical values in *Silybum marianum* oil and Sunflower oil when they were used for 5 days within 20 min for frying at 180-190 ℃ temperature range. An increased in peroxide index was recorded during the frying process in the range of 16.17 to 81.40 meq/kg of oil and 18.60 to 85.50 meq/kg of oil in *Silybum*

marianum oil and Sunflower oil respectively. The peroxide value was lager in *Silybum marianum* oil than Sunflower oil. Abdulkarim and coworkers (2007) recorded rise in peroxide value in distinct oils such as *Moringa oleifera* seed oil, palm olein and soybean oil in the entire frying procedure. The change in peroxide value for each day of heating was 0.72, 0.69, 0.67 and 0.62 % in canola oil, soybean oil, palm olein and *Moringa oleifera* seed oil respectively. Largest values 5.08 meq/Kg of oil after 2 days, 4.0 meq/Kg of oil after 4 days, 4.42 meq/Kg of oil after 2 days, 4.92 meq/Kg of oil after 4 days of peroxide value were obtained in palm olein, canola oil and soybean oil respectively. The results depict the rapid increase of peroxide value in soybean oil and canola oil was due to instability of the oil that occurred in C18:3 fatty acid. The instability in canola oil was as a result of oxidation whiles that of the soybean oil as a result of lager quantity of C18:3 fatty acid in the oil.

Manral and coworkers (2008) investigated the peroxide value of sunflower oil used to fry fish at 180 ℃ for 14 h. At 12 h period there was an increase in peroxide index from 0.1 to 28.98 meq/Kg of oil. The peroxide value reached 24.88 meq/Kg of oil after 14 h frying time. The reduction in peroxide index may be as a result of instabilities of peroxide (Fritsch, 1981). Siddique and coworkers (2011) evaluated the peroxide values of different blended oils which are, palm olein: canola oil, palm olein: soybean oil and palm olein: Sunflower oil with ratio of 50:50. An increased in the peroxide value was recorded in all the blended oils but peroxide value was much more higher in palm olein: canola oil due to the oil instability to oxidation.

Effect of Frying Process on iodine value

The amount unsaturated compounds present in the oil can be indicated by the measurement of the iodine value. It also determines how unsaturated an oil can be. The double bonds of the unsaturated compounds in the oil can be destructed by means polymerization and oxidation as heat is applied to the oil through frying method this leads to the reduction in the iodine value of the oil. The iodine value reduces whiles time of heating increase. Tynek and coworkers (2001) stated that this could be associated with changes in fatty acids that occurred during the frying process.

Goburdhun and coworkers (2001) measured the disappearance of cis double bonds in sunflower oil using Fourier Transform Infrared (FTIR) spectroscopy and some other oxidation indexes, such as the iodine value at 180 °C for 600 min after the sunflower oil has been used to fry potato chips. It was observed from their result that as the iodine value decreases the free fatty acids in the oil also increases. The reduction in iodine index indicates the loss of unsaturation. Naz et al. (2004) affirmed that iodine value linearly decreased with prolong time of heating the oil, this result from the destruction of unsaturated fatty acids and lipid oxidation. Sunflower oil was used to fry samosa and potato chips at a temperature of 190 °C in both laboratory frying and commercially frying by Rehman and coworkers (2006). The iodine value was found to have decreased more in the commercial frying as compared to the laboratory method. Hence, the laboratory frying was better than commercial frying method.

Abdulkarim et al. (2007) studied the iodine value in soybean oil, canola oil, palm olein and *Moringa oleifera* seed oil after these oils is used to fry for 5 consecutive days. Their results indicated a decrease in iodine value in soybean

oil and canola oil at 116.9 – 111.8 and 109.9 –103.0 g/100g and smaller decreases occurred in *Moringa oleifera* seed oil and palm olein at 65.9 – 62.2 and 56.8 – 53.7 g/100g. deterioration in canola oil and soybean oil was more pronounce as compared to that of the palm olein and *Moringa oleifera* seed oil. There was also decrease in the iodine value of sunflower oil when evaluated by Manral and coworkers (2008) after it been used to fry fish for 14 h at 180 ℃. A range of 126.44 to 117.42 g/100 g decrease was recorded. Guillen and coworkers (2012) reported the iodine value in sunflower oil at a temperature of 190 ℃ using an industrial fryer. These results from the two studies showed that the iodine value decreased when heat is applied to the frying oil. Ghosh et al. 2012 also studied various frying techniques such as shallow frying, par frying and deep frying and how these methods can affect the iodine value of soybean oil. It was observed that the decreased iodine value was in the range of 102.6 g/100 g oil for par frying, 101.07 g/100 g oil for shallow frying and 98.56 g/100 g oil for deep-frying method. The result showed that oxidation occurred more with deep frying.

Changes in Color during Fat Frying

Oil quality can be assessed by observing the color changes as it is used for frying. These color changes can be qualitative assessed by visual means (Nayak et al., 2016).

The development of pigments such as the nonvolatile decomposition products [NVDPs] and the α-, β-unsaturated carbonyl compounds in the oil causes the darkening of oil color despite the presence of traces of carotenoids in the oil. Also, fatty acids degrade into the oil by means thermal decomposition and oxidation during frying procedure. The lightness value of the oil reduces

because of the presence of caramelized scorch product in the oil (Maskan., 2003). Sulieman et al. (2006) assessed a preliminary comparative studies on the physicochemical characteristics and antiradical performance of vegetable oil when it is used fry French fries, darkening of the oil increases gradually as observed during the frying process. Increasing the frying time increases the darkening of the vegetable oil. This results from brown pigment decomposing from the fried food into the oil.

Irwandi et al. (2000) stated that the darkening of the frying oil was as a result of both polymerization and oxidation of unsaturated fatty acids present in the frying oil. The authors compared their result to the three distinct sample of vegetable oil and stated that the darkening in color was due to higher amount of linolenic in the oil. darkening in color in palm olein was rapid during frying than oil containing the oxidation product as it was observed. However, reports have indicated that darkening in palm olein oil throughout the frying procedure do not affect the fried food (Jurid et al. 2020). Darkening is very important in oil during frying method because a change in color indicates deterioration of the edible oil and hence must be discarded.

Methods for Evaluating Frying Oil Parameters

Many techniques are utilized to investigate the quality of edible oils and fats used for frying food material. The most widely used technique in assessing trans fatty acids and fatty acid ratio (Song et al., 2015) is Fourier-transform infrared (FTIR) spectroscopy and Gas Chromatography (GC). Kandhro et al. (2008) reported that the GC techniques was use for the assessment of fatty acid profile which include trans fatty acids in biscuits, oils and margarines. Toxic organic solvents and chemical are required to enhance the derivatization of the

oil in the GC techniques. Hence, it requires a lot of labor work and is highly costive when compared to FT-IR. This is because FT- IR is a good alternative analytical technique to GC because of its simplicity, very rapid, and less extensive sample preparation (Van de Voort et al., 1994). Many oxidation parameters such as peroxide index, iodine index, conjugated diene and conjugated triene, free fatty acids, index of saponification of oil and fats can be assessed by the use of FT-IR spectroscopy consequently oil analysis like as main fat groups, fatty acid composition, trans fatty acids can also be measured (Brühl, 2014; Van de Voort et al., 1994). Changes in the peroxide index, unsaturation, carbonyl compounds, free fatty acids content, dielectric constant, density and the quantity of polar compounds are product which diffuse into the oil as a result of the oil used for frying, The FT-IR technique can be used to assess these products. (Moreno et al., 2009). Moreover, Yu et al. (2007) stated that, oxidation products such as trans fatty acids, iodine value, free fatty acids, frying temperature, peroxide value and unsaturated fatty acids can also be evaluated using the Infrared (IR), NMR (nuclear magnetic resonance) and UV-Vis.

A well-established standard American Oil Chemical Society (AOCS) iodometric procedure can be used to assess the level of hydroperoxides (AOCS, 2009), and the resulting peroxide value is expressed as meq/Kg of the oil. Both direct and indirect measurement of hydroperoxides which was based on spectrophotometry has been stated to be use to assess the peroxide value of oil (Yu et al., 2007). Yu et al. (2007) reported that the hydroperoxides developed in oil can react stoichiometric with triphenylphosphine (TPP) to form triphenylphosphine oxide (TPPO). The FT-IR spectroscopy technique can be

used to evaluate the reaction product which results in accurately assessing the hydroperoxide present in the oil.

Chemical Indices of Orange Seed Oil

Saponification Number (value)

Saponification is the processing which triglycerides (fats and oils) react with a strong base to form soap (fatty acid metal salts) of the oil. The amount of potassium hydroxide measured in mg needed to saponify 1.0 g of oil and fats (Onyeike & Oguike, 2003). It also evaluates the molecular mass of the triacylglycerol in the sample. As the carbon chain of the fatty acid present in the oil increases, the oil records low saponification number whereas a record of higher saponification number indicates lower weight of the fatty acid chain are present in the oil. The saponification value is of interest especially in the industry for the production of soaps and shave creams.

An increased in saponification value suggests an industrial used whereas a low value of saponification number renders it not useful as an industrial purposes (Amoo et al., 2004). The higher saponification number, the better the oil can be used to make soap (Sutheimer et al., 2015). Plant extracts, such as vegetable oils, and essential oils are mostly added to soaps to enhance quality of soap. In the formation of soaps not all the fatty acids are saponified. Inorder to render the purity a soap, certain unsaponifiable matter such hydrocarbons, sterols and aliphatic alcohols of high molecular mass can be extracted from oils and fats (Schröder & Vetter, 2012). Unsaponifiable matter is defined as substances soluble in oil but remain insoluble in water after saponification. Figure 7 shows the hydrolysis of fat (triglyceride) to produce the various components.

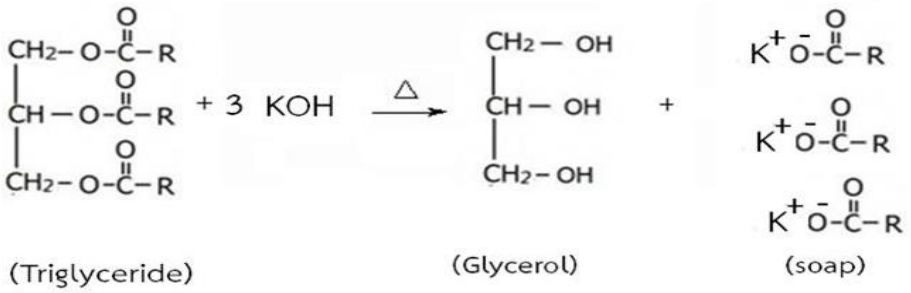


Figure 7: Saponification reaction of triglyceride

Reference: Schröder & Vetter (2012)

Free Fatty Acids

Hydrolysis results to the formation of free fatty acids in the oils and fats. Glycerol and free fatty acids hydrolyzed into the oil because this process occurs at the junction of the fatty acids and the glycerol portion of the triglyceride. A triglyceride, diglyceride and monoglyceride molecules disintegrates into the these fatty acids by either by enzymatic or chemical hydrolysis (Frega et al., 1999; Twumasi & Osei., 2013). The free fatty acid measures fats and oil rancidity caused by lipase. The level of free fatty acid depends on temperature, time, moisture content, and other factors such as processing and storage. Half of acid value of the oil represent the free fatty acid of the oil. The acid value is an index used to evaluate the degree of rancidity (freshness) of the fat and oil (Hussain et al., 2013). Also, the acid value evaluates how by means of lipase glyceraldehyde diffuses into the oil. Free fatty acid content of the of the oil account for it nutritive purpose. A limit of 0.0 to 3.0 % free fatty acid content of the oil is ensures especially in the tropics where vegetable oils are the most means of dietary lipid. In the storaging process of the oil, free fatty acid is obtained as either the raw material from which the fat is obtain or the oil itself

deteriorates. This process is also known as the hydrolysis of the triglyceride present in either sample. In preparation of edible fats, the free fatty acid must be removed.

An increase in moisture content of oil obtained from seeds enhances enzyme activity and shortened the shelf-life of oil (Akintayo, 2004; Twumasi & Osei., 2013). An increased in moisture content and enzymes activities results in the degradation an oil especially vegetable oils (Twumasi & Osei., 2013). Generally, low free fatty acid content of oil from 0.5 to 5.0% is an indication of oil purity for commercial use (Japir et al., 2017). Determination of free fatty acid content of oil is therefore a measures oil quality by commercial oil refiners.

Iodine Value/Number

The amount of iodine measured in gram needed to add to 100 g of oil and fats. This parameter evaluates the degree of unsaturation of fat or oil. The rancidity of the oil can also be monitored using the iodine value (Amoo et al., 2004). Iodine value monitors the shelf life of the oil and fats since unsaturated fatty acids which diffuses into the oil affect the oil shelf-life. The ability of an unsaturated fatty acid to absorb iodine is also evaluated using the iodine value. The double bond of the fatty acid present in the oil is very reactive because it contains unsaturated organic compounds and has the ability to form addition reaction with halogen compounds. As the halogen is added to the double bond, a halogenated compound is formed. Assessing the quantity of halogenated compounds formed measures the quantity of double bonds present in the unsaturated fatty acid.

The larger the iodine number implies high polyunsaturated fatty acid composition in the oil and hence high susceptibility to rancidity. Low iodine

number depicts low content of unsaturation in the oil. Nwobi and coworkers (2006) used a standard method of analysis to assess the iodine index of sweet orange seed oil (Nwobi et al., 2006). The value obtained was 108 and the result confers a high degree of unsaturation that indicated oxidative rancidity of the oil. Iodine value is therefore an important parameter in classifying oils and fats.

The Peroxide Value

The determination of the peroxide index is a useful parameter for determining the oxidation ability of fats, lipids, and oils. Lipid oxidation of food leads to undesirable odor, loss of fat-soluble vitamins and the production of toxins which have great effect on human health. Some fixed oils like sunflower oil, coconut oil, wheat germ oil, nettle seed oil, hazelnut oil and grape seed oil are utilized in diet, medicine and cosmetics. Estimating of peroxide content in an oil is a very important analytical process in assessing food quality (Dermis et al., 2015). The peroxide number (value) evaluates both peroxidation and peroxides in food substance such as an oil. It is one of the identified causes of degradation and production of poisonous compounds in food substances. The quality and oil storage are much influenced by its peroxide value.

