EXTRACTION AND PHYSICOCHEMICAL CHARACTERIZATION OF PORK OIL

BY

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NIGERIA

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DEPARTMENT OF CHEMISTRY AHMADU BELLO UNIVERSITY, ZARIA NIGERIA

DECEMBER, 2012

DECLARATION

I declare that the work in this thesis entitled Extraction and Physiochemical Characterization of Pork Oil has been performed by me in the Department of Chemistry under the supervisor of Prof Ajibola and Prof. (Mrs.) Agbaji the information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

_____	_____	_____
Name of Student	Signature	Date

CERTIFICATION

The thesis entitled "Extraction and Physicochemical Characterization of Pork Oil (LARD) by Samuel Nathaniel Sunday meet the regulations governing the award of the degree of master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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God bless

DEDICATION

This project is dedicated to God and to the blessed memory of my uncle Mr. Stephen Joseph Adeyemo.

ABSTRACT

Pork oil was extracted through dry rendering from pork obtained from a local slaughter house in Samaru, Zaria. The physicochemical properties (refractive index, saponification value, percent free fatty acid content, iodine value, acid value, peroxide value, melting point, moisture content, hydroxyl value, unsaponifiable matter and ash content) were assessed using standard procedures. The result obtained compare favourably with recommended physicochemical properties of edible oils, the fatty acid composition of the oil determine by Gas Chromatography-Mass spectrometry (GC-MS) shows the major constituent to be oleic acid (46.49%), palmitic acid (28.19%) and stearic acid (19.45%). The thermal degradation of the oil was studied using Fourier Transform Infrared Spectroscopy (FTIR). These were carried out by heating oil at different temperatures) for duration of one hour each. Spectra were recorded from a film of each oil sample between two disks of NaCl. Changes in the value of the frequency of most of the bands of the spectra were observed. The shifts of the frequency value of specific bands allowed for distinction between the different stages of the oxidation process and to establish the degree of oxidation each oil sample. The elemental analysis of the oil ash using Neutron Activation Analysis (NAA) shows the presence of Al 2136 ± 175ppm, Ca 4005± 62ppm, Ti 155± 40ppm, V 1.9± 0.3ppm. Mn 87± 9ppm, Na 2022± I13ppm, K 1986 201ppm, As 0.15± 0.01ppm, Br 1.8± 0.1ppm, La 0.46± 0.05ppm, Tb 0.5± 0.1ppm, Sc 0.16± 0.02ppm, Cr 7± 2ppm, Fe 3762± 200ppm, Zn 227± 15ppm and Ba 3611± 345ppm.

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CHAPTER ONE

INTRODUCTION

The word oil has little specific meaning as it is applied to wide range of substances that are quite different in chemical nature. They are derived mainly from two main source, plants and animals. In Animals, they occur mainly in form of adipose tissue and as component of cells while in plants they are mostly found in storage organ like seed (Deuel Jr., 1954).

Fats and fatty oil are water insoluble substances of plants and animal Origin which consist mainly of glycerol esters of long chain fatty acids (Kirschenbauer, 1960). As a matter of fact, there is no scientific differentiation between edible oils and fats and the two terms may be used interchangeable. The common distinction between them is to consider solid product as fats and liquid ones as oils. This difference is temperature dependent at high temperature all edible oils and fats are liquid and at low temperature they appear to be solid. Even this latter statement is only apparently true. As most natural fats while appearing solid at ambient temperature are strictly blends of solid and liquid component (Hopkins, 1973). The products which form this subject could equally well be referred to either as fatty oil or simply fats, irrespective of consistency at ambient temperature.

1.2 CLASSIFICATION

The term oil has been applied in general usage to describe certain physical characteristics of various substances rather than their chemical nature or composition. This common use has led to confusion over the nature of oils and it is essential from the onset to understand their true classification.

Oil and fats can be divided into groups according to both their origin and their chemical nature.

1.2.1 Fixed Oil and Fat

Animal fats derived from milk and body tissue of animals including marine animals.

1.2.2 Vegetable Oil and Fats

These are obtained from the fruits and seeds of a wide range of plants. These oils and fats are esters of fatty acids (specifically glycerides) and are non-volatile.

1.2.3 Mineral Oil

These are distilled from petroleum and shale deposits this group of material include paraffin oil, fuel oil and most lubricating oils.

1.2.4 Volatile Or Essential Oil

Oil of lemon, oil of clones and anamous oil these are obtained mainly from plant source. Although, some are derived from animals. These oils are complex mixtures of aldehydes, ketones, hydrocarbons alcohol, acid, and short chain esters. They are used for flavours, perfumery and pharmaceutical purpose.

I.1.5 Crude Fat

These are completely untreated, as isolated from the oil bearing tissue. Crude oils are unpalatable and hence inedible in this state.

1.4.6 Natural Fat

This may have been treated to remove the impurities which make them unpalatable but otherwise have not been altered.

1.4.7 Modified Fat

These are fat or oil which has been changed by chemical treatment or physical separation to produce a fat which is different from the original material.

1.4.8 Synthetic Fat

These are fats which have been chemically made from various source materials. Some have been produced by oxidation of hydrocarbons to fatty acids which are then esterified with glycerol. This process was operated on a large scale in Germany. However, not only was it costly, but the fats produced contain unnatural fatty acids which were reported, to give rise to toxic effects (Christie *et al.,* 1972)

1.5 USES OF ANIMAL OIL

Animal Oils, like most vegetable oil and fat have found useful application in the industry and commerce and as edible oil in several food preparations (Ekpeyong, 1989). Industrially, they are used for example in foam, paint and surface coatings lubrications, adhesive plasticizers, textile dyes, pharmaceutical and cosmetic formulation and many others (Terill and Ault, 1975; Burger, 1994).

1.6 JUSTIFICATION

Oils and fats are important parts of human diet and more than 90 percent of the world production from vegetable, animal and marine source is used as food or as an ingredient in food products; oils and fats is rich source of dietary energy and contains more than twice the caloric value of equivalent amount of sugar. Their functional and textural characteristics contribute to the flavour and palatability of natural and prepared foods. They contain certain fatty acids which play an important role in nutrition and are also carriers of fats soluble vitamin (MMAF, 2005).

Many people in developing countries especially children under the age of five years suffer from acute and chronic protein and energy deficiencies. At the current trend of population increase, it is projected that, by the year 2020 there will be as many as 300 million chronically undernourished people in the sub-Saharan Africa (Harsch, 1997). There is definitely a need for

food production to keep pace with the increase in world population. In order to achieve this national development strategies in many agriculture-based tropical countries are now biased toward increasing the diversity of consumable food productions in order to alleviate malnutrition and stress on promotion and broadening of agricultural based industries to ensure that their product are both whole some and safe.

The dietary role of edible oils and fats are highly recognised. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have recommended an average daily intake of 55g fat per capita to complement the requirement for energy (Kabyemela *et al.,* 1992) and a 20-30% conversion rate for fat to energy to ensure good health (WHO, 1994).

Pork oil therefore, an edible oil obtained from any source is justifiably an area worthy of quality research time.

1.7 AIM AND OBJECTIVE

The aim and objective of this research work include the following;

a. To extract oil from a pig carcass obtained from a local dealer by dry rendering.
b. To investigate some physiochemical properties of the extracted oil in (a) above.
c. To investigate the fatty acid composition of the extracted oil in (a) using Gas chromatography-Mass spectrometry (GC-MS)
d. To investigate the thermal stability of the extracted oil using Fourier Transform Infrared Spectrometry (FTIR). Identify shift and changes in the oil bands.
e. To determine trace elements available in the extracted oil ash using Neutron Activation Analysis (NAA).

CHAPTER TWO

LITERATURE REVIEW

2.1 SOURCES OF RAW MATERIAL

One of the fundamental issues of development is economic development which according to Meier (1976) is a process of cumulative change that results from positive forces raise productivity (Edozium, 1979). Accordingly, one of the development forces in economic theory of production is availability/distribution of scarce raw materials for the manufacturing industry (Wells, 1970; Scott, 1985).

i. Agriculture Source

ii. Fossil source

iii. Inorganic source

2.1.1 Agricultural Source of Industrial Raw Material

This are animal and vegetable (plant) matter, products and by product such as meat, hides/skin milk from the animal matter, wool, gems, resins and tannins, fats and oil from animal and vegetable matter to mention a few (Tedder *et al.,* 1975; Mohammed, 1981).

2.1.2 Fossil Source of Industrial Raw Material

Fossil are dead bodies of animal and plant that have been buried in the desert or frozen ice or covered sand or mud in ponds or at the bottom of the sea (Hatch and Matar, 1977). Various chemical reaction take place for several year leading to formation of petroleum associated gas and coal from which various chemicals and petro chemical are obtained to feed other industries.

2.1.3 Inorganic Source of Industrial Raw Material

These are the mineral elements and their compound from the earth crust. They also include the gases from the atmosphere (Parker, 1978). They include; iron, copper, tin, and aluminium to mention a few while from the atmosphere such gasses as CO, O_2, N_2 are obtained (Hill, 1992). Generally the agricultural and fossils source provide the bulk of raw material for the process and manufacturing industries (Chang, 1978; Monts and Kahlkase, 1978). This further justifies this particular research on pork oil.

2.2 PIG PRODUCTION IN NIGERIA

Pigs have been described as one of the most prolific and fast growing livestock that can convert food waste to valuable products the excel above other red meat animals such as cattle, sheep and goat in converting feed to flesh (Ironkwe and Amefule, 2008). And their annual growth rate (3.8%) is higher than that of the human population (2.30-2.80%). The indigenous pigs have been recommended as good alternative source of cheap, high quality animal protein that suits escalating human population. They have relatively low cost of production and their growth rate is fast (Osaro, 1995). They also have short generation interval, high production potential, high prolificacy and high carcass yield. They adapt early to environment conditions (ILCA, 1992). Pig production has therefore been advocated as a short term measure toward alleviating the animal protein and calorie deficit, especially where there are no religious edict preventing their production and consumption (Eusebio, 1984).

Nigeria is estimated to have 4.4million pigs about 78% of these are found in the sub-humid zones of Northern and Southern Guinea Savannah (Shuaibu *et al.,* 1997). Most of the pig are reared in the extensive system and their productivity has been reported to be low (Okorie, 1978). The pig is not only a source of animal protein it also serve as an investment alternative and source of additional income especially in the rural areas. In the southern part of Nigeria, Pig

farming is kept in commercial quantities because there are no ethics or Nigerian taboos forbidding its production. But most time pig keeping has been reported to be a secondary enterprise and represent, some preposition for the income earned by households, most especially women (Holness, 1991).

2.3 PORK OIL (LARD)

Lard is the fat rendered from fine clean tissue from pig in good health at the time of slaughter and fit for human consumption. The tissue does not include bones detached skin, head skin, ears, tail organs, wind piper, large blood vessels scrap fat, skimming's, settling pressing, and the like and reasonably free from muscle tissue and blood. Lard is a soft, creamy, white solid or semisolid fat with butter like consistency obtained by rendering or melting the fatty tissue of pig. The major fat depot in pig is the subcutaneous depot which forms a tube around the body and consists of 70-90% triglycerol. There is considerable intramuscular fat but the internal cavity fats are much less developed than in cattle and sheep. The major fatty acid present are oleic, palmitic and stearic but there may, also be large quantities of linoleic acid, thus distinguishing lard from beef or mutton tallow in general, although there is some overlap at the lowest concentration (Spencer *et al.,* 1976). Lard is much softer than tallow not only because of the presence of more, linoleic acid, but also because of the higher concentration of oleic acid and lower concentration of stearic acid. The ranges of fatty acid given by Spencer et al. (1976) appear to be somewhat high for palmitic acid and stearic acid based on recent reports in literature of the composition of pig carcass fats. However, Hubbarb and Pockclington (1968) reported similar ranges for the fatty acid composition of various fats deports from European and Canadian pig carcasses. In view of

the number of factors which affect the fatty acid composition of pigs it is not possible to ascribed specific causes for the composition of samples of unknown origin.

2.3.1 Depot Site and Fatty Acid Composition

Henriquess and Hansen (1901) demonstrated that the internal fat has a higher melting point and a lower iodine value than subcutaneous fat, and that the inner subcutaneous fat layer adjacent to the skin. Before the advent of GLC it was established laboriously that the latter differences arose from higher concentration of palmitic and stearic acid in the inner layer and more oleic acid in the outer layer (Bhattacharya and Hilditch, 1931; Bank and Hilditch, 1932). Across each subcutaneous layer the fatty acid composition is virtually consistent (Dean and Hilditch 1933). But there is a distinct discontinuity at the connective tissue sheet separating the two layers and there are changes adjacent to the skin and muscles (Christie et al 1972). The differences between the two layers are small of the order of 2% or less for each fatty acid involved, most consistently for oleic and stearic acids. Linoleic acid is usually about 2% higher in the outer layer until its concentration reaches 30%, when the differences may be lost and even reversed at greater concentration (Wood, 1973), although Koch et al. (1968) observed that the differences was retained up to 38% linoleic acid. The factors which affect the variation in relative concentration of the other major fatty acids in the two layers are not clear. As the proportion of linoleic acid increases, the different in stearic and palmitic acids between the two layers decreases (Marchelo *et al.,* 1983) but in pigs which were virtually devoid of linoleic acid, the difference were quite small (Anderson *et al.,* 1970).