Peroxidation leads to the formation of radical species which results from decomposition of fatty acids and other oxidation products in the oil or fat. The most used chemical index for monitoring these oxidation products food such as the oil or fat is the peroxide number (value) because these materials causes diseases such as cancer (Dermis et al., 2015). Fats and oils undergo a spontaneous reaction (Auto oxidation) in the presence of atmospheric oxygen, enzymes (enzymatic oxidation), and heat during deep-fat frying (thermal oxidation) and photo-oxidation. Free unsaturated fatty acids and triglylcerols

are prone to such reactions. Vitamins such as vitamins A and E are damaged as a results of these processes (Baião & Lara, 2005). It affects, and also decreases the nutritive value of the oil is reduced as a result of this process this is because the aroma, flavor, color and texture has been affected. Primary compound such as aldehydes, ketones, hydrocarbons and volatile organic acids are produced as results of oxidation of hydroperoxides, epoxy compounds are also produced as secondary oxidative products.

The primary oxidative products are assessed together with the free radicals to detect deterioration of fats and oils. In order to achieve a peroxide value of an oil, potassium iodide is made to react with oil first hence, any peroxide presence in the oil then oxidize the iodide to iodine followed by a titration of the solution mixture with sodium thiosulphate. The quantity of iodine produced is measured after the completion of the titration method. The degree of oxidation of the fat or oil can be determined by the peroxide value (Gary & Jerry, 2016).

Standards for oil quality showed that, the peroxide value of good oil should not exceed 10 mill equivalents of peroxide.

Refractive Index

Index of refraction estimates the ratio of speed of light in vacuum to the speed of light of an unknown substance. When beam of light is passing through a thin film of melted fat, an angle is made when the light is bent. The angle measured is the refractive index of the fat or oil which is attributed to the triglycerides present in the fat. Different of fat or oil have a specific index of refraction and this feature characterize an oil. Index of refraction is related to

the amount of unsaturation present in the oil however other oxidation index like fatty acid content as well as heat treatment can be used as an evaluation.

Index of refraction is constant for a particular type oil within certain limits and is used in the determination of lipids (Koman & Danielová, 1976). Index of refraction is means of identification of fats and oils measurement of its purity because each oil has a characteristic feature as the refractive index (Cho et al., 2013). Higher refractive index indicates a higher degree of unsaturation as well as lower molecular weight of the fatty acids. Iwuagwu et al, (2018) used a refractometer at 25 ℃ to study the refractive indexes of an oil obtain from seeds of orange (*Citrus sinensis* L.) and pumpkin (*Cucurbita pepo* L.). The results obtained during the study were 1.47 and 1.46 and these values showed that the oils were less thick.

Proximate Composition

The nutritional values that form the basis of the food to be accepted in market includes protein, carbohydrate, ash, fat, crude fibre and moisture content. These values are also known as the proximate composition of a food (Joseph, 2016). The evaluation of these nutritional values in food is an application of food science and technology.

Moisture Content

Moisture is an important factor in food quality that measures the water content in food. Moisture content determines the resistance to deterioration or preservation of food. To determine the nutritive value of oil, the moisture content must also be known, because the moisture content is very important for many economic, scientific and technical reasons. Also, on analytical basis, the moisture content when determined in food meets the compositional standards

or laws. If the determined analytical value does not increase in a simple manner or vary in linear with a high amount in dry matter content (Joseph, 2016), then analytically, it is important to determine the weight of the samples on a given moisture basis. Oil or fat with very low moisture content has good resistance to deterioration or better storage properties (Abdellah & Ahmed Ishag, 2012). Okoye et al. (2011) reported moisture contents of oil obtained from both papaya and orange seeds. An oven dry method was used to evaluate the moisture content of the oils. The moisture content of the oil from the pawpaw was 0.18 percent while that of the orange seeds was 6.43 percent. The results indicated less moisture content in both oil and hence the oil can be preserved for a longer time since shelf-life increases as the moisture content in oil is reduced.

Ash Content

Any inorganic components that remains when the organic material part or other volatile portion of the food has been burnt off or oxidized and evaporated is known as the ash content that food. Ash content in food gives information about the mineral element present. To determine the ash content in food, normally the dry food sample is weighed and then heated at elevated temperature (550°C). Ibrahim and Yusuf (2015) reported the ash content of *Citrus sinesis* seed oil by calcination in a muffle at 550°C in line with the Official Methods of Analysis of Association of Official Analytical Chemists (AOAC) International (2009). Ash components found in the seed oil was 5 % and this value suggested high amount of mineral in the oil which confirmed that orange seed oil is an important source of micronutrients (Ibrahim & Yusuf, 2015).

Crude Fat Content

Fat and oil are very essential in human diet and are classified as lipid (Winkler-Moser & Mehta, 2015). They are commercially important and averagely in the world, 90 % of the produced fat and oil are utilized as food or as an ingredient in the preparation of food (Winkler-Moser & Mehta, 2015). Most cells in the body utilized fatty acids as means of energy. Fats are concentrated form or source of energy and the energy supplied is more than the same weight of protein or carbohydrate.

The brain uses fatty acid derivative as source of energy during fasting. Fat and oil are essential nutrient and hence, increases the palatability of food by retaining the flavours in the manufactured food. Fat soluble vitamins A, D, E are adsorbed and transported rapidly by dietary fat (Kono & Arai, 2015). Adewole et al. (2014) investigated the crude fat content of orange peels obtained from unripe oranges. The fat content was found to be 14.35 %. Five grams of the sample was extracted in a soxhlet apparatus using petroleum ether as the extracting solvent in order to obtain the crude fat. The high value indicated high fat content in the orange peel (Adewole et al., 2014).

Energy

Oils from seeds are used as energy foods because they contain energy example, oil from sesame seeds gives about 600 kcal/g of energy in foods (Prasad et al., 2012). There are variations in the fatty acid composition from the sources of the oils for instance, oils from peanuts contains Monounsaturated fatty acids (MUFA) whereas or Polyunsaturated Fatty Acids (PUFA) are contain in sunflower seeds oil. Some oils from seeds contain an important

amounts of the essential fatty acids, n-3 fatty acid, linoleic acid (LA), and n-6 fatty acid (Kuhnt et al., 2012).

Some components of food such as vitamins, minerals and water do not provide any energy in the form of kilocalories to the body. Protein, carbohydrate and fat are the three main nutrients that supply energy to the body. However, fats provide the body with most of the energy in the form of weight for weight (Lupton et al., 2002). A kilocalorie which is sometimes written as Calorie is defined as the amount of heat energy that is needed to increse the temperature of one kilogram of pure water by 1 ℃. One gram (1 g) of pure carbohydrate has an energy index of 4.0 kCal. One gram (1 g) of pure fat has an energy index of 9 kCal or 38 kJ and One gram (1 g) of pure protein has an energy index of 4 kCal or 17kJ (Lupton et al., 2002). Depending on the physiological state of adult human, the daily energy requirement is 10,500-12,600 kJ while that of infants is 3094.68 kJ (Rahma & Gafar, 1999).

Infrared Spectroscopy

Infrared spectroscopy (IRS) can be measured when a sample absorbed infrared light. IR spectroscopy detects molecular structures of known or unknown sample; this is because there is a link between some chemical structures of compounds and the peak positions in the spectrum of IR. Spectra, peak position, heights and widths of samples can be investigated in order to differentiate between samples.

Different radiations from the electromagnetic spectrum have different wavenumbers (Smith, 2011). Radiations from the electromagnetic spectrum ranges at frequencies between 14000 and 4 cm^{-1}, these wavenumbers are related to the molecular vibration. Three different regions can be obtained from the the

infrared spectrum region: near, mid and far regions with frequencies of 14000 to 4000 cm^{-1}, 4000 to 400 cm^{-1} and 400 to 4 cm^{-1} respectively (Derrick, 2000).

ATR-FTIR

Attenuated total reflectance (ATR) FTIR uses the principle of internal reflectance. This is where a beam of light is produced from a radiation source, the light transmits through a high refractive crystal placed near margin of low refractive index sample (Smith, 2011). Any angle between the surface normal and the refracted light represent the refractive angle (Smith, 2011). Total internal reflectance occurs when the angle of refraction is 90. Minimum angle of incidence required to effect total internal reflectance represent the critical angle (Smith, 2011). Evanescent wave which forms part of a beam of wavelength are in contact with the sample. When the light wavelength is transmitted beyond the reflecting surface, the sample absorbs part of the rays of light, whiles the rest is being detected by the detector (Smith, 2011). ATR measures the attenuated degree of the total reflectance infrared rays that has been absorbed by the sample. The spectrum of FT-IR located in the mid infrared region consists of characteristics bands like particular functional groups. ATR-FTIR measures multiple kinds of sample and is non-destructive method. ATR-FTIR is cost effective and hence required little sample preparation.

The Approach in Fatty Acid Research

To effectively identify the key fatty acid compounds that contribute to lipids of foods, some key approaches are important. It starts from the isolation of key fatty acid compounds and the identification of these active compounds. Each step is necessary for determining the final results.

Isolation of Fatty Acid Compounds

Isolation of fatty acid compounds from the food matrix is the first step in lipid research and is vital because no analytical method will be valid unless the isolates represent the food or bulk materials being investigated (Amri et al., 2017). Fatty acid mixture represents the true characteristics of the food and one of the techniques in the isolation of fatty acid is the solvent extraction.

Solvent Extraction

Solvent extraction is one of the efficient and simplest approaches in isolation of fatty acid compounds (Morais et al., 2010). In Solvent extraction which is also known as liquid-liquid extraction, organic chemical are used to extract the fatty acid compounds from the non-aqueous phase. Fatty acid compounds are continuously extracted by the liquid-liquid extractor. An example is the soxhlet extractor (Zhang et al., 2018). The extraction is carried out in batches. In the extraction of the fatty acid from the food sample, the selectivity, boiling point and high purity of the chosen solvents are considered. Among many kinds of solvent, Petroleum ether is the most commonly used solvent because it has high extraction efficiency of the fatty acid in the food matrix. However, it also has a relatively low boiling point.

Separation of Fatty Acid Compounds

After the isolation of the fatty acid compounds by the above-mentioned technique, the compounds need to be separated and identified for characterization. An indispensable instrument called Gas chromatography (GC) is the first choice used to separate the compounds and this is a separation technique, particularly for analysis of fatty acid compounds (Fisk et al., 2014). In GC, samples are vaporized and transported in a carrier gas (mobile phase) to

the column. Separations take place in the column (Asfaw et al., 2019). The carrier gas for GC analysis is inert and normally Helium gas is used. Nitrogen and Hydrogen gases can also be used. After separation has taken place at the end of the column, a detector is used to detect the separated compounds. Basic components of a GC system are injector, column and detector.

Detecting of Fatty Acids

Effluents from the column of GC system are detected by the detector. The signals are in the form of chromatogram which are recorded on the detector. The signals from the detector can be used to quantify each compound present in the sample. Flame ionization detector (FID) is the detector used and this is coupled with GC. FID is simple, reliable, good linearity and relative highly sensitive for organic compounds. Upon combustion of hydrogen and air, the flame produced is used by the FID (McNair et al., 2019). The reaction of the hydrogen and air produces fewer ions however; more ions are produced when organic compounds are injected into the flame via the FID jet. A current is produced during FID system because a voltage is applied and the ions will then be attracted by the FID collector. When a polarized voltage is introduced into the system, a current is produced upon which the ions are attracted by the FID collector (McNair et al., 2019). The current produced is correlated to the amount of unknown sample in the flame.

Chapter Summary

In this chapter, a discussion is made on morphology of *Citrus sinensis*, scientific classification of *Citrus sinensis*, phytochemistry of citrus fruits, uses of orange seed oil in the industry, nutritional composition of orange/orange seed such energy, fat, ash and moisture contents, antioxidants properties of *the Citrus*

sinensis, the composition of fatty acid and fatty acid properties of the orange seed oil, effects on the physiochemical properties of the oil after continuous use for frying, chemical indices of the orange seed oil, Infrared spectroscopy, the approaches in fatty acid research, isolation of fatty acid compounds and the use of Flame ionization detector (FID) in detecting fatty acids.

CHAPTER THREE

RESEARCH METHODS

Introduction

In this chapter, the experimental procedures, chemicals, materials and Instruments or equipment used in present study are discussed.

Sample Collection, Identification and Preparation

Ripen sweet oranges (fruit) was harvested from the farm at Elmina, in the Central Region, Ghana, in November 2018 and was cut to remove the seeds. After that a clean tap water was used to wash the seeds and then dried in in an oven for 24 h at temperature 40 ºC. The outer coats of the seeds were removed and the dried seeds was milled into fine powder.

Extraction from The Powdered Orange Seed

The seed was milled and 920.96 g of it was weighed and divided into five separate parts. Separately, each part was extracted using the soxhlet extractor which was fitted with a condenser and round bottomed flask. 0.50 L of 40-60 % petroleum ether was used as the extraction solvent the extraction process. A heating mantle was used as source of heat for the extraction and each extraction last for 6 hours.

Percentage of Yield Calculation

The percentage of yield of the dried crude extract was calculated per weight of sample after the weight of dried crude extract was measured (Chen et al., 2018). The percentage yield of crude extracts was calculated using the formular:

$$Percentage\ Yield = \frac{weight\ of\ extracts}{weight\ of\ samples}\ x\ 100$$

Physicochemical Analysis

The following analyses were carried out on the oil: index of iodine, saponification index, peroxide index, acid value, moisture content, relative density, ash content, pH, free fatty acid, and refractive index using standard methods and appropriate formulae (Rasel, 2016).

Saponification Value

The weight of the oil sample (2.0 g) was introduced into a 200 mL erlenmeyer flask. 25 mL of 0.5 M ethanolic KOH was introduced to the conical flask to dissolve the oil. The mixture was refluxed on a water-bath coupled with condenser for one hour. The mixture was then titrated with 0.5 M HCl after it was cooled at room temperature. The indicator used during the titration was Phenolphthalein indicator (1%). A blank titration was carried leaving the the oil (Abubakar et al., 2014; Ibrahim & Yusuf, 2015).

Saponification index was calculated using the formular:

$$Saponification\ value = \frac{(B - S) \times 56.1 \times M}{Mass\ of\ oil\ sample\ use}$$

Where M = molarity of HCl, S = titre value of the sample and B = titre value of the blank

Determination of Iodine Value

Pre-weighed oil sample of 5.0 g was introduced into a clean dry 250 ml erlenmeyer flask and 20 ml of carbon tetrachloride was also introduced to

dissolve the oil. Then 25 ml of a prepared Wij's solution was introduced to the mixture after that, the flask was corked. The flask was placed in a dark room at 25 ℃ for 30 minutes. A mixture of 20 ml of 10 % potassium iodide solution and 100 ml of distilled water was introduced to the mixture. The resultant mixture was titrated against sodium thiosulfate solution using starch as an indicator (Ibrahim & Yusuf, 2015). A blank titration was also conducted under the same conditions without the sample.

$$\text{Iodine value} = \frac{(B - S) \times M \times 12.69}{Mass\ of\ oil\ sample\ used}$$

Where M = molarity of $Na_2S_2O_3$, S = titre value for sample and B = titre value for blank

Preparation of The Wejis Solution

Iodine monochloride (ICl) of 8.0 g was weighed and dissolve in 200 mL of glacial acetic acid to form a mixture. Also, an iodine solution was prepared by dissolving 9.0 g of iodine crystals in 300 mL of carbon tetrachloride (CCl_4) which was then added to the mixture. (100 % v/v) Glacial acetic was added to bring the solution up to the mark (Ibrahim & Yusuf, 2015).