The perirenal and leaf fat of pigs contains higher proportion of saturated fatty acids and lower proportion of unsaturated fatty acids than the subcutaneous deports, stearic acid is 3.5% higher than in the inner subcutaneous fat whereas palmitic acid is raised by a smaller amount

(Christensen, 1963). Oleic acid is approximately 2% lower as in linoleic acid (Sink *et al.,* 1964) although greater decreases of oleic acid have been reported (Dermarne *et al.,* 1977) whereas Wood *et al.* (1986) found 21.9% linoleic acid in perirenal fat compared with 19.6% in the inner subcutaneous fat of the loin the latter authors also found the inter muscular fat of the shoulder to closely resemble the outer loin subcutaneous adipose tissue in fatty acid composition.

2.3.2 Effect of Diet on Lard Fatty Acid Composition

The potential for dietary variation of the fatty acid composition of pig depot fats is much greater than that for cattle and sheep because of the absence of the rumen in the digestive systems (Christie *et al.,* 1972). Hence, unsaturated fatty acid as well as saturated fatty acid, pass through the digestive system essentially unchanged and are deposited in the depots. The simple stomach of the pig also allows it to accept much higher concentrations of fats in the diet with the result that less common acids presents in some feeds can appear in the depot fat.

In the absence of significant amount of fat in the diet palmitic, stearic and oleic acid make up more than 90% of the adipose tissue fatty acids (Leat *et al.,* 1964) with smaller amount of pamitoleic, myristic and arachidic acid. When fat is present in the diet it is deposited in the tissue together with the fatty acids which can be synthesised *de novo.* This is readily detected by the appearance in the fat depots of plant derived linoleic acids which cannot be synthesised by the pig. When linoleic acid is present in large quantities in the diet, its deposition in the fat lowers the melting's point to the extent that the lipid becomes oily (Ellis and Isbel, 1926). Linoleic acid (Beadle *et al.,* 1948) and the longer chain, polyunsaturated fatty acid of marine oils (Brown, 1931; Garton *et al.,* 1952) are also readily deposited although their presence in the diet in significant amount is usually avoided because of the oxidative instability they impart to the lard. The concentration of linoleic acid in the adipose tissue lipids is proportional to the quantity in the

diet or more strictly, to the proportion of dietary calories which it supplies (Leat, 1962; Babatunde *et al.,* 1968).

Oleic acid is normally abundant in porcine depot lipids and changes in its deposition resulting from different dietary levels are often consisting of more than 10% fat in which oleic acid is present at about 60% are fed, the subcutaneous fat may contain approximately 55% oleic acid (St John *et al.,* 1987).

Feeding large quantities of saturated long chain fatty acids, unlike their unsaturated counterpart, fails to increase their concentration in adipose tissue (Roberts and Enser, 1988; Walker, 1972). Thus, the upper range of there saturated fatty acids is likely to exceed that quoted by (Spencer *et al.,* 1976) as a result of vagaries in the supply of dietary saturated fatty acids.

The biochemical mechanisms which regulate their concentration are not understood and poor digestion cannot account fully for the findings. However, studies with labelled saturated fatty acids clearly indicate that they are absorbed into the blood stream and deposited in adipose tissue (Cunnigham, 1968).

2.3.3 Non Fatty Acid Containing Component

Total unsaponifiable matter in lard was reported to be 0.18% ± 0.03% (Dahl, 1958) so that cholesterol accounts for over 50% of the total. However, William and Pearson (1965) observed that only 17% was cholesterol but the rest appeared to have been oxidised. Thus, vitamin A plus squalene fraction at 19% seems high as other studies show squalene is present at very low level 3mg /100g (Fitelson, 1943) although it constitutes 89.6% of the hydrocarbon fraction of lard (Maritano de correche, 1985). Somewhat surprisingly the latter authors also reported a phytol derivative in the alcohol fraction of lard, although the quantity of chlorophyll is low in pig feed. Palmitoyl alcohol and stearoyl alcohol were present but more of the materials were quantified.

Paul and Southgate (1978) reported only traces of vitamins in lard. Actual values determined by (Piironen *et al.,* 1978) were and α-tocopherol 0.7mg/100mg and α-tocotrienol0.2mg/100mg. vitamin A is present at 40-200U/100g in lard (Herb *et al.,* 1953).

2.4 OCCURANCE AND FORMATION OF FATS AND OIL

There is only a physical distinction between a fat and oil. If liquid at ordinary temperature the substance is termed oil. If solid, it is called fat (Kirschenbauer, 1960).

2.4.1 Catabolism of Fats and Oil

The formation of fat in the plant is obscure. The carbohydrate matter which is synthesised by the plant from carbonic acid and water is apparently converted into fat (Armstrong and Allan, 1924). The intermediate reactions are not known. In seeds, only a very small amount of fats is present after the fall of the flower. As the seed ripen, there is an increase in fat and a decrease in carbohydrate (Deuel Jr., 1954). Hilditch (1951) opined that during the later phase of the development the unsaturation of the fat increases while the free fatty acid content decreases. It appears therefore that the saturated acids are formed primarily from carbohydrate, then this is followed by esterification with glycerine, and that at a later stage desaturation takes place. Recent evidence appears to indicate however, that saturated and unsaturated acid are formed independently.

The importance animal fat depots are subcutaneous tissue, the abdominal cavity, the liver and intermuscular connective tissue the bones (bone grease) and particularly the feet and shinbones of cattle (neat and foot oil) and other animals also contain appreciable amount of fat of technical importance. Fat in the animal body may originate from ingested fat, from carbohydrate, and from protein but the mechanism of conversion of carbohydrate and protein into fat in the animal body have not yet been satisfactorily explained. Feeding experiments on rats and pigs indicate that

linoleic, linolenic and arachidonic acids cannot be synthesized in animals but are derived from ingested fats only (Shorland, 1955).