Determination of Peroxide Value

Exactly 3.0 g of the oil sample was transferred into 250 mL flask and a solvent mixture of glacial acetic and trichloromethane (2:1) and 1.0 g of powdered potassium iodide (KI) were added. Complete dissolution was achieved after the reaction mixture was place on water bath for some few minutes. 20 mL of 50 percent potassium iodide were added to the mixture. The mixture was then titrated with $Na_2S_2O_3$. A blank titration was also conducted

under the same conditions without the sample (Abubakar et al., 2014; Ibrahim & Yusuf, 2015). The indicator used was a starch solution.

$$Peroxide\ value = \frac{(BxS)\ \times\ M}{Mass\ of\ sample\ used\ in\ the\ test}$$

Where M = molarity of $Na_2S_2O_3$, S = sample titre value and B = blank titre value

Where (VTest) and (V Blank) stand for Volume of sodium thiosulphate solution used in the test sample and the blank in term of titre values, respectively.

Determination of Relative Density

A specific density bottle was washed, dried and weighed (W1). It was filled with distilled water and weighed (W2). The water was poured off and the bottle was dried to its previous constant weight and then filled with the oil sample and weighed (W3). The procedure was repeated three times.

$$Relative\ density = \frac{W2 - W3}{W2 - W1}$$

Determination of Acid Value

Pre-weighed oil sample of 5.0 g was introduced into a clean dry 250 ml erlenmeyer after that 25 ml of ethyl alcohol was introduced followed by one to three drops of phenolphthalein indicator. The mixture was shaken in water bath (60 degree Celsius) for 10 minutes and then cooled (Sara Mohamed Elmustafa Fregon, 2015). 0.1 M KOH solution was titrated with the above solution until pink colour endpoint was reached that persisted for 30 seconds. The acid value was calculated using equation below and was expressed in mg of KOH:

$$Acid\ value = \frac{V \times M \times 56.1}{Weight\ of\ sample}$$

V= ml of KOH used, M = molarity of KOH

Determination of The Refractive Index

This was done using Abbe-60 refractometer. The machine was calibrated with water first. The surface of the prism was totally cleaned with (100 % v/v) isopropyl alcohol and allowed to dry. The refractometer scale knob was turned on to get a clear interface between the dark region and illuminated region. Two drops of the oil sample were placed on the prism and the index of refraction was read using the telescope scale at normal temperature.

Proximate Composition

Determination of Moisture Content

Glass crucible was weighed and five grams of the sample was introduced into it. The glass crucible and its content were placed in an oven (FS Tupola Plant- Wageningen) thermostatically controlled at 105 degrees for 5 hours. A desiccator was used to cool the sample after that, the final weight of the sample was taken. The above procedure was repeated until consistent weight value was recorded (AOAC, 2009). The change in weight was calculated as a percentage of the previous weight of sample which gave the moisture value expressed in percentage using the formula:

$$\text{Moisture (\%)} = \frac{weight\ of\ wet\ sample - weight\ of\ dry\ sample}{Weight\ of\ wet\ sample} x\ 100$$

Determination of Ash Content

A Crucible was washed and dried in an oven and then allowed to cool in a desiccator. 5.0 g of the oil was put into the crucible and the weight of the crucible containing the sample taken. The content was heated in a muffle burner (Binder, Model FD 115 model, Germany) until there was no more smoke. It was then heated at a temperature of 550 °C – 570 °C for 2 hours to burn all organic matter in the muffle furnace until it turned into ash. The crucible was taken out of the muffle and placed in a desiccator to cool and its weight was taken (AOAC, 2009).

$$Ash\ content\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample} x\ 100$$

Determination of Crude Fat

250 mL round bottom flask was subjected to an oven (FS Tupola Plant-Wageningen) after that, the weight of the dried flask was weighed. 2 grams of the sample was weighed into a 22 ×80 mm paper thimble and then cotton was placed into the thimble to prevent sample lost. The sample was extracted using the soxhlet extractor which was fitted with a condenser and round bottomed flask. 150 mL of 40-60 % petroleum ether was utilized as the extraction solvent throughout the extraction process. A heating mantle was used as source of heat for the extraction and each extraction last for 6 hours.

A distillation method was used to recover the solvent after the thimble have been removed immediately after extraction. Evaporation of the residual solvent from the fat was achieved by placing the extract on water bath. The

sample was then placed in an oven (model) for further drying at 105 ℃ for 30 minutes and finally cooled in a desiccator (AOAC, 2009).

$$Crude\ fat(\%) = \frac{weight\ of\ fat}{Weight\ of\ sample} x\ 100$$

Determination of Energy Content

In order to obtain the energy content of the oil, the individual mean values of the total carbohydrate, crude protein and crude fat should be multiply by their respective factors by the factors of 4, 4 and 9. The products were summed up and the results were expressed as kilocalories per 100 g sample (Garrett & Grisham, 2007; Sherwood, 2015). The formula used was:

$$Energy\ (kCal/100\ g) = 4 \times \%\ protein + 4 \times \%\ carbohydrate + 9 \times \%\ fat$$

Attenuated Total Reflection (ATR- IR)

IR determination was performed using Fourier Transform Infrared Spectrometer (FTIR) (Bruker Alpha Platinum ATR), the operating technique used was Attenuated total reflection and the analysis wavelength range was 3500- 500 cm^{-1}.

The surface of the diamond pellet of the ATR machine was cleaned with a cellulose tissue and isopropanol. Small amount of the oil was smeared on the Platinum crystal surface and ensured a good contact or fastened with the probe (Rohman, & Man, 2010). The IR spectrum was observed and recorded.

Determination of Free Fatty Acid Value

Pre-weighed oil sample of 5.08 g was introduced into a clean dry 250 mL erlenmeyer and 25 mL of neutralized alcohol was introduced into the flask.

The mixture was swirl until the sample was complete dissolution was obtained and bubbles were seen. The solution was warmed in water bath followed by one to three drops of phenolphthalein indicator. 0.1 M KOH solution was titrated with the above solution until pink color endpoint was reached which lasted for 30 seconds. The index of acid was calculated using the equation below:

$$\%\ free\ fatty\ acid\ (FFA) = \frac{V \times N \times 28.2}{Weight\ of\ sample}$$

V= ml of KOH used, N = normality of KOH

Determination of pH

The laboratory pH meter (Hanna HI5522, 230V, 50/60HZ, AC) electrode was rinse with distilled water and then wiped clean with a free tissue. In order to calibrate the pH meter, the rinsed electrode was submerged into buffer solution (pH = 7) to ensured that the pH meter read 7 before it was used to determine the pH of the oil.

A quantity of oil was taken into a beaker and the tip of the pH meter was introduced into the oil at 28 °C and the value was recorded.

Unsaponifiable Matter and Total Fatty Acid

The crude orange seed oil (3.86 g) was weighed and dissolved in ethanolic potassium hydroxide (20 mL 96 % ethanol and 0.8 g/ml KOH). The content was refluxed for 1 hour. The hot solution was cooled to room temperature and (40 mL) distilled water was added. The solution was extracted in a separating funnel with diethyl ether (3 x 10 mL). The ether extract containing the unsaponified matter was separated from the saponified material

of the soap solution in a separatory funnel. The ether extract was washed with 0.1M NaOH (3 x 1mL), followed by two drops of 12 M HCl, distilled water (3 x 1mL) and dried over anhydrous sodium sulphate. It was evaporated under pressure and weighed (Barku et el., 2014).

Procedure for Frying of the Orange Seed Oil

Fresh tuber of yam was purchased from local market of Cape Coast, Ghana. The yam tuber was peeled, washed and cut into pieces of 0.5 cm thickness and 2.5 cm wide. Small frying pan was used to execute frying operations. The volume capacity of the frying pan was about 15 ml. The yams were fried in batches at 15 min intervals for 6 h per day at constant frying temperature. After 2 hours of each frying, about 5 ml of the oil was fetched from the fryer and capped in the vials at 4 ℃ awaiting further analyses.

Preparation of Fatty Acid Methyl Ester for GC-FID Analysis

1 mL of 10 % Sulphuric acid mixed with methanol was introduced to 0.921 g of the oil in 5 ml centrifuge tube. The mixture was swirled for some few minutes. Nitrogen gas was then introduced to the mixture in the centrifuge tube for three minutes then was cool at 25 ℃. 5ml of hexane was added to the mixture for three consecutive times. The mixture allowed to settle down after it had been shaken well. Because the fatty acids methyl esters (FAME) was suspended in the hexane, with the used of pipette, the upper layer was collected and introduced into another centrifuge tube. This layer subjected to evaporation leaving the FAME. A syringe was used to introduce 1 μl of the sample into the GC –instrument for assessment.

GC-FID Analysis

A GC machine (GC-2010 Plus series, Shimadzu, Japan) was coupled with a flame ionization detector (FID). The GC machine was equipped with SP-2560 capillary. The capillary tube was also fused with silica column with a dimension of 100 m × 0.25 mm ID and a film thickness of 0.25 µm. Initially, the column temperature was adjusted to 140 °C, then was isothermally raised to 240 °C for 15 min with a rate of rise of 4 °C/min. Nitrogen was use as a carrier gas with a flow rate of 1 ml /min^{-1}. The injector temperature was 260 °C. 100 mg/mL FAME was used as basics samples quantification, after 1µL of sample was introduced and mix C8-C24 analytical standard.

Antioxidant Activities

Determination of Total Antioxidant Capacity (TAC)

Preparation of Standard Stock Solution

A 2000 ppm in distilled water stock solution of ascorbic acid was prepared into a volumetric flask and this serve as reference standard used for the study.

Varying concentrations of 20, 40, 60, 80 ppm from the stock solution were prepared in distilled water and used to assess the antioxidant potentials.

Preparation of the total antioxidant capacity reagent

The total antioxidant capacity (TAC) reagent solution was prepared by forming a mixture of 28 Mm sodium Phosphate, 4 mM Ammonium molybdate 0.6 M H_2SO_4 in an empty 500 ml volumetric flask. Distilled water was used to filled up to the mark of the 500 ml volumetric flask.

Estimation of Total Antioxidant Capacity (TAC) By Phosphomolybdenum Method

To 12 mL centrifuge tube, different concentrations of 6 mL each (200, 400, 600, 800, 1000 ppm) of the oil was mixed with 1 % DMSO. 6 mL of the TAC reagent was also introduced into the centrifuge tubes and the incubated for 90 min at 95 ℃. The absorbances at 695 nm of the reaction mixture was measured after they have been allowed to pre – cooled for 15 min. A blank was prepared by introducing 6 mL of the reagent solution and 6 mL of the 1 % DMSO in a 12 mL centrifuge and then taken through same experimental procedures as the test sample. this was used as the negative control. A calibration curve of concentration against absorbance was obtained using five different concentration (20, 40, 60, 80, 100 ppm) of the ascorbic acid. Gram equivalent of ascorbic acid/g of dry oil weight (g AA/E100g) was used to express the antioxidant potential of the oil (Mensah et al., 2014). Antioxidant capacity was calculated using the formular below:

$$TAC = \frac{C\ X\ V}{M} X\ 100$$

where TAC is the total antioxidant capacity in gAAE/100 g of the oil, C is the concentration of ascorbic acid (ppm), M is the mass of the oil, V is the volume of the reaction mixture (Mensah et al., 2011).

Antioxidant Activity of 1, 1 diphenyl-1-picrylhydrazyl (DPPH)

100 mL methanol was used to dissolve a pre-weighed DPPH powder (3.9 g) to make 0.1 mM solution of DPPH. 100 mg of the oil was introduced into a 100 mL volumetric flask after that, a methanol was used to filled to add

up to the mark, that serve as the stock solution of the oil. Various concentrations of (0.2, 0.4, 0.6, 0.8, 1 mg/mL) of the oil were prepared from the already prepared stock solution. 3 mL of each samples concentrations was introduced to (1 mL) of the prepared DPPH stock. The mixture was shaken and allowed to stand at 25 ℃ for 30 min after which the absorbance of each sample was obtained at 517 nm using the spectrophotometer (UV-VIS Shimadzu). Three absorbances was obtained for each concentration to ensure consistency. Vitamin C was used as a standard.

Different concentrations of (0.2, 0.4, 0.6, 0.8 and 1 mg/mL) of vitamin C was obtained and 1 ml of DPPH solution was introduced to 3 ml of Vitamin C at different concentrations and to stand for 30 minutes after which the absorbance was measured (Adetuyi et al., 2018).

Antioxidant Activity of (2, 2'- azino-bis (ethylbenzthiazoline-6-sulfonic acid (ABTS $^{+}$)

The ABTS cation was obtained by introducing 7 mM ABTS stock solution to 2.45 mM potassium per sulfate in a 100 mL volumetric flask to form a mixture of ratio 1:1. The mixture was allowed to stand for 12 hours till reaction took place to generate the ABTS free radical ($ABTS^{\bullet +}$)(Gupta, 2015). In order to calibrate the radical solution, 0.5 mL of the free radical solution was diluted with 25 ml of methanol and an absorbance of 0.7 at 734 nm was measured. Different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) of 20 µL oil sample was introduced to the 1 mL of the diluted radical solution. The mixture was incubated at 30 ℃ for 10 min after that, the absorbance was read at 734 nm. A blank which serve as a control was also run in each assay. The radical

scavenging capacity was obtained and compared with Ascorbic acid as percentage of the control sample (free radical solution minus plant extract) as:

$$(\%)inhibition = \frac{(Acontrol - A\ sample)}{Acontrol} x\ 100$$

Where $A_{control}$ is the absorbance of radicals' solution and A_{sample} is the absorbance of radicals' solution + sample (oil /standard).

Chapter Summary

This chapter presents and outlines the chemicals, materials or apparatus and all instrumental procedures employed for analyzing the samples. The instruments such oven, Fourier Transform Infrared Spectrometer, and analytical Gas Chromatography coupled with a flame ionization detector (FID).

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

This chapter consists of results and discussions of the present study which includes physiochemical analysis, proximate composition of the orange seed oil, GC-FID analysis for fatty acid composition, the IR spectra analysis of the orange seed oil and antioxidant activities of the oil.