2.4.2 Metabolism of Fats

The digestion of fats is brought about by the action of the bile with the assistance of enzymes. Because of the strongly acidic nature of the gastric juice, which does not permit existence of sufficient amounts of lipolytic (fat-splitting) enzymes, no appreciable digestion of the fat takes place in the stomach (Bergstrom and Bergstrom, 1955). Hydrolysis of fats therefore is mainly carried out in the small intestine by the combined effect of the bile and the pancreatic enzymes steapsin (Kirschenbauer, 1960). The enzymes catalyse the splitting action and the bile salts appear to serve two fold purpose. Primarily they assist in the emulsification of the fats and secondarily, they form soluble complexes with the split fatty acids at least to a large extent, recombine into fats, which then enter the lymph stream (Mattson, 1956).

The lymph carries the fatty matter through the thoracic duct into the blood stream. Mattson (1956) claimed that only about 60% of the ingested fat can be traced in the lymph. Apparently, the remainder is directly conveyed to the liver by way of the portal system. There is evidence indicating that the ingested fats rebuilt in the liver. In this organ the fat which originate from carbohydrate and protein, also appear to be synthesised. From the liver the fats conveyed to various parts of the body by means of the blood streams. Evidently there is a continuous exchange of fatty matter between the liver and the depots. The latter are constantly supplied by the liver with newly formed or resynthesized fats. The fats taken up by the body serve these purposes:

i. They are stored for future use and reserve food in adipose tissue.

ii. They combine with protein in formation of cellular protoplasm, cell membrane etc.

iii. They may be oxidised immediately to carbon dioxide and water. The energy then liberated in used to produce muscular work and maintain body temperature. The calories per unit of fat metabolised are more than twice those produce by either protein or carbohydrate.

2.5 CONSTITUENCE AND COMPONENETS OF FATS AND OIL

A detailed treatment of the definition of lipid was first carried out by (Bloor, 1920) in essence, he described lipid as a major class of biological substance including fatty acids their naturally occurring compound and other substance related to them chemically or found naturally in association within them.

Bloor (1925) further characterised lipid as follow; lipid are insoluble in water but soluble in fat solvents such as diethyl ether, chloroform, benzene and boiling ethanol. These were their most distinguishing properties in contrast with the other main group of biological substance. These properties were however, recognised as not being absolute i.e. lecithin forms dispersion in water that approach true solutions. On the other hand, some lipids were known to be relatively insoluble in some fats solvent e.g. lecithin in acetone, caphalin in alcohol and sphgnomylin and glycolipid in diethyl ether.

Bloor (1925) classifications of the lipid has found wide acceptance. He distinguishes:

i. Simple lipids

ii. Compound lipids

   a. Phospholipids: lecithin, cephalic, sphgnomylin

   b. Cycolipids: cerebrosides

   c. Amino lipids: sufolipides

iii. Derived lipids

2.5.1 Simple Lipids

The simple lipids include both fats and waxes the major and minor constituent of fats and oil include

2.5.1.1 Glyceride

The number of triglycerides in a given natural fat is a function of the number of fatty acids present and specifity of the enzymes systems involved in the particular fat synthesis reactions. In his excellent treatise on triglyceride analysis Litchfield (1972) points out that many plant seed fats have the potential to provide 125-1,000 different triglyceride, animal fats contain potentially 1,000-64,000 triglyceride species and butter fats could generate 2,863,286 triglyceride from 142 different fatty acids. On saponification or hydrolysis undecomposed fats yield about 10-14% of triglyceride. In addition to being as a by-product of the soap and fat splitting industries, glycerine has been prepared commercially by fermentation of sugar solution (Scott, 1958).

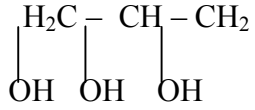


Fig. 2.1 Glycerol Structure

2.5.1.2 Fatty Acids

The higher fatty acid occurring as glycerides in natural fats are of saturated and unsaturated character. They are monocarboxylic straight chain acid possessing an even number of carbon atom. However, the following cases are exceptions.

Isovaleric acid is a branched chain acid with an uneven number of carbon atom that occurs in dolphin and porpoise fats (Gunstone, 1957). Branched chain acids have been found in wool wax in certain bacilli lipids, and in small amount in various fats, particularly the fats of ruminants. Acid containing a cyclopentene ring, particularly lydnorcarpic, chalmogric, and gorlic acid, occur in large amounts in plants fats that have been used in the treatment of leprosy (Hanson and

Shorland, 1954). Odd numbered straight chain acids probably are present in many fats. Ox, sheep and butter fats may contain as much as 2 per cent of these acids (Gunstone, 1957).

Most of the fatty acids in fat are esterified with glycerol to form glycerides. However, in some fats, particularly were abuse of raw material has occurred leading to enzymatic activity, considerable (75%) free fatty acids (FFA) is found. As in the case of triglycerides, the number of known fatty acids is very large. In 1965 it was noted that about 900 vegetable and 500 animal fats had been analysed. Seed lipids researches in the USDA laboratories (Wolff, 1966) as well as the unusual fatty acids in plants (Smith Jr., 1970) have been reviewed.

Table 2.1 Some Saturated Fatty Acid.

No of carbon Atoms	Systematic Name	Common Name
4	Butanoic	Butyric
6	Hexanoic	Caproic
8	Octanoic	Capylic
10	Decanoic	Capric
12	Dodecanoic	Lauric
14	Tetradecanoic	Myristic
16	Hexadecanoic	Palmitic
18	Octadecanoic	Stearic

$CH_3CH_2CH_2COOH$

Fig. 2.2 Butanoic Acid Structure

2.5.1.3 Antioxidants

The most commonly occurring anti-oxidant in vegetable fats is the tocopherol (Vitamin E). There derivative of 6-chromanol are not synthesized by animals and occur in their fats only through ingestion of plant material and vegetable fats (Rossel, 1988). Antioxidants tend to protect fats by inhibiting auto oxidation and subsequent rancidity. A certain percentage of the tocopherol is removed in some refining steps and is recovered as by products of fats and oil processing (Bauernfeind, 1977). Tocopherol was found necessary for normal reproduction in many animal species. It consist of four closely related alcohols α, β, γ and δ tocopherol. α-tocopherol has the greatest biological activity and δ- tocopherol is the most active anti-oxidant (Dam, 1955).

Another compound of consequence is sesamol, a sterol found in sesame oil. The lecithin and gossypol (a pigment of cotton seed oil) also have been reported to have antioxidant characteristics, and this may reflect an ability to interact with heavy metals.

2.5.1.4 Pigments

The major pigments of fats are carotenoids. The carotenoids are also known as lipodromes. They are yellow and red pigments of the fats. The carotenoids belong to four groups; hydrocarbons (carotenes), ketonic or hydroxylic derivatives xanthophyles, carotenoids acid and xanthophylls esters. They are highly unsaturated and owe their colour to a long conjugated system of double bonds (Dam, 1955).