Physicochemical Analysis

The result obtained from physiochemical analysis of *Citrus sinesis* seed oil is presented in Table 1. Petroleum ether extraction of 920 g of orange seed produced oil volume of 933 ml and a percentage yield of 71 %. The oil yield in this result is higher than the oil yield (56 %) reported from Sokoto Nigeria (Ibrahim & Yusuf, 2015). Similarly, 43.10% yield of orange seed, 40.10 % papaw seed, and 53.20 % of egusi seed oil reported in Northern Nigeria (Abubakar et al., 2014) are far lower than this current result. The result is also higher than the value reported by Nwobi et al. (2011) and Okoye et al. (2011). The high percentage oil yield favours the use of *Citrus sinensis* seed oil for industrial purposes. Plant varieties, climate, ripening stage of the fruit, geographical, environmental conditions could account for the differences in the oil yield from the various regions. The high percentage oil yield favours the use of *Citrus sinensis* seed oil for industrial purposes.

The presence of carotene in the oil gives the oil a medicinal and antioxidant properties (Amiri, 2012). Current dietetic commendations highlight an increase in the intake of fruits and vegetables to be the main dietary sources of vitamin A and good sources of antioxidants. An activity of carotene with β-

ring can undergo cleavages to give rise to vitamin A. Foods rich in carotenoid are linked with reduced risk of contracting certain types of diseases, cancers, heart disease, and other human diseases. β-carotene also has as antioxidant potential and may interfere in free radical oxidation.

The refractive index of the *Citrus sinensis* seed oil was found to be 1.457 which is the same for citrus seed oil (1.46) reported by (Anwar et al., 2008). The result is also close to 1.466 for sunflower oil, 1.44 for olive oil, 1.47 for rapeseed oil, 1.43 -1.471 for coconut oil, 1.47 for corn oil, 1.45-1.48 for cotton seed oil, 1.47 for soyabean oil and lemon oil to be 1.47 at 25°C (Okoye et al., 2011). These results are similar to the results obtained by Ibrahim and Yusif, 2014; Ofoegbu Nwobi, and similar to that of Farooq Anwar et.al (Anwar et al., 2008; Ibrahim & Yusuf, 2015; Nwobi et al., 2011). The refractive index in the crude oil will be different when it is refined. Continuous removal of impurities from the oil leads to a decrease in the refractive index. On the other hand, if the refractive index of the citrus seed oil remains the same after refinery process, it implies the oil is pure.

The refractive index 1.457 obtained for the oil in this research was measured in its crude form and a decrease in refractive index is expected if the oil contains impurities. The amount of impurities in the oil affect the rate at which the light rays are reflected during the evaluation of the index of refraction of the oil (Turek & Stintzing, 2013). The refractive index value obtain for *Citrus sinensis* seed oil in this work could be and an indication that the oil pure even though not refined and qualify the oil as edible oil. Index of refraction is used to measure any change in the unsaturation as the fat/oil is hydrogenated. Index of refraction of oil depends on the molecular weight of the oil/fat, fatty acid

chain length, degree of conjugation and the degree of unsaturation (Ouilly et al., 2017). Pure oils are identified with a mark index of refraction and density thus any change in oil from its true value indicates either it is pure or impure.

The relative density of the *Citrus sinensis* seed oil was found to be 0.8814 g/cm^3 at 25 °C This result is the same as the results obtained by Ibrahim and Yusif, 2014; Ofoegbu Nwobi, and similar to that of Farooq Anwar et.al (Anwar et al., 2008; Ibrahim & Yusuf, 2015; Nwobi et al., 2011). This makes the oil to be of high purity and qualified as edible oil.

Acid value of *citrus sinensis* seed oil was 27.96 ± 0.29 mg KOH/g which is lower than 47.12 and 51.40 for pawpaw and orange seed oil respectively. However, this value is higher than 0.43 and 5.31 of Sorghum and Jatropha seed oil respectively (Jonas et al., 2020).

Abubakar et al. (2014) found the acid value for egusi to be 4.30 mg KOH/g, pawpaw to be 9.46 mg KOH/g and sweet orange seed oil to be 7.59 mg KOH/g which is also lower than 17 mg KOH/g of olive oil and 1.20 mg KOH/g reported for Jatropha seed oil. The value, 27.96 ± 0.29 mg KOH/g in this current report is higher than that of olive oil and jatropha oil but in turn smaller than that of orange seed oil reported by Ved Padam et al. (2013) which indicates a maximum purity and suitability of the oils for soap production. The measure of acid value is an important parameter in oil analysis for both nutritional and industrial purposes. This parameter directly measures the percentage amount of free fatty acids in a specific quantity of oil. This value helps to measure the degree to which the triglycerides in the citrus seed oil have been disintegrated by the action of lipase into free fatty acids. The acid index obtained from the analysis largely depends on the degree of rancidity which is used as an index of

oil quality (Esfarjani et al., 2019). It is commonly known that this parameter measures the degree of spoilage of oil; hence we reported that the value obtained is not too high and this reflects the freshness and edibility of the crude oil if refined.

The pH of the *Citrus sinensis* seed oil was found to be 5.05, which is an indication of the acidic nature, and fatty acids present in the oil. This makes the oil useful as industrial raw material. Measuring the pH of the oil evaluates the acidity or alkalinity of its content. If the value of the pH is less than 7 then the content of the oil is acidic also if the value of the pH greater than 7 then the content of the oil is alkaline. The measure of the hydrogen concentration shows that the *Citrus sinensis* seed oil is highly acidic. This suggests that the oil contains large quantity of fatty acids making them useful for industrial purposes. Higher concentrations of free fatty acids (FFA) are not needed in edible vegetable oils, this is because, this can reduce the shelf-life and palatability of the oil (Choe & Min, 2006).

Free fatty acid for *Citrus sinensis* seed oil was 13.96 ± 0.8 which is lower than 25.70 reported by Koye for orange seed oil (Okoye et al., 2011). Free fatty acid measures the amount of oxidative products in the oil and is a measure of oil quality (Okoye et al., 2011). Thermal degradation and oxidation of unsaturated fatty acids, hydrolysis and pyrolysis as a result of the cleavage of triglyceride results in the formation of free fatty acids. The low contents of fatty acid of the oil compared with other results in literature presents *Citrus sinensis* seed oil as edible when refined.

The unsaponifiable matter extracted from *Citrus sinensis* seed was 0.65 % which is more than (0.3–0.5 %) for citrus seed reported by Anwar et al.

(2008). The value in this current paper is slightly higher than the one reported for olive oil (0.41 %), soybean oil (0.25 %) and corn germ oil (0.97 %) (Schwingshackl et al., 2018). Most oils and fats contain in the range of 0.2 – 1.5 unsaponifiable components. The unsaponifiable matter in vegetable oil consists of variety of non-glyceridic bioactive compounds containing variable mixtures of aldehydes, alcohols, ketones, sterols, fat soluble vitamins that may occur during oil processing or degradation of oil or naturally formed fat soluble vitamins.

The unsaponified material components in the oil were steroids, hydrocarbons, pigments, free sterols, diterpenes, tocopherols and Triterpene. These components that do not form soaps when reacted with caustic soda constitute remains in the oil. These substances are insoluble in the soap but are soluble in common solvents of the oil. The greater than percentage of unsaponifiable matter, the less the value of the fat and the oil. The accepted percentage in fats and oils is 1 % (Baiao & Lara, 2005). Most refined oils have the range of unsaponified extract to be much smaller than the unrefined fats and oils. From the result above, the content of unsaponifiables is less and falls within the accepted range for fat and oils that are used as edible oils.

Table 1: Physicochemical properties of *Citrus sinensis* seed oil.

Parameter	Value
Mass of sample used (g)	920.96 ± 0.02
Colour of oil	Light yellow
Percent yield (% wt/wt)	71 ± 0.02
Refractive index at 25°C	1.457 ± 0.01
pH at 28°C	5.05 ± 0.03
State at 28°C	Liquid
Mass of unsaponified extract (g)	0.65 ± 0.01
Peroxide value (meq/kg)	1.770 ± 0.01
Iodine value (g/100g)	34.06 ± 0.01
Saponification value (mg KOH/g)	165.971 ± 0.01
Relative density (g/cm^3)	0.8814 ± 0.01
Free fatty acid (mg KOH/g)	13.96 ± 0.83
Acid value (mg KOH/g)	27.96 ± 0.29
Unsaturated fatty acid (%)	96.97 ± 0.03
Saturated fatty acid (%)	3.088 ± 0.04

Values are means ± standard deviations of triplicate determinations.
Source: FAO (2003)

The saponification value for the *Citrus sinesis* seed oil was found to be 165 mg KOH/g. The value is lower than the saponification values 190.32 mg KOH/g of orange seed oil reported by (Ibrahim & Yusuf, 2015), 192 mg KOH/g by Ofoegbu (Nwobi et al., 2011), 194 mg KOH/g by (Okoye et al., 2011). The result is again lower than that of cotton seed oil (199.42 ± 0.53 mg KOH/g), neem seed oil (213 mg KOH/g) and coconut oil (253.2 mg KOH/g) obtained in Nigeria (Hassan et al., 2015). It is however higher than saponification value of

106 mg KOH/g of orange seed recorded by Abdul Hamid Abubakar, and 24.13 mg KOH/g of pawpaw seed oil from Northern Nigeria and 79.38 mg KOH/g of pawpaw seed oil from Enugu state of Nigeria (Reazai et al., 2014b). Even though the saponification value of 165 mg KOH/g is low as compared to other results obtained from Nigeria, to some extent, it is larger than that of beeswax (93 mg KOH/g), which is mostly utilized in soap production (Abubakar et al., 2014). Atasie and Akinhanmi (2009) reported that saponification values for groundnut oil which is mostly used in soap making falls within the range of 188-196 mg KOH/g oil (Atasie & Akinhanmi, 2009). Reasonably high saponification value is an indication that sweet orange (*Citrus sinesis*) seed oil could be used in soap making, production of shampoos, lather shaving cream, liquid soaps and detergents (Nzikou et al., 2010; Rahma & Gafar, 1999).

The iodine value of *Citrus sinesis* was 34.06 ± 0.03 g100 g which is higher than 30.20 g/100 g of pawpaw seed oil (Okoye et al., 2011) and lower than 49.190.3 g100 g of Egusi oil, 37.08 ± 0.0419 g100 g of orange seed oil (Abubakar et al., 2014). The degree of unsaturation of carbon to carbon in oil or their derivatives is determined by the iodine value which is the measure of g of iodine absorbed by 100 g of oil. It is useful in studying oxidative rancidity of triacylglycerols. There is greater possibility of rancidity when unsaturation in oil is higher (Esfarjani et al., 2019). The value obtained for *Citrus sinesis* seed oil qualifies it to be less prone to oxidative rancidity and non-drying oil since the iodine value is below 100 g/100g.

This nondrying quality means the oil can be used effectively in the paint industry. To know the unsaturation nature of an oil is to measure its iodine value. The determination of the iodine value is very important parameter which is

widely used in oil analysis to characterize fats and oils. The iodine parameter could also be used as a measure of the amount of the carbon double bonds present in fats and oils which shows the tendency of the oil to get oxidized when exposed to other biological and environmental factors. The determination of iodine value is based on the capability of hydro peroxides to oxidize iodide to iodine followed by reaction with thiosulphate. The higher the iodine value (IV), the more unsaturated the fat or oil nature is. In the process of frying the oil, the iodine value significantly decreases with time. This significant decrease in iodine value (IV) can be due to the fact that the double bonds have been distracted by polymerization and oxidation which occurred simultaneously during the flying process (Abdulkarim et al., 2008).

The peroxide value for sweet orange was found to be 1.770 ±0.4 meq/kg which is similar to the result obtained on four citrus species in Pakistan with peroxide values of 1.97 ± 0.08, 1.55 ± 0.12, 1.67 ± 0.10, and 2.40 ± 0.15 of Mitha (*Citrus limetta*), Grapefruit (*Citrus paradisi*), Mussami (*Citrus sinensis*), and Kinnow (*Citrus reticulata*) (Anwar et al., 2008) respectively. It is also similar to (1.6–2.4 meq/kg of rape seed oil) (Hicham et al., 2014) and 1.27 ± 0.05 4 meq/kg of Pera-rio orange variety (Pereira et al., 2013; Reazai et al., 2014a). The result is also not far different from Peroxide value of 2.21 ± 0.464 meq/kg and 2.33 meq/kg reported from northern Nigeria and Tunisia respectively (Abubakar et al., 2014; Saloua et al., 2020). The result is however higher than 0.91 ± 0.054 meq/kg of Hamlin orange variety, 0.44 ± 0.064 meq/kg of Natal orange variety and 0.63 ± 0.054 meq/kg of Valencia orange varieties (Pereira et al., 2013). The results indicate that the oil is stable to oxidative rancidity since high peroxide value is associated with high rancidity rate

(Abubakar et al., 2014; Ibrahim & Yusuf, 2015) . The low peroxide value qualifies the oil to be used in diet because high level of peroxide in oils have been a threat to human health (Dermis et al., 2012). Standards for oil quality showed that, the peroxide value of good oil should not exceed 10 ml equivalents of peroxide.

Proximate Composition

Moisture Content

Moisture content was found to be 5.803 ± 0.1 which is lower than 6.43 reported by (Okoye et al., 2011), 13.5 % reported by Waheed et al. 2009 but higher than 4.0 reported by (Ibrahim & Yusuf, 2015). The low moisture content will help in prolonged storage of the oil as it is less susceptible to microbial attack (Akintayo, 2004). The current result is however similar to the moisture content for pumpkin seed which is 5.20 ± 0.28 reported by Gohari Ardabili et al., 2011. High moisture content can be accountable for rapid deterioration in the quality of the oil by enhancing enzyme activity which shortened the shelf-life of the oil (Akintayo, 2004; Joseph & Abdullahi, 2016; Twumasi & Osei., 2013). The low moisture content will help in prolonged storage of the oil (Akintayo, 2004). It is necessary to know the nutritive value of an oil by evaluating their moisture content. Due to economic, scientific and technical means it is crucial to evaluate the moisture content of foods. Moisture content in food helps to know the ability of the food to withstand degradation, preservation and as well as it quality (Joardder et al., 2014). food with relatively low moisture content should have good storage ability (Vera et al., 2019). The longer the shelf of food should contain fewer moisture content. Since the result (5.803 ± 0.1) obtained from the analysis agrees with Okoye et al. (2011) it

indicates that the orange seed oil can be stored for a longer time without deterioration.

The moisture content reported for edible seed oil such as sunflower seeds (6.58 %), cotton seeds (6.46 %), palm kernel (5.31 %), sesame (4.60 %), peanuts (4.58 %) and soybean (11.07 %) (Baiao & Lara, 2005) has a close relationship with the current report. The reported moisture content for palm kernel, sesame, peanut, sunflower and cotton seed oil as edible oils is much closer to (5.803 ± 0.1) for *Citrus sinensis* seed oil in this current report. Moisture content is a generally used index in diet testing and validations. It is a key parameter to study the activity of water in many food (Ehi-eromosele, 2013). High moisture content can be accountable for rapid deterioration in the quality of the oil by enhancing enzyme activity which shortened the shelf-life of the oil (Li & Wang, 2018).

Ash Content

The ash content was 0.2 ± 0.1 and this is an indication of presence of minerals in *Citrus sinensis* seed oil. The percentage ash content of 0.2 ± 0.1 in *Citrus sinensis* seed is much higher than 0.63 and 0.49 for Benue and River States Oranges respectively (Joseph & Abdullahi, 2016). Studies on *Maclura pomifera* seed oil gave an ash content of 6.72 % (Saloua et al., 2020) whereas Farooq et al. (2008) reported on four citrus species to have ash content of 5.50 ± 0.11, 5.03 ± 0.1, 4.60 ± 0.13, 5.60 ± 0.09, for *citrus limetta, citrus paradisi*, Mussami *citrus sinensis*, and Kinnow *citrus reticulate* respectively (Anwar et al., 2008). The importance of ash content is that, it gives an idea of the amount of mineral elements present in the oil food (Monti et al., 2008). (Monti et al., 2008). It is also an important index to determine the quality of feeding materials

often used by animal feed producers for poultry and cattle. The ash of an agricultural material is the inorganic residue remaining after the organic material has been burnt off (Monti et al., 2008).

Crude Fat Content

In the global market, fats and oils Signiant group of substances. National Research Council (2000) classified fats and oils as lipid. More than 90 percent of the entire world production utilized fats or as ingredient in food production because, they are very important in the human diet (Khan & Abourashed, 2011). They increase the nutritive ability of food by retaining flavors. They are used in the industries for food products, cosmetics and drugs. Soya bean oil, sunflower oil, peanut oil, rapeseed oil, cotton seed oil, colza oil, palm oil, olive oil, coconut oil and grape seed oil also have similar multipurpose function as in the pharmaceutical industries, cosmetics industries and other food industries (Sicari et al., 2017). The higher the fat content in an oil the higher the nutritional value and the energy content. The result from the orange seed oil indicated a higher fat content of 89.25 %. The value obtained is far higher than the fat content of soya bean oil (20.10 %), sunflower oil, pumpkin seed oil (45.4 %), melon seed oil (37.8 %) and cowpea oil (15.0 %) (Ezeagu et al., 2004; Lazos, 2000). By this result the orange seed oil can be described to be highly nutritional and with high energy content.

Energy Content

Lipids consist of the key energy reserve of all animals with the largest caloric value among all other nutrients. Fowomola et al. (2010) investigated the energy content of a mango seed. The energy content was obtained by

multiplying respectively the average values of crude protein, crude fat and total carbohydrate by the factors of 4, 9, and 4. The value obtained (453.92 ± 4.32 Kcal/100) shows that mango seed is a good source of carbohydrate, protein and fat. In this study, the energy content investigated in orange seed oil using crude fat only without the components of carbohydrate and protein, was 803.261 Kcal/100g. The result shows that orange seed oil contains reasonable amount of energy which can be utilized by the body.

Table 2: Proximate Composition

Nutrients	Values
Percent moisture (%)	5.803 ± 0.10
Percent fat (%)	89.251 ± 0.20
Ash content (%)	0.23 ± 0.15
Energy content (KCal/100g)	803.261 ± 0.41

Values are means ± standard deviations of triplicate evaluations.
Source: FAO (2003)

GC-FID Analysis of Fatty Acid Composition

Gas chromatography equipped with flame ionization detector (GC-FID) was utilized in characterization of 12 fatty acids (FA) composition expressed as percentage of the total methyl ester of fatty acids (FAMEs) of sweet orange seed oil. Among the 12 fatty acids characterised 8 are saturated fatty acids (SFA) and the remaining 4 are unsaturated fatty acids. The fatty acid composition in the oil is as shown in Table 3 below.

Table 3: Percentage Fatty Acid Methyl Ester Composition of Orange Seed Oil

Fatty acid methyl ester (FAME)	Retention time	Area	Concentration (mg/ml)	Percentage of fatty acid (%)
Methyl Decanoate (C10:0)	9.641	1140	0.012	0.024
Methyl Laurate (C12:0)	14.781	5945	0.111	0.130
Methyl Tetradecanoate (C14:0)	19.515	4531	0.075	0.090
Methyl Palmitate (C16:0)	23.670	9409	1.568	2.060
Methyl Octadecanoate (C18:0)	27.823	2371	0.051	0.051
Methyl Arachidate (C20:0)	31.575	2467	0.019	0.053
Methyl Docosanoate (C22:0)	37.403	1010	0.042	0.020
Methyl Lignocerate (C24:0)	42.668	3061	0.956	0.660
Methyl Palmitoleate (C16:1)	23.236	2574	107.008	56.390
Methyl Linoleate (C18:2)	26.770	1821	105.851	39.820
Cis-9-Oleic acid Methyl ester (C18:1)	27.399	2593	0.573	0.560
Methyl Erucate (C22:1)	36.097	9500	0.400	0.200
TOTAL		4572		

Source: FAO (2003)

Among the eight (8) saturated fatty acids (SFA) are Lignoceric acid (C24:0), Decanoic (C10:0), Lauric (C12:0), Tetradecanoic (C14:0), Palmitic (C16:0), Otadecanoic (C18:0), Arachidic (C20:0), Docosanoic (C22:0). The unsaturated fatty acids (UFA) are Cis-9-Oleic acid (C18:1), Palmitoleic acid (C16:1), Linoleic acid (C18:2) and Erucic acid (C22:1).

The dominant fatty acids are Palmitoleic (C16:1), Linoleic and Palmitic acids (C16:0) with percentages of 56.39 %, 39.82 % and 2.06 % respectively. Some of the fatty acids analyzed in the oil are unsaturated which are present the oil as very useful edible oil (Reazai et al., 2014a). The *citrus sinensis* seed oil contains 96.97 % total unsaturated fatty acids and 3.088 % total saturated fatty acids. Palmitoleic acid (C16:1) was identified as the highest unsaturated fatty acid (56.39 %). The percentage composition of Palmitic acid (C16:0) was found to be higher than all the citrus species analysed by Sicari et al. (2017). However, the current result shows some similarities in the linoleic acid content for lemon seed oil (33.7 % - 36.3 %) and sour orange (32.3 – 36 %) (Adeyeye et al., 2015; Reazai et al., 2014a). Malacrida et al. (2011) reported high contents of unsaturated fatty acids in some citrus seed oils ranging from 67.55 % (orange) to 74.72 % (lemon). This result was comparable to the unsaturated fatty acid content obtained from this study 56.39 % (sweet orange).

The differences in the results compared with literature results from other geographical locations agree with the assertion that constituent of fatty acids changes due to some factors such as seed variety, growing area of the fruit and ripeness of the fruit (Takenaga et al., 2014).

The oil can be said to be mixed triglyceride because of the presence of more than one fatty acid group and from the nutritional point of view, the orange seed oil could increase the unsaturated fatty acids intake in humans when used as edible oil (Nwobi et al., 2006). The data obtained for the composition of fatty acid of the *citrus sinensis* seed oil is of importance because, it can be utilized to assess how stable and nitrous the oil is. If the unsaturation is more in the oil, then the oil is prone to oxidative degradation. To prevent coronary heart

infections and other diseases, it is highly recommended to increase in the intake of monounsaturated, n-3 polyunsaturated fatty acids and n-6 polyunsaturated fatty acids in human foods. On the other hands it is also advice to reduce the quantity of saturated fatty acids in foods.

The stability of the *citrus sinensis* seed oil might be due to the fact that, linoleic acids are not present in the oil. The absence of linoleic acid in the citrus sinensis seed oil prevents the formation of several oxidative products in the oil. reports suggest that the peroxides of linoleic acid are hydrolyzed into the oil and other polyethenoid acids speeds up the autoxidation of oleic acid that enhances rancidity in fats and oils (Nwobi et al., 2011).

Table 4: IR for Unsaponified Extract

Peaks	Wavelength	Bond	Functional groups
1	3330.77	-OH	Alcohol
2	2958.94	$-CH_2-$	Alkanes
3	2925.41	$-CH_2-$	Alkanes
4	2854.17	$-CH_2-$	Alkanes
5	1637.34	C=C	Olefins
6	1547.98	-CN- stretching, -NH- bending	Amide
7	1464.46	-CH- bend	Olefins
8	1406.51	C-C stretch (rings)	Aromatics

Source: FAO (2003)

Table 5: IR for Orange Seed Oil at 25°C

Peaks	Wavelength	Bond	Functional groups
1	3008.26	-C=C-H stretch	Alkanes
2	2921.73	$-CH_2-/-CH_3-$	Aliphatic
3	2852.70	$CH_2-/-CH_3-$	Aliphatic
4	1742.86	C=O (stretch)	Carbonyls
5	1709.23	C=O (stretch)	Carbonyls
6	1462.08	-CH- bend	Olefins
7	1412.69	C-C stretch (rings)	Aromatics
8	1377.80	CH –rock	Olefins
9	1279.58	CH –wag (CH_2X)	Alkyl halides
10	1167.66	C-O (Fingerprint stretching)	Ester
11	937.77	OH bend	Carboxylic acid
12	721.56	C-H rock	Aliphatic
13	462.72	C=C bend	Olefins

Source: FAO (2003)

Table 6: IR for Orange Seed Oil Used for Frying Yam for The First Time

Peaks	Wavelength	Bond	Functional group
1	3007.92	C=C-H stretch	Alkenes
2	2920.98	-CH_2-/-CH_3-	Aliphatic
3	2852.05	-CH_2-/-CH_3-	Aliphatic
4	1742.72	C=O (stretch)	Carbonyls
5	1708.68	C=O (stretch)	Carbonyls
6	1462.38	CH bend	Olefins
7	1413.15	C-C stretch (rings)	Aromatics
8	1377.43	CH –rock	Olefins
9	1272.56	CH –wag (CH_2X)	Alkyl halides
10	1166.26	C-O (fingerprint stretching	Ester
11	937.81	OH bend	Carboxylic acid
12	721.69	C-H rock	Aliphatic
13	688.45	-CH- Bend	Aromatic

Source: FAO (2003)

Table 7: IR for Orange Seed Oil Used for Frying Yam for The Second Time

Peaks	Wavelength	Bond	Functional group
1	3007.72	C=C-H stretch	Aliphatic
2	2921.99	$-CH_2-/-CH_3-$	Aliphatic
3	2852.66	$-CH_2-/-CH_3-$	Aliphatic
4	1742.36	C=O (stretch)	Carbonyls
5	1460.52	-CH- bend	Olefins
6	1376.88	CH –rock	Olefins
7	1236.25	C-O-stretch	Carboxylic acid, esters and ethers
8	1160.59	C-O (finger print stretching)	Ester
9	967.87	CH=CH (trans)	Alkene
10	721.74	C-H rock	Alkanes
11	461.88	C=C bend	Olefins
12	421.04	Alkanes (fingerprint)	Alkanes

Source: FAO (2003)

Table 8: IR for Orange Seed Oil Used for Frying Yam for The Third Time

Peaks	Wavelength	Bond	Functional group
1	3007.64	C=CH-Stretch	Aliphatic
2	2922.03	-CH_2-/-CH_3-	Aliphatic
3	2852.73	-CH_2-/-CH_3-	Aliphatic
4	1742.14	C=O (stretch	Carbonyls
5	1459.97	CH bend	Olefins
6	1376.84	CH –rock	Olefins
7	1236.51	(C-O stretch)	Ester
8	1160.60	C-O (finger print stretching	Ester
10	967.87	CH=CH (trans)	Alkene
11	721.87	C-H rock	Olefins
12	457.82	C=C bend	Olefins
13	427.30	Alkanes (fingerprint)	Alkanes

Source: FAO (2003)

ATR-IR

The IR spectrum of the orange seed oil showed peaks at 3008.26 cm^{-1} as a result of cis-double bond of C-H stretching vibration 2921.73 cm^{-1} is an asymmetrical vibrational mode of an alkane (–CH_2) group and 2852.70 cm^{-1} is symmetrical vibrational mode of (–CH_2) group with sharp bands. The peaks at 1742.08 cm^{-1} and 1709.23 cm^{-1} represent the triglycerides and ester carbonyl stretching vibrations of which are strong and sharp. The bending vibrations of the CH cis-olefinic groups are seen at 1462.08 cm^{-1} and 1377.80 cm^{-1}. The peak seen at the frequency, 1163.66 cm^{-1} is finger print of the vibrational stretching of the (C-O) ester group (Coates, Khalil & Khalil, 2017) and the OH –bend of

the carboxylic acid group is found at a frequency 937.77 cm^{-1}. The frequency 721.56 cm^{-1}, and 462.72 cm^{-1} represent CH- rock of an alkane, and C=C (Olefins) respectively (Anang et al., 2019). The result obtained agrees with those obtained by Keifer et al, 2019 when they applied ATR-FTIR spectroscopy to authenticate and classify passion fruit oil.

The oil was used to fry pieces of yam and the used oil was reused for repeated times (three times) at increased temperatures at atmospheric conditions. The chemical and physical characteristics of the oil were assessed. An example of the parameters considered in this was the colour of the oil after first, second and third use. The colour of the oil changed from light yellow to light brown when used for the first time and the colour intensified to deep brown after subsequent uses. Fatty acids in oil normally undergo physical and chemical deteriorations that affect the quality of oil as noticed from its bad smell. The changes in the quantity of fatty acids stated at the time of commercial frying are as a result of deterioration due to oxidation that results to a reduction in the total amount of unsaturated fatty acids and enhances saturated fatty acids content. A presence of oxygen, moisture in the yam and higher temperature used in the process of frying the yam allow oil to undergo three deteriorating reactions through hydrolysis, oxidation and polymerization and as a result free fatty acid, mono- di-acylglycerols, glycerols, peroxides and hydro peroxides are formed leading to off-flavor of the oil used. This also decreases the iodine value of the used oil but increases the peroxide value. Hydrolysis of the fatty acids results in the formation of free fatty acids, peroxides and hydro peroxides which are frequently formed in the presence of oxygen. The frying process also enhances the formation of trans fatty acids, enhances the quantity of saturated fatty acids

and decreases unsaturated fatty acids level and makes the oil unhealthy for use in diet (Abdulkarim et al., 2008) (Song et al., 2015). The process of hydrolysis of lipids also results in the formation of bad aroma and unpleasant taste. The formation of Trans fatty acids depends largely on, temperature, nature of oil and the frying time (Romero et al., 2000) (Liu et al., 2007). The used oil becomes unstable with shorter shelf life.