Carotenes occur in the lipoids fraction of green and yellow vegetables, palm oil and certain animal oil. Three isomers of this hydrocarbon are known:

α, β and γ- carotene. Many seed oils particularly if processed from mature seeds also contain significant levels of chlorophyll pigment that lend a greenish tinge to the fats. Cotton seed oils is

heavily coloured by gossypol type pigments most of these pigments are removed in the alkali refining and bleaching processer.

2.5.1.5 Vitamins

The principle component is vegetable fats with vitamin activity are the tocopherols. Vitamin A is found in butter fat and in fish oil. The carotenes (pro-vitamin A) are found at significant level in palm oil in butter fat and as traces in other fats. Vitamin D is found mainly in some fish oil. While vitamin K is found in green vegetables in the liver fat of many mammals and in egg yolk.

Vitamin A is essential for growth, normal vision and proper functions of the epithelial tissues. The so called night blindness is one of the first symptoms of vitamin A deficiency. Vitamin K prevents anaemia and haemorrhage. It is administrated for the prevention of haemorrhage in the new-borns and treatment of human obstructive jaundice.

2.4.1.6 Sterols

Most of the unsaponifiables in vegetable and animal fats are sterols. The animal fats predominantly contain cholesterol and most vegetable fats contain only traces of this sterol. Plant sterols collectively called plytosterols are made up mainly of sitosterols and stigmaterol but some individual vegetable fats contain additional plytosterols.

The pattern of typical sterols has been suggested as useful in detecting adulteration of oil with another (Eisner and Firestone, 1963). Sterols are of minor importance in the technology of fats. Normal there removal processes have been utilized as a source of certain plytosterols that are used as raw material in the pharmaceuticals industry. A good summary of current knowledge on sterols are vividly elaborated in (Seher *et al.,* 1976).

2.4.1.7 Minor Constituent

In addition to the materials listed above, waxes, hydrocarbon, ketone, aldehydes and mono and diglycerides are found in fats and oil at varying levels. The waxes in some seed oils (e.g. corn sunflower and safflower) are troublesome and are removed in processing to prevent haze formation in damage and can causes flavour and odours in fat. The mono and diglycerides result pose particular problem in end products. The hydrocarbons are mainly analytical curiosities and are of no technological consequence.

2.6 THE STRUCTURE AND COMPOSITION OF FATS

2.6.1 Glyceride Structure

As glycerine is a trihydric alcohol, there may exist as monoglycerides, diglycerides and triglyceride. The glycerides which occur naturally in the fresh undecomposed state are triglyceride (Vander Val, 1955).

If the fatty acid radical of a fat molecule are alike, the ester is termed a simple glyceride. If at least two different acid radicals are contained in a fat molecule, the ester is called a mixed glyceride. They three possible places which a fatty acid radical may have in the glyceride molecule are designated as alpha, beta and gamma or more recently as 1, 2, and 3.

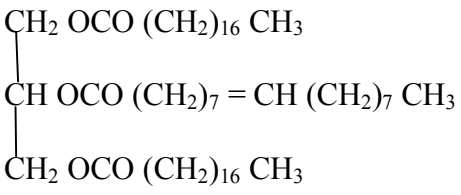


Fig. 2.3 Beta – oleo – alpha, gama– distearin or 2 – oleo – 1, 3 distearin

If the fatty acid radicals occurring in the alpha and gamma position are taken by different radicals, the glyceride is term asymmetrical.

The natural fats and oil contain only small percentages of simple glyceride. As there are at least three and commonly many more different fatty-acid radicals occurring in a particular fat or oil, the possibilities of isomerism are numerous (Quimby, 1953). A fat derived from 3 acids may consist of 10 different glycerides, and a fat containing 10 different acids may possess 220 isomeric glycerides. In the majority of fats, particularly in the case of most seed fats and marine animal fats, there exists a tendency toward even and complete distribution of fatty acid radical in the glyceride molecules (even distribution). The fats of the large land animals, on the other hand represent a heterogeneous mixture, the distribution of the fatty-acid radicals being of poor order (random distribution) (Quimby, 1953). A third class, which with regard to fatty acid distribution fall between two groups comprises the fruit-coat fats and all those animal fats which contain large percentages of linoleic acid namely bird, rodent and pig fats. This difference in the glyceride studies also explain why two fat may differ widely with regard to their physical properties (Malkin and Bevan, 1957).

2.6.2 Classification and Fatty Acid Composition

Fats and oils have been classified in a number of ways. For instance thy have been grouped according to unsaturation as measured by their iodine value. Thus, there are non-drying (iodine value below 90) semi-drying (iodine value 90-130), and drying oil (iodine value above 130). A more fundamental classification was undertaken by Grun and Haldens, who grouped the fat on the basis of their biological origin. Later Hilditch (1954) made a detailed classification according to the major component fatty acids and characteristic fatty acid based on botanical and zoological families from the many fact that justify this classification, a few may be mentioned thus;

Marine oils are composed of a great variety of unsaturated acids and contain only small amount of saturated acids. The two classes of fish Teleostei (having a bony structure) and Elasmo branchii (no bony structure) show a pronounced difference in fatty acid composition. Elasmo branchii possess appreciable amount of C_{24} acid known as selacholeic acid. Teleostei do not contain this acid but have large proportions of C_{20} unsaturated acid (Vander Val, 1955). Between the component acid of fresh-water fish and those of the sea fish, there also exist typical differences. The former have a higher percentage of C_{18} unsaturated acid and a lower content of C_{20} and C_{22} unsaturated acid than the latter (Vander Val, 1955).

The fatty acid composition of the fats of higher land animal is distinguished from that of the marine oils by its simplicity. Palmitic acid, oleic acid and stearic acid are the only major component acid. The difference between the Herbivora and carnivora is not as pronounced as might be expected (Kirschenbauer, 1960). Palmitic and oleic acid are also the major component acid of animal milk fats. In addition, the milk fats of many animals, particularly that of ruminates, contain relatively large amounts (5-30 percent) of short fatty acids (C_4 to C_{10}) the component fatty acid of amphibians, bird and rodents occupy and intermediate portion between the two preceding classes. The oil of amphibians and reptile contain appreciable amount of C_{20} and C_{22} unsaturated acid and have other characteristics of marine oil (Malkin and Bevan, 1957). In the study of their oil birds and rodent appear more closely related to the higher land animal, than fish (Kirschenbauer, 1960).