Heating process affect the structure of oils, based on the temperature, the amount of air to which the oil is exposed to and the heating process time. For instance, the concentration of polyunsaturated fatty acids can be reduced in deep-fat frying process this is because the volatile compounds are lost as steam is produce and they oxidize over time. The primary products are decomposed quickly leading to the formation of secondary products, such as ketones, aldehydes, hydrocarbons, polymers and alcohols as observed from the infra-red spectra for the used oil and hence, the oil utilized for deep-fat frying process must therefore be changed often. The presence of the peak with intensity1742.14 observed from the spectrum for used citrus seed oil indicated the presence of the carbonyls (aldehyde and ketones), 1459.97 and 427.30 indicate the presence of CH-bend of an olefin group and 1376.84 indicates the CH-rock of the hydrocarbons. Table 5, 6, 7 and 8 depict ATR-IR spectra of the *C sinensis* oil at different times: 25 ℃, frying for the first time, frying for the second time and frying for third time.

The oil could be described to inhibits hydrolytic decomposition and lipid oxidation to prevent the degradation in the quality of the oil because there were no significant changes in bands among the spectra after the spectra analysis of

the oil at room temperature, frying for the first time, frying for the second time and frying for third time.

However, in the spectra table 6 and 7, the spectra bands trend was similar to the oil at frying for the second time and frying for third time with the absence of 1709.23 and 1708.68 cm^{-1} that were highly intense. An absorption at 1708 cm^{-1} to overlapped with the vibration at 1742 cm^{-1} of ester carbonyl group of triglycerides, this could result from the secondary oxidation resultant product or present of a saturated aldehyde functional group. The disappearance of absorption band between 968-966 cm^{-1} was due vibrational mode of (E)-HC=CH. The absence of (E)- HC=CH functional groups in all the unsaturated fatty acid chains is very important because report has been stated that (E)-HC=CH functional groups are responsible for heart complications (Housecroft, & Constable., 2006). Isomerization occurs in the unsaturated fatty acid chains because of the continuous heating the oil as used in frying and hence, an absorption at 967 cm^{-1} indicates this phenomenon probable occurring as shown Figure. 8.

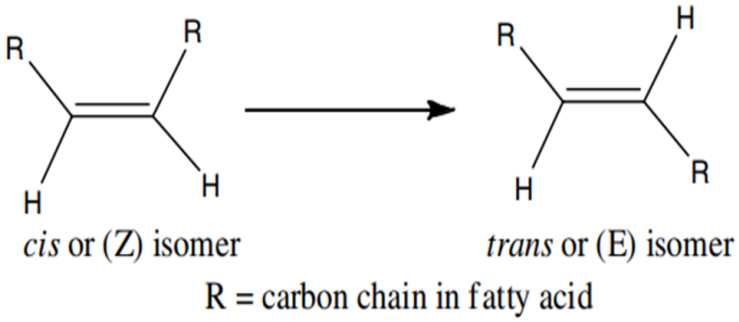


Figure 8: Isomerization in unsaturated fatty acid chain
Reference: Housecroft & Constable (2006)

The unsaponified matter was 0.65% which is more when compared to 0.31 ± 0.04 of Mitha (C*itrus limetta*), 0.39 ± 0.03 of Grapefruit, 0.39 (*Citrus*

paradisi), Mussami, 0.50 ± 0.04 of Mussami (*Citrus sinensis*), 0.48 ± 0.05 of Kinnow (*Citrus reticulate*). The IR result of the Unsaponifiable extract indicated the presence of an alcohol group at a frequency of 3330.77 with a broad band which might be as a result of flavonoids and other polyphenol compounds in the oil.

The asymmetrical and symmetrical modes of vibration of $–CH_2-$ exhibited very strong and sharp bands at 2925.41 cm^{-1}, 2854.59 cm^{-1} and 2925.41 cm^{-1}. And the rest of the peaks were found at frequencies 1637.34 cm^{-1}, 1547.98 cm^{-1}, 1464.46 cm^{-1} and 1406.51 cm^{-1} which represent (C=C) of the olefins, (CH-bend of an alkene and CH_2/CH_3 bending in that order (Anang et al., 2019; Boughendjioua & Djeddi, 2017; Khalil & Khalil, 2017).

Antioxidant Activities

Antioxidant capacity of *Citrus sinensis* seed oil

Total antioxidant (TAC), DPPH and ABTS results showed that the citrus seed oil can exhibit antioxidant capacity which increases with increasing concentration of the oil showing the scavenging property of the oil.

DPPH Antioxidant Assay

Plant materials or fruits and vegetables have been stated to scavenge radical (Najafabad & Jamei, 2014). A plant extract or its formulation could reduce DPPH radical which can indicates its antioxidant property. Odd electron are responsible for the radical's activity and as such seed extract may contain chemicals that are capable of releasing hydrogen to the free radical to enhance its removal (Gangwar et al., 2014). From literature, radical scavengers and antioxidants are substance that able to reduce DPPH stable nitrogen -centered free radicals by donating electron or hydrogen and as such a colour change from

violet to yellow shows radical reduction (Benmehdi, 2013). The DPPH assays measures the property of the antioxidant in these fruits and vegetables to reduce the DPPH free radicals.

The scavenging property of *Citrus sinensis* seed oil was assessed by obtaining the reduction in absorbances of the oil at different concentrations and the percentage inhibition at each concentration was measured and compared to a standard Ascorbic acid. At sample concentration (0.2 0.4 ,0.6, 0.8 and 1.0) mg/ml, the percentage inhibition was 21.11 %, 22.05 %, 22.05%, 22.56 %, and 22.82 % respectively. As the concentration of the sample increases gradually the percentage inhibitions also increases. Maximum inhibition of *Citrus sinensis* oil and Ascorbic acid are 22.821 % and 89.487 % respectively in 1.00 mg/ml. *Citrus sinensis* seed oil showed antioxidant potential over DPPH assay. The reduction in absorbance of DPPH radical was as a result of molecules of antioxidant which reacted to reduce the DPPH radicals by donating hydrogen to stabilize the radical which was noticed by colour change from purple to yellow (Qaiyum et al., 2013).

ABTS Antioxidant Assay

Significant decrease in ABTS assay was observed as a result of scavenging ability of the oil. Various concentrations of 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml and 1.0 mg/ml of the oil and Ascorbic acid standard, percent inhibition of the oil was measured as 0.847 %, 1.977 %, 18.079 %, 55.367 % and 58.192 %. The maximum percentage inhibition for the oil and the standard was 58.192 % and 74.576 % in that order in 1.0 mg/ml. *Citrus sinensis* seed oil showed greater scavenging capacity for DPPH antioxidant assay as compared with ABTS. Total Antioxidant Capacity (TAC)

This study also reported the antioxidant potentials of *C. sinensis* seed oil. The total antioxidant capacity (TAC) was principled on the conversion of Molybdenum (VI) to Molybdenum (V) by the extract and conversion of green phosphate/ Molybdenum (V) complex in acidic medium. TAC assessed both fat-soluble antioxidants and water-soluble antioxidants. The total antioxidant capacity was 6.22 ± 1.56 gAAE/100g of the crude oil. Ascorbic acid was used as a reference standard antioxidant capacity and it compares with the oil capacity because Aliyu et al. (2013) has stated that antioxidant potential of plant extract can be compared with antioxidant potential of ascorbic acid. The citrus seed oil, with this result, can be said to be a potential source of antioxidants.

Chapter Summary

This chapter presents the main points or results of this research. All the findings from this research has been presented in this Chapter. It includes and discusses the results obtained from the physiochemical assessment, proximate composition of the seed oil, GC-FID assessment of composition of the fatty acids, IR spectra evaluation of the orange seed oil and antioxidant activities of the oil.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Overview

The specific objectives the study is to evaluate the physicochemical assessment of the *Citrus sinensis* seed oil, examine the nutritional value of orange seed oil, study the antioxidant potentials of the seed oil, identify the composition of the fatty acids of orange seed oil, Investigate the potential uses of the seed oil in soap production and determine if repeated use of *Citrus sinensis* seed oil for frying will affect the oil quality.

Soxhlet extraction technique was utilized to extract the crude oil. GC-FID was used to analysis fatty acid composition, physico-chemical assessment, nutritional composition of the *Citrus sinensis* seed oil was investigated using standard procedures. ATR-IR spectrum of *Citrus sinensis* seed oil was assessed before and after use for frying. Antioxidant potentials of the oil was also assessed utilizing the DPPH assay, ABTS assay and phosphomolybdenum assay.

This chapter is the conclusion of the report. It deals with the summary of the entire work. Conclusions and recommendations are also stated in this chapter.

Summary

The results indicated 71 percent of oil content which is in higher amount. The physico-chemical qualities of the oil revealed index of refraction of 1.457, saponification index of 165 mgKOH/g, peroxide index of 4.12 meq/kg, iodine index of 34.06 Wijs, moisture (5.803 ± 0.1%) and ash (0.23 ± 0.15%). Crude fat

(89.251 ± 0.2) with energy contents in the oil was 803.261 Kcal/100g. The ATR-IR spectrum of oil depict a significant band which was characteristics of unsaturated fatty acid. GC-FID analysis of fatty acid composition of *Citrus sinensis* seed oil indicated dominant fatty acids: Palmitoleic acid (C16:1), Linoleic acid and Palmitic acid (C16:0) with percentages 56.39 %, 39.82 % and 2.06 % respectively. The fatty acid with the highest percentage composition (56.39 %) is Palmitoleic acid (C16:1) a monounsaturated fatty acid. The *Citrus sinensis* seed oil showed greater degree of unsaturation forming 96.97 % of the total fatty acids and 3.088 % of saturated fatty acids.

Citrus sinensis seed oil showed antioxidant potential over DPPH and ABTS assay. Also, the Total Antioxidant Capacity (TAC) of *Citrus sinensis seed* oil indicated potential antioxidant capacity of the oil because it compares well with vitamin C capacity which was utilized as the reference standard antioxidant. These results suggest that sweet orange seed oil could be utilized for both industrial purposes and human consumption.

Conclusions

The percentage yield of oil from *Citrus sinensis* seed could be an alternative source of vegetable oil for the industrial sector. Results from the analyses show that crude fat was the major nutrients in the oil and this constituted to the energy contents in the oil. Result from the study of the seeds of *Helianthus annuus* was rich in crude protein (28.25 %) and crude lipid (19.80 %) respectively. The value of the crude lipid is high; therefore, the seed should be given attention as a potential oil seed.

The other parameters which were present in lower percentages or minor constituents include moisture and ash. The seed moisture was below 10 percent,

an ideal index for the extraction of vegetable oils. 13.5 % and 3.83 % moisture content were reported in *Citrus sinensis seeds* and *Citrus sinensis* embryo, respectively.

Linoleic acid, which is the second-highest 39.82 % was the only one essential fatty acid recorded after the study (Table 2). In the Ghanaian market, edible oils for example the palm oil, palm kernel oil and coconut oil contain lower values of the linoleic acid. Linoleic acid (36.26 %) and α-linolenic acid (3.44 %) were stated as the only two major essential fatty acids after a study from Pakistan was conducted. The nutritional values of oils and fats relies on their fatty acid composition, especially the quantity of erucic acid, oleic acid, linolenic acid and linoleic acid. The fatty acid profile and the functional groups present in this report prove the citrus seed oil to be rich in fatty acids and that the oil can have useful applications in the pharmaceutical, soap and industrial sectors.

The total antioxidant capacity together with DPPH and ABTS assay projects the C*itrus sinensis* seed oil compared to ascorbic acid to be a promising source of antioxidants to be considered in diets and drugs. Thus, harnessing of C*itrus sinensis* seeds for economic value rather than treating them as wastes is suggested. Findings from this work could be used to educate famers on both medicinal and economic benefits of the orange seed oil.

Recommendations

It is recommended that:

Extraction of this oil for industrial use. The profit status of the orange seed oil can increase especially in the fruit juice making industries and the oil

industries when these oils are being extracted and commercialized and that can increase the sustenance of the seedy species of sweet orange fruit cultivations.

The Unsaponifiable fraction should further be analyzed to identify the compounds present.

One of the most energy-rich sources of renewable fuels available in nature is orange seed oils. Many seed oils are in the form of triacylglycerols that can be changed into biodiesel by converting their acyl chains into methyl esters of fatty acid. To meet the growing demand for biodiesel, oils from orange seed can be investigated for use as biodiesel.

The estimation of some phytochemicals such as tocopherols, carotenoids and phenolic contents of the orange seed oil. The information obtained might support or contribute to the usage of the citrus seed oil in food and pharmaceutical industries as a cheap raw material for antioxidant supplement.

In order to make use of citrus seeds, it is necessary to investigate other lipids composition such as phospholipids and sterols.

There is lack of publications on the oxidative stability of most orange seed oils (Malacrida, 2012) and hence it is highly recommended to conduct studies on the stability and shelf life of the oil. Such study might give other information on the orange seed oil qualities.