Fats obtain from fruit flesh or fruit coatings, such as palm oil, olive oil and Chinese vegetable tallow, contain palmitic, oleic and often linoleic as major component acid (Kirschenbauer, 1960). Seed fats generally contain palmitic, oleic linoleic, and/or linolenic acid as principal acid. Some seed fats contain a characteristic component acid that occurs only in certain botanical families.

For instance, petroselinic acid is typical of the families of *Umbelliferae* and *Araliaceae*: tariric acid is typical of the *Sunaronbaceae*, erucic acid is found only in *Cruciferae* and T*ropaeolaceae* eleostearic acid is typical of *Alearites chordate* and *Aleurites fordii* and ricinoleic acid of *Ricinus cummons* and some other specie of the *Euphobiaceae* and *Oleaceae* (Kirschenbauer, 1960).

2.7 THE NATURE OF FATS AND FATTY ACIDS

2.7.1 Physical Properties

2.7.1.1 Colour and spectral properties

Pure fatty acids and their glycerides are colourless and therefore do not have spectral properties in the visible range. The colour of natural fats is due to the presence of small amount of fat soluble pigment such as carotenoids and chlorophyll, or sometimes to oxidation and polymerization products of the fatty acids (Holman, 1954). These pigments have absorption patterns and the spectrophotometric method of evaluating colour of fats has been used to an increasing extent: although, they transmit visible light readily. Unsaturated acids and their derivations absorb in the ultraviolent region, and double bonds in conjugated position have characteristic absorption spectral. The usefulness of this property has been increased greatly by the development of a method for shifting unconjugated double bonds of fatty acid in conjugated position by treatment with alkali at elevated temperature (Farmer, 1942). Infrared spectra have found usefulnessss in connection with the estimation of the amount of trans acid present in a mixture of fatty acid or their derivatives the strong and distinctive absorption band of trans acid is associated with the frequency of interatomic vibration. Infrared spectra have been increasingly used for the study of reaction mechanism such as the dying of oils (Kaufmann, 1987)

2.7.1.2 Refractive index

The refractive indexes of glyceride are higher than of the corresponding fatty acid. They indexes of fatty acids increases with increasing molecular weight and the degree of unsaturation. Conjugated acid have higher values than the corresponding non conjugated acid.

2.7.1.3 Odour and flavour

The natural odour and flavour of fats, except those derived from very short chain acids are generally due to the presence of fractions of non fatty matter, for instance, the fragrances of palm oil is partly due to β-ionone and the odour of coconut oil is stated to be largely due to nonyl methylketone (Swern, 1955).

2.7.1.4 Solubility

Fat are insoluble in water and with the exception of castor oil, they are only poorly soluble in lower alcohols. On the other hand they are readily soluble in ethyl ether, hydrocarbons benzene, carbon disulfide and the common halogenated solvents (Kirshenbauer, 1960). Solubility properties of certain oil have been applied on the basis for many analytical tests such as the Valenta and Crismer method. Solvent extraction which utilizes of the solubility of fats in certain solvents, has found increasing application in their commercial production. The solubility of the fatty acids is greater than that of the corresponding glycerides. They are soluble in all the common polar and nonpolar organic solvents, the lower members are also soluble in water, with increasing chain length the acids become quite insoluble in water (Harwood, 1952).

2.7.1.5 Isomerism

If double bonds occur in the chain, stereoisomeric acid may be formed the physical properties of the latter unusually are quite different from those of the naturally occurring acids. As an example of this steroisomerism oleic and elaidic acid may be mention.

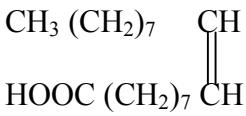


Fig. 2.4a Oleic acid (cis form)
M.P 16.3^0C

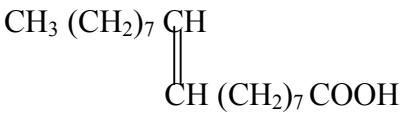


fig. 2.4b Elaidic acid (trans form)
M.P 43.7^0C

The double bound restrains from rotation. This fact is responsible for the existence of two different forms of the acid, according to Van't Hoff's theory. The naturally occurring cis form can be converted to elaidic acid by treatment with nitrous acid, sulphuric acid, sulfour, or selenium. The rearrangement is halted when equilibrium of two third fluids eladic acid and one third oleic acids has been reached. This same ratio is obtained in the synthetic preparation of oleic acid.

In addition to cis-trans also known as geometrical isomerism, other form may exist; chain, position and optical isomerism. Chain isomerism does not exist extensively in natural fatty acid except in the few cases previously mentioned. The bulk of the fatty acids are straight chain, unbranched compound (Kirschenbauer, 1960).

2.7.2 Chemical Properties

The reactions of fatty acid and their glycerides may be regarded conveniently as either reaction of the carbonyl group or the hydrocarbon chain that is attached to the carbonyl group. The latter may be a plain paraffinic chain or it may contain unsaturated centres or substituent group(s).

Although it is convenient to consider separately different parts of the same molecule, regards their respective chemical properties, it should be kept in mind that there exist an important relationship between carbon chain and carbonyl group which greatly modifies their respective activities. The carbonyl group exercise a particularly powerful effect on the reactivity of the hydrocarbon chain, owing to its electron configuration. Example of this is chlorination and sulfonation, which proceed more or less by favouring alpha substitution in the case of saturated acids (Woodbridge, 1958). Another example is the selectivity of hydrogenation of certain unsaturated acid while the corresponding esters react highly selectively (Eckey, 1956).

2.7.2.1 Hydrolysis

In the presence of water or steam, fats are hydrolysed to free fatty acid and glycerol. The reaction is catalysed by acid, enzymes, and compounds that form fatty acid soaps. The effect of enzymatic action is well-illustrated by some commercial grades of palm oil which, if produced by crude nature methods, at the time of their arrival on the American market contain 50 percent or more of free fatty acids (Harwood, 1952). The presence of enzymes and moistures responsible for this hydrolytic decomposition industrially hydrolysis is carried out on a large scale

2.7.2.2 Esterification

Esters of fatty acids may be prepared by direct esterification or by inter esterification. In the former instance the fatty acid are heated with mono or polyvalent alcohol in the presence of catalytic amount of free mineral acid or other catalyst. Inter esterification may be either one or three reactions: alcoholysis, where an ester react with free fatty acids; and ester interchange, when two esters are made to react as in the presence of alkali ion (Eckey, 1956). The methyl ester or other short-chain alcohol esters are often prepared in the laboratory because they have lower boiling point than the corresponding free fatty acids.

2.7.2.3 Saponification

When fats are heated with alkalis such as caustic soda, salts of fatty acid and glycerine is liberated. Free fatty acid may be reacted with soda ash or other suitable carbonates to yield soap. For nearly two thousand years, until recently water soluble soap has served as the principal detergent (Harwood, 1952). Potassium, ammonium, triethanolamine, or other organic base soaps are also made iindustrially but on a much smaller scale. Water insoluble but oil soluble soaps such as calcium, magnesium and lead, soap are preferably made by reaction two suitable water soluble salt i.e. Magnesium chloride and sodium oleate. The oil soluble soap is used as lubricants, oxidation catalysts and for many other purposes.

2.8 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Infrared spectroscopy allows the qualitative determination of organic compound because the characteristic vibrational mode of each group causes the appearance of band in the infrared spectrum at a specific frequency, which is further influenced by the surrounding functional groups. It is also possible to carry out quantitative analyses as the intensity of the band is proportional to the concentration of the functional group, according to the Lambert-Beer's law. In the past, due to the major difficulty for the dispersive instruments to obtain fast reproducible results, infrared spectroscopy has scarecely been used in food studies.

However, with the incorporation of the Fourier transformation, the application of this technique has increased in all fields (Guillen *et al.,* 1992, 1995) including foods (Guillen and Manzanos, 1996; Guillen and Cabo, 1997 a, b). One of the advantages of the Fourier transform technique is the high accuracy and reproducibility in the measurement of the frequency values.

Likewise, the degree of unsaturation of edible oil and fats has been relate to the intensity of specific bands. Pioneer studies (Sinclar *et al.,* 1952) have shown the existance of close

relationship between the number of double bonds in mixtures of cis fatty acid and the ratio between the absorbance of the streching vibration bands of the -CH_2- group, at 2920 cm^{-1} and the difference between the absorbance of this band and that at 3020cm^{-1} due to the streching vibration band of =CH- cis group high (low) value of this ratio indicates high (low) unsaturation in the sample subject of study.

Mid infrared spectra have been used to characterised edible oil and fats (Batlet and Mahon, 1958; Ahmed *et al.,* 1986; Guillen and Cabo, 1997a) because they show differences in the intensity and the exact frequency at which the maximum absorbance of the band appears, according to the nature and composition of the sample under study with the utilization of multivariate method such as principle component analysis (Safar *et al.,* 1994; Dupuy *et al.,* 1996) or discrimant analysis (Lai *et al.,* 1994). The difference between spectra has been emphasized and the classification of different commercial edible oil and fat has been carried out. Recently, Guillen and Cabo (1997a) have observed close relationship between the frequency of some bands of the fingerprint region and the proportion of mono-and poly unsaturated and saturated acyl groups in 13 samples of several edible oils and lard, which may be useful for predictive purpose. Infrared spectroscopy has also been widely applied to the determination of trans unsaturation by means of the qualification of the intensity of the band at 967cm^{-1} assigned to isolated trans double donds vibrational mode, and the method has been standardized by IUPAC (1987), AOCS (1989) and AOAC (1990). In addition, some methods have been developed from the FTIR spectra data for the fast accurate determination to some classical indices of edible oil and fat such as iodine value and saponification number (Van de Voort *et al.,* 1992), free fatty acid (Ismail *et al.,* 1995) cis and trans content (Van de Voort *et al.,* 1995) and solid fat content (Van de Voort *et al.,* 1996).

The usefulness of infrared spectroscopy, together with other technique, for characterization pure compound formed by oxidation of fatty acids and ester is well known (Frankel *et al.,* 1977, 1982; Neff *et al.,* 1982; Thomas and Prior, 1980; Wu *et al.,* 1992). In a little past, Van de Voort *et al.* (1994a) studied the oxidation process of cottonseed and safflower oil, carried out on a heated horizontal ATR crystal or heated in a heating mantel, by measuring band height at preselected frequency in FTIR spectra, using standards these authors have proposed a quantitative approach, based on the measurement of the absorbance of specific bands. For determining the oxidation state of oil, defined in terms of the percentage of hydro peroxides, alcohol, and the total carbonyl content.

2.9 GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GCMS)

GCMS is a method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. Application of GCMS includes; drug detection, fire investigation, enviromental analysis explosive investigation and identification of unknown sample.

The use of mass spectrometer as the detector in gas chromatography stated during the 1950s by Rowland and Mchafferty (Gohlke, 1959). These sensitive devices were bulky, fragile and originally limited to laboratory settings. The developments of affordable and miniatiurised computer has helped in the simplification of the use of this instument as well as allowed great improvements in the amount of time it takes to analyse a sample.

The GCMS is composed of two major building blocks. These two components used together; allow a much finer degree of substances identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample

while a gas chromatography using a traditional detector e.g. (flame ionisation detector) detects multiple molecules that happen to take the same amount of time to travel through the column (i.e. have the same retention time) which result in two or more molecules to co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragment in a mass spectrometer (mass spectrum). Combining the processes reduces the possibility of error, as it is extremely unlikely that two different molecular will behave in the same way in both a gas chromagraph and a mass spectrometer. Therefore, when identifying mass spectrums appear at a characteristic retention time in a GCMS analysis, it typically lends to increased certainty that the analyte of interest is in the sample.

2.9.1 Analysis Processes

For the analysis of volatile compounds a purge and trap (P&T) concentrator system may be used to introduce samples. The target analytes are extracted and mixed with water and introduced into an airtight chamber. An inert gas such as nitrogen (N_2) is bubbled through the water, this is known as purging. The volatile compounds move into the head pace above the water and are drawn along a pressure gradient (caused by introduction of the purge gas) out of the chamber. The volatile compound are drawn along a heated line onto a trap. The trap is a column of absorbent material at ambient temperature that holds the compounds by returning them to the liquid phase. The trap is then heated and the sample compounds are introduced to GCMS column via a volatile interface, which is a split inlet system. P&T GCMS is particularly suited to volatile organic compounds (VOCs) and compounds associated with petroleum.

2.9.2 Identification

The primary goal of instrument analysis is to quantify an amount of substance. This is done by comparing the relative concentration among the atomic masses in the general spectrum. Two

kinds of analysis are possible comparative and original. Comparative analysis essentially compares the given spectrum to a spectrum library to see if its characteristics are present for some sample in the library. This is best performed by a computer because there are a myriad of visual distortion that can take place due to variation in scale. Computers can also simultaneously correlate with data (such as the retention times identified by GC), to more accurately relate certain data.

Once a chemical formula has been matched to spectrum, the molecular structure and bonding can be identified and must be consistent with the characteristics recorded by GC-MS. Typically, this identification done automatically by programs which come with the instrument, given a list of the elements which could be present in the sample.

2.10 NEUTRON ACTIVATION ANALYSIS (NAA)

Activation analysis (AA) is an analytical technique for qualitative and quantitative determination of elements in a sample base on the production of radioactive nuclei from stable nuclei in the sample subjected to irradiation by a flux of activating species such as neutron, proton, gamma ray and so forth. Specifically, Neutron Activation Analysis (NAA) is an activation analysis employing neutrons as the activating specie. The neutron employed in NAA may be thermal (energy 0.025 ev) as is obtained in reactors or fast (energy > 1mev) as is obtained from neutron generators and isotopic source of neutron as the activating specie produce by by neutron induced reaction following the exposure of the sample, both to a flux of neutron. By characterising and measuring the radioactivity produced in a sample both qualitative and quantitative analysis can be performed.

NAA is particularly powerful as a means of trace analysis of geological, archaeological, biological, clinical, and forensic materials and also materials of metallurgical origin and other industrial material.

2.10.1 Miniature Neutron Source Reactor (MNSR)

Neutron activation analysis is a technique which in its instrumental form, makes it possible to determine large number of elements in different types of matrices. Thermal neutrons are used for the activation analysis in MNSR. The principles of the non-destructive relative method i.e. instrument neutron activation analysis. The MNSR is a tank pool type reactor which has a core with highly enriched uranium as fuel, light water as the moderator, and metallic beryllium as the reflector and uses natural circulation for heat removal negative reactivity temperature effect and very small excess reactivity. All those make the MNSR very safe and reliable. The start-up and shutdown of the MNSR can be controlled by a microcomputer, making the MNSR easy to operate. Besides there two “fast sample transfer system” coordinated with the MNSR. All these make the MNSR have unique feature when it is used as neutron source for neutron activation analysis compared with other neutron sources and large and medium reactors.

2.10.2 Analysis

In NAA, following are the irradiation of a sample and formation of many radionuclides in the radioactive product, the radionuclides are separated in some manner for quantitative analysis. There are two basic approaches to this; in one the separation is done by taking the product through a sequence of chemical processes to isolate the individual radionuclides of interest. NAA employing this radio chemical separation technique: radiochemical NAA (RNAA). In the other, the separation is done by exposing the product to a discriminatory counting device such as a gamma-ray spectrometer. In the gamma-ray spectrometer the separation is done by taking

advantage of the differences in gamma – ray energy and half lives, and using a high resolution detector to resolve the gamma ray photo peaks NAA employing this technique of radionuclides separation is called instrumental NAA (INAA).

The activity of a given radionuclide induced in sample depends on several factors. Consider a sample irradiated for a time t_r allowed to decay for the time t_d and then counted for the time t. the induced activity $A_i(t)$ for a given radionuclide is at a time t, after the irradiation given by:

$$A_i(t)=\frac{f_i w_i Na \sigma_i \Phi}{m_i}[1-\exp(-\lambda_i t_r)]\exp(-\lambda_i t) \quad \text{.....Equation 1}$$

Where

w_i= Weight of Element
f_i= Fractional abundance of the Element
Na= Avogadro's number
σ_i= Microscopic absorption cross section for the reaction of interest for "i".
Φ= Neutron Flux
m_i= Atomic Mass
λ= Decay constant

The total concentration of an element in a sample is measured by quantifying the corresponding radio nuclide in irradiated samples. The amount of the radio nuclide in the irradiation sample is directly proportional to the amount of its parent isotope in the original sample.

2.10.3 Absolute Method

In this method, the absolute flux of the activating particles and the absolute distinct ratio rate are required to determine the absolute activity of the samples. The flux is measured with a flux monitor during irradiation. This method is more complicated than the relative method and it's not popular.

2.10.4 Relative Method

In NAA, the relative method is based on the comparism of an isotope in the sample with the activity of an isotope in a known standard produced under identical irradiation, cooling and counting conditions. The standard material is necessarily closely similar both chemically and

physically to the sample material, so that given the condition of irradiation; a lot of the parameters in equation 1 are not measured as they are taken care of in the equation;

$$\frac{\text{Specific activity of element A in sample}}{\text{Specific activity of element A in standard}} = \frac{\text{Concentration of element A in sample}}{\text{Concentration of element A in standard}} \quad \text{.....equation 2.}$$

Equation 2 is used in quantifying the concentration of element of interest in a sample. Standard reference material SRM of known elemental concentration can be used as activation standard.

2.11 FATS AND OIL DEGRADATION

The oxidative stability of oil could be defined as their resistance to oxidation. This is an important indicator of performance and shelf life and depends on the composition of the sample and on the conditions to which it is subjected. Any study of the oxidative stability of oil require the establishment of the conditions under which the sample is oxidised, knowledge of the oxidation process and a method to determine the rate at which this process take place; the same requirements should be taken into account in studies of anti-oxidant activity. Oxidative condition can include a determined flow of air of oxygen, the heating of the sample at a determined constant temperature, the presence or absence of light and catalysers, the exposure of the sample to a determined light frequency etc. Some of these oxidative conditions are used in the well-known accelerated test of oxidative stability (Frankel, 1993). However, it must be taken into account that the oxidative condition can influence mechanism of the degradation process.

Although other degradation mechanisms are possible, the oil degradation process has been generally established as being a free radical mechanism yielding hydro peroxide, also into aldehydes, ketones, lactones, alcohol, acid etc. or secondary oxidation products.

The methods used to determine the rate at which the oxidation process advances are related to the measurement of the concentration of primary or of secondary oxidation products or both, or

to the amount of oxygen consumed during the process. Among those based on the evolution of the concentration of primary oxidation products, peroxide value (PV) which measure hydro peroxide concentration is one of the most widely used. Different approaches have been proposed for its determination, most of them based on chemical reactions (Gray, 1978).

Traditional method for establishing the oxidative state of edible oils and fats are chemical method based on the measurement of the concentration of the main products generated in the process. That is true for peroxide value, the most commonly used which is based on chemical determination of hydro peroxide concentration and which can be considered as an indicator of the initial stages of oxidation but cannot be indicative of the current extent of oil or fat oxidation because hydro peroxide decompose rapidly, nor is peroxide value useful for monitoring the degradation of oil without hydro peroxide formation as intermediate compounds. Infrared spectroscopy has been used to establish methods with high reproducibility for peroxides value determination after previous calibration with standards, (Van de Voort *et al.,* 1994b; Ma *et al.,* 1997).

Other chemical methods to establish the oxidation degree in edible oils are based on the determination of secondary oxidation products such as anisidine value, which basically measure the concentration of aldelydes considered mainly responsible for the off flavours in oxidised