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APPENDICES

Appendix A: ABSORBANCE AND PERCENTAGE INHIBITION OF DPPH ANTIOXIDANT CAPACITY

Absorbance of DPPH and sample

Concentration(mg/ml)	1	2	3	Vitamin C
0.2	0.315	0.298	0.31	0.296
0.4	0.306	0.303	0.303	0.051
0.6	0.304	0.303	0.305	0.044
0.8	0.302	0.302	0.302	0.043
1.0	0.301	0.301	0.301	0.041

Source: FAO (2003)

Percent inhibition of DPPH

Concentration(mg/ml)	1	2	3	%inhibition of extract	%inhibition of Vitamin C
0.2	20.513	23.59	20.513	21.111	24.103
0.4	22.308	22.308	22.308	22.051	86.923
0.6	21.795	22.308	21.795	22.051	88.718
0.8	22.564	22.564	22.564	22.564	88.974
1.0	22.821	22.821	22.821	22.821	89.487

Source: FAO (2003)

Appendix B: ABSORBANCE AND PERCENTAGE INHIBITION OF ABTS ANTIOXIDANT CAPACITY

Absorbance of ABTS and sample

Concentration(mg/ml)	1	2	3	Vitamin C
0.2	0.116	0.117	0.118	0.051
0.4	0.115	0.116	0.116	0.043
0.6	0.097	0.095	0.098	0.043
0.8	0.052	0.053	0.053	0.046
1.0	0.05	0.049	0.049	0.03

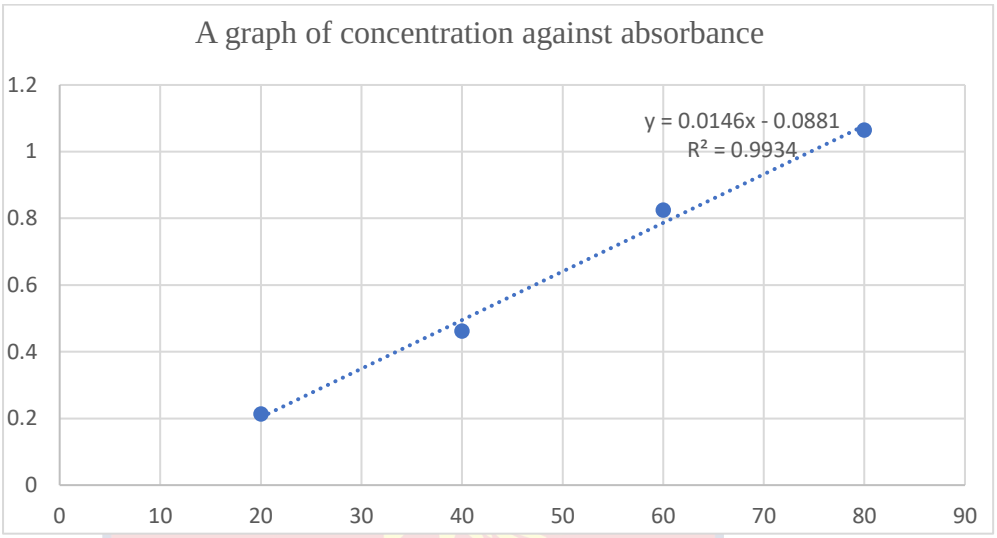
Source: FAO (2003)

Percent inhibition of ABTS assay

Concentration(mg/ml)	1	2	3	%inhibition of extract	%inhibition of Vitamin C
0.2	1.695	0.847	0	0.847	56.780
0.4	2.542	1.695	1.695	1.977	63.560
0.6	17.800	19.492	16.950	18.079	63.560
0.8	55.932	55.085	55.085	55.367	61.017
1.0	57.627	58.475	58.475	58.192	74.576

Source: FAO (2003)

Appendix C: CALIBRATION CURVES FOR DETERMINATION OF ASCORBIC ACID EQUIVALENT OF THE OIL



Appendix D: TOTAL ANTIOXIDANT CAPACITY

PHOSPHOMOLYBDENUM ASSAY (PM)

Conc/ppm	1	2	3	Average	AAE/ppm	TAC (g/AAE/100g)
200	0.221	0.220	0.220	0.137	15.422	15.422
400	0.224	0.224	0.224	0.141	15.662	7.831
600	0.262	0.262	0.263	0.180	18.300	6.100
800	0.271	0.2715	0.271	0.188	18.886	4.721
1000	0.461	0.461	0.461	0.377	31.888	6.378

Source: FAO (2003)

Appendix E: RESULT FROM ANTIOXIDANT ACTIVITY

	TAC= (AAE g/100g) ± SEM
C.sinensis	6.22 ± 1.56

Appendix F: RELATIVE DENSITY (R.D)

Concentration(mg/ml)	W1	W2	W3	R. D
1	26.898	76.5772	70.7448	0.882607
2	26.89910	76.5771	70.7441	0.879151
3	26.89812	76.5769	70.7444	0.882586
Average	26.898	76.577	70.7444	0.881448

R. D = Relative density = 0.881 ± 00

Source: FAO (2003)

Appendix G: MOISTURE CONTENT (M.C)

Concentration(mg/ml)	W1	W2	W3	Moisture
1	38.386	43.936	43.610	5.873874
2	41.697	46.797	46.322	5.647367
3	28.652	33.753	33.416	5.802784
Average				5.802784±0.1

Source: FAO (2003)

Appendix H: TITRATION DATA FOR PHYSIOCHEMICAL ANALYSIS

Free Fatty Acid Content (FFA)

	Initial volume	Final volume	Volume used
1	0.00	25.3	25.3
2	25.3	49.1	23.8
3	29.1	54.3	25.2
Average			

FFA=13.96 ± 0.83
Source: FAO (2003)

Titration Data (A) For Determination of Acid Value of the Oil

	Initial volume	Final volume	Volume used
1	0.00	25.3	25.3
2	25.3	50.1	24.8
3	29.2	53.99	24.79
Average Titre			24.963

Source: FAO (2003)

Titration Data (B) For Determination of Acid Value the Oil

	Initial volume	Final volume	Volume used
1	0.00	25.29	25.29
2	25.29	50.09	24.8
3	50.09	74.88	24.79
Average Titre			24.960

Source: FAO (2003)

Titration Data (C) For Determination of Acid Value of the Oil

	Initial volume	Final volume	Volume used
1	0.00	25.3	25.3
2	25.3	50.1	24.8
3	29.2	53.99	24.79
Average Titre			24.963

Source: FAO (2003)

Acid Value (AV) For the Oil

Average tire	A. V1	AV2	A. V3	Average acid value
24.962	27.960	27.960	28.00	27.97 ± 0.29

Source: FAO (2003)

Titration Data (A) For Determination of Iodine Value (IV) For Blank

	Initial volume	Final volume	Volume used
1	0.00	27.50	27.50
2	27.50	56.50	29.00
3	56.50	82.00	25.50
Average titre			27.33

Source: FAO (2003)

Titration Data (B) For Determination of Iodine Value (IV) For Blank

	Initial volume	Final volume	Volume used
1	0.00	27.51	27.51
2	27.51	56.51	29.00
3	56.51	82.01	25.51
Average titre			27.34

Source: FAO (2003)

Titration Data (C) For Determination of Iodine Value (IV) For Blank

	Initial volume	Final volume	Volume used
1	0.00	27.50	27.50
2	27.50	56.50	29.00
3	56.50	82.00	25.50
Average titre			27.33

Source: FAO (2003)

Titration Data (A) For Determination of Iodine Value (IV) Of Oil Sample

	Initial volume	Final volume	Volume used
1	0.00	5.80	5.80
2	5.80	11.55	5.75
3	11.55	17.55	5.80
Average titre			5.783

Source: FAO (2003)

Titration Data (B) For Determination of Iodine Value (IV) Of Oil Sample

	Initial volume	Final volume	Volume used
1	17.55	23.34	5.79
2	23.34	29.80	5.80
3	29.80	35.55	5.78
Average titre			5.780

Source: FAO (2003)

Titration Data (C) For Determination of Iodine Value (IV) For Oil Sample

	Initial volume	Final volume	Volume used
1	0.00	5.80	5.80
2	5.80	11.55	5.75
3	11.55	17.55	5.80
Average titre			5.783

Source: FAO (2003)

Iodine Value for The Oil (I.V)

IV1	1V2	IV3	Iodine value
34.06	34.07	34.06	34. 06 ±0.003

Source: FAO (2003)

Titration Data (A) For Determination of Peroxide Value (P.V) For Blank

	Initial volume	Final volume	Volume used
1	0.00	3.01	3.01
2	3.01	7.56	2.56
3	7.56	10.36	2.80
Average titre			2.79

Source: FAO (2003)

Titration Data (B) For Determination of Peroxide Value (P.V) For Blank

	Initial volume	Final volume	Volume used
1	10.36	13.37	3.00
2	13.37	16.17	2.80
3	16.17	18.72	2.55
Average titre			2.78

Source: FAO (2003)

Titration Data (C) For Determination of Peroxide Value (P.V) For Blank

	Initial volume	Final volume	Volume used
1	10.36	13.37	3.00
2	13.37	16.17	2.80
3	16.17	18.72	2.55
Average titre			2.78

Source: FAO (2003)

Titration Data (A) For Determination of Peroxide Value (P.V) Of the Oil Sample

	Initial volume	Final volume	Volume used
1	0.00	2.00	2.00
2	2.00	4.01	2.01
3	4.01	6.20	2.10
Average titre			2.036

Source: FAO (2003)

Titration Data (B) For Determination of Peroxide Value (P.V) Of the Oil Sample

	Initial volume	Final volume	Volume used
1	6.20	8.20	2.00
2	8.20	10.30	2.10
3	10.30	12.30	2.00
Average titre			2.030

Source: FAO (2003)

Titration Data (C) For Determination of Peroxide Value (P.V) Of the Oil Sample

	Initial volume	Final volume	Volume used
1	12.30	14.30	2.00
2	14.30	16.30	2.00
3	16.30	18.50	2.00
Average titre			2.06

Source: FAO (2003)

Average Peroxide Value (PV) of The Oil Sample

P1	P2	P3	Peroxide value
1.769	1.758	1.784	1.770 ±0.013

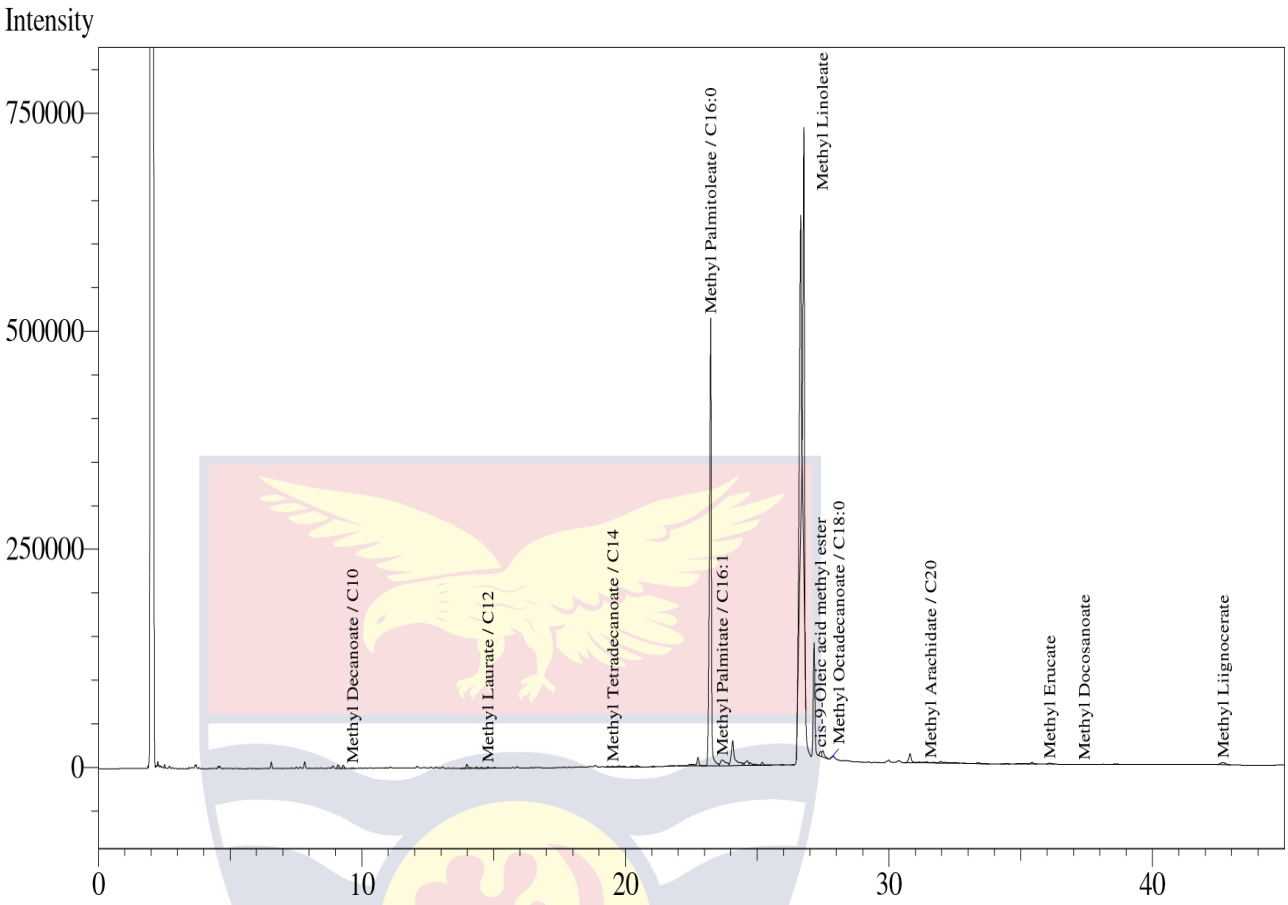
Source: FAO (2003)

Titration Data for Determination of Peroxide Value (P.V) Sample C

	Initial volume	Final volume	Volume used
1	0.00	2.00	2.00
2	2.00	4.01	2.01
3	4.01	6.20	2.10
Average titre			2.036

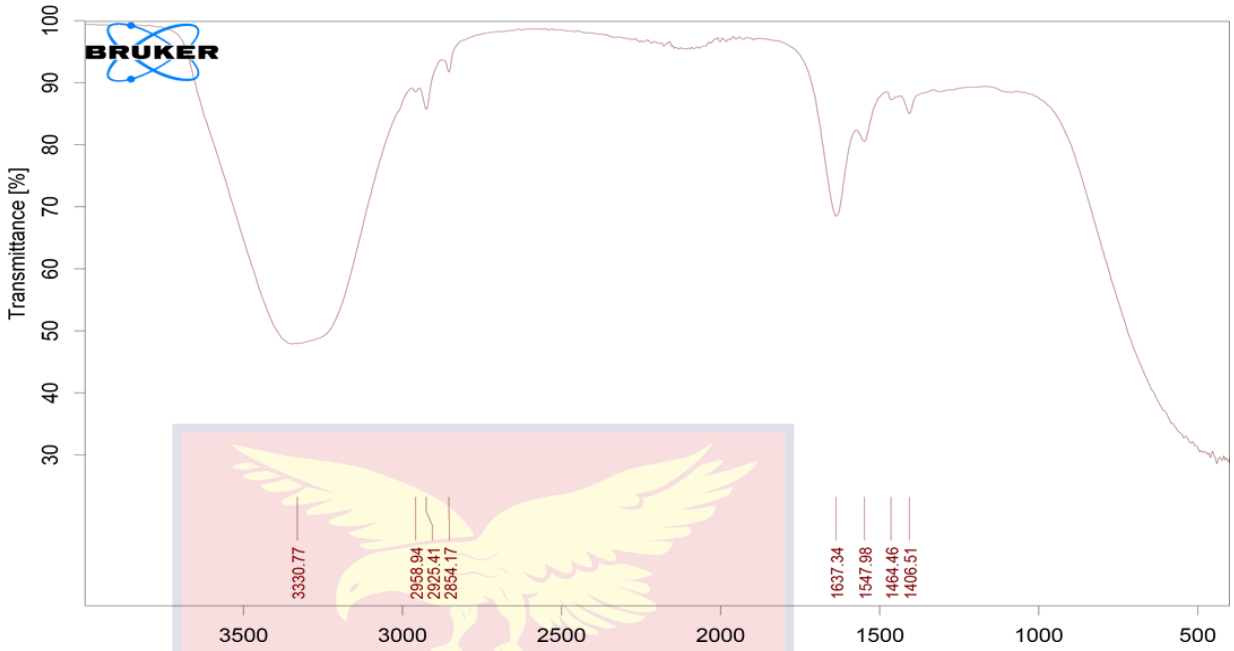
Source: FAO (2003)

Appendix I: GC-FID SPECTRUM OF ORANGE SEED OIL



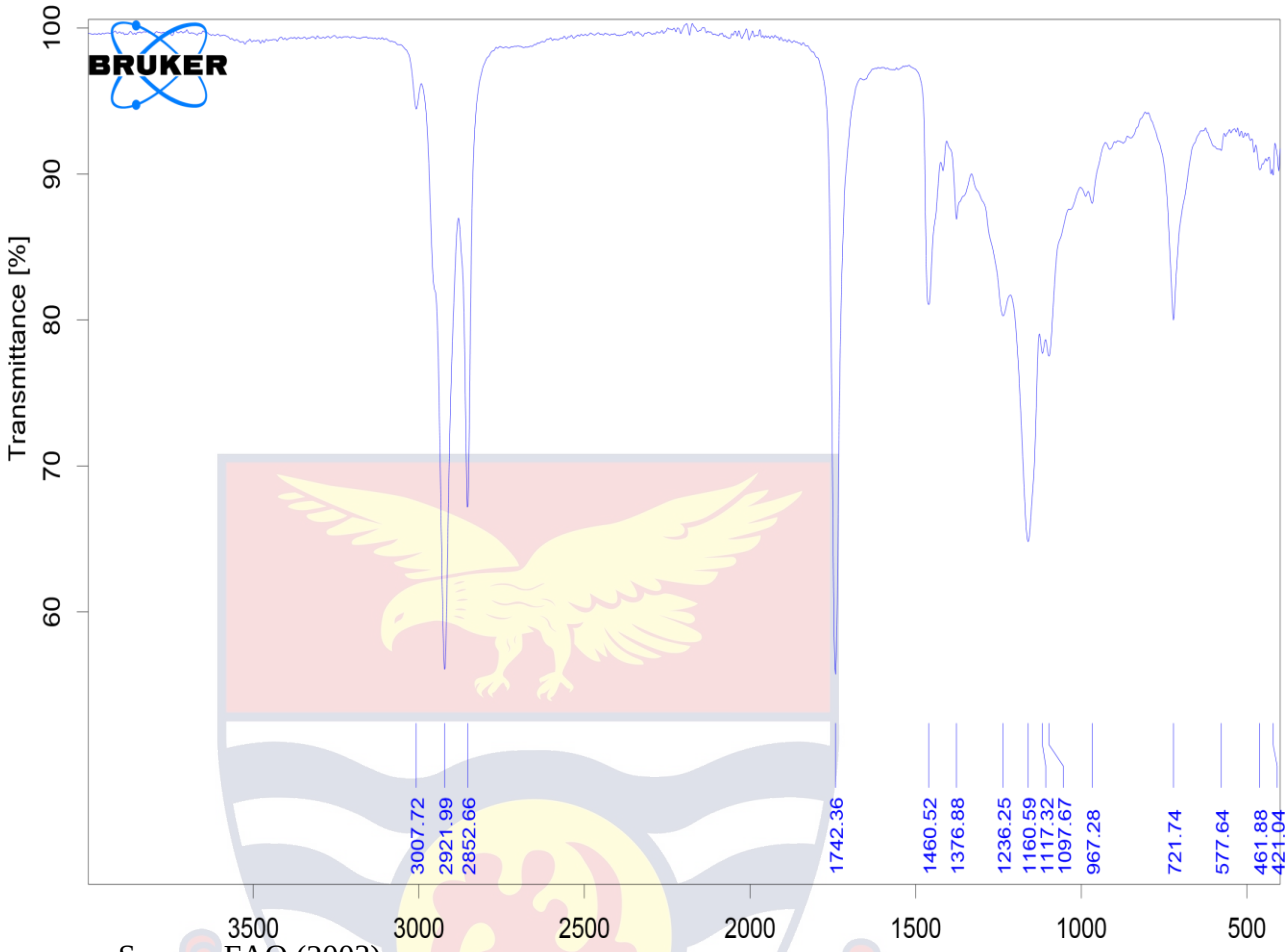
Source: FAO (2003)

Appendix J: IR-SPECTRUM FOR UNSAPONIFIED EXTRACT



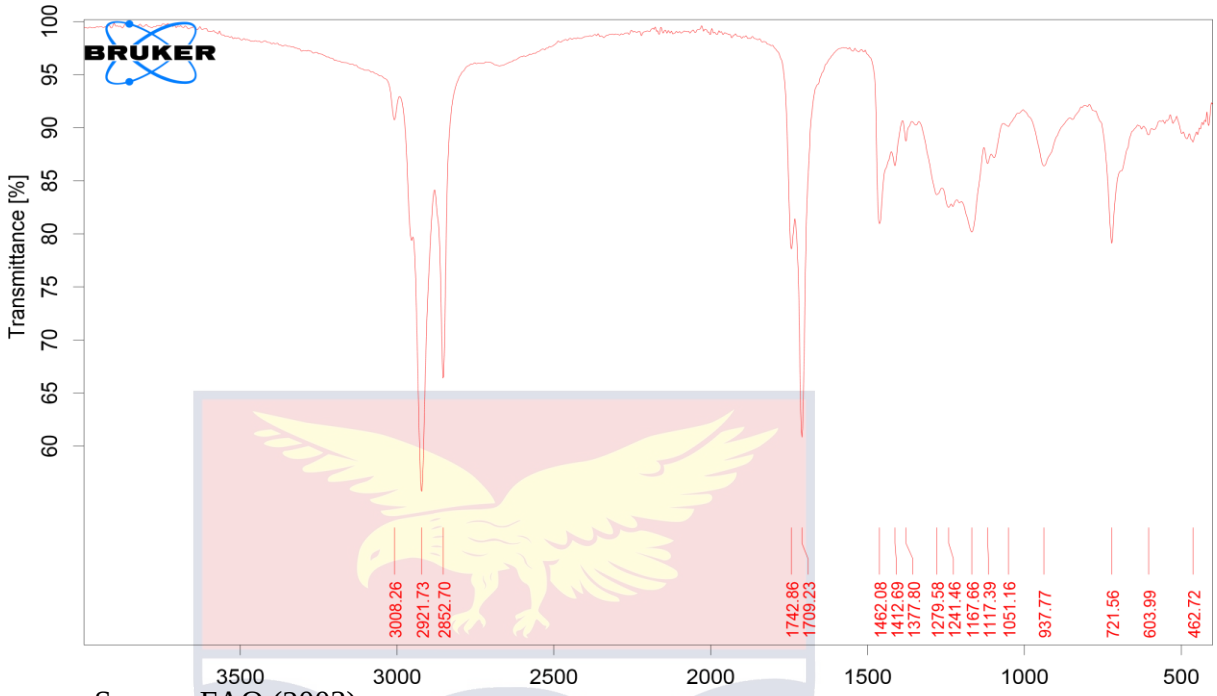
Source: FAO (2003)

Appendix K: IR SPECTRUM FOR UNUSED ORANGE SEED OIL



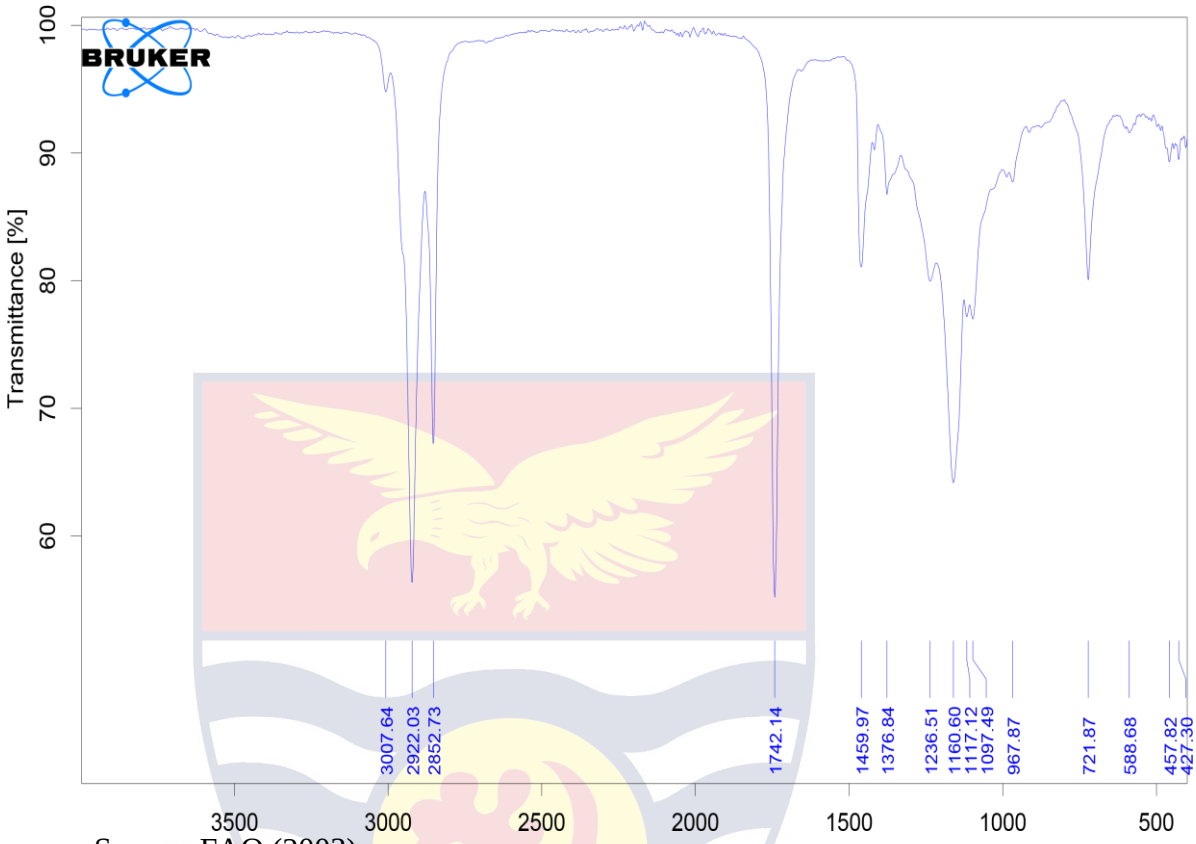
Source: FAO (2003)

Appendix L: IR SPECTRUM FOR ORANGE SEED OIL AFTER FIRST USE



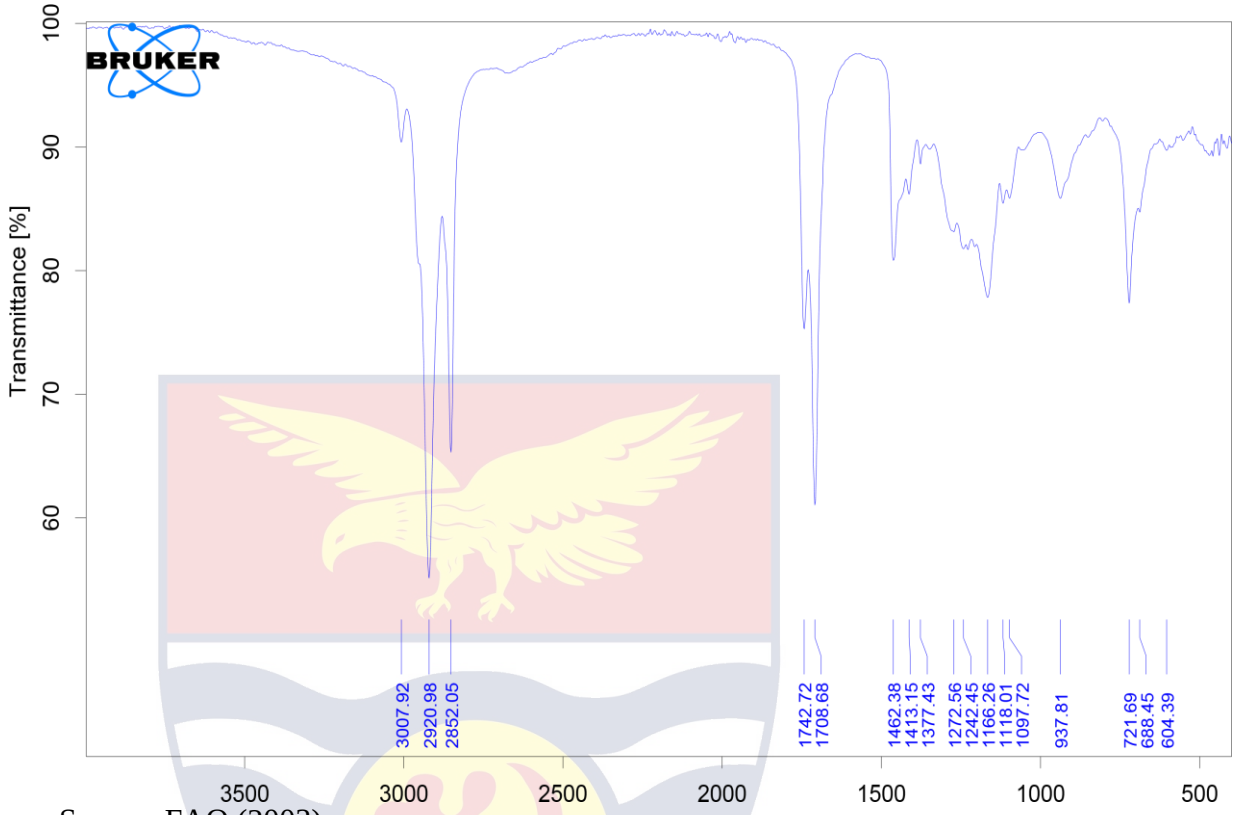
Source: FAO (2003)

Appendix M: IR SPECTRUM FOR ORANGE SEED OIL AFTER SECOND USE



Source: FAO (2003)

Appendix N: IR SPECTRUM FOR ORANGE SEED OIL AFTER THIRD USE



Source: FAO (2003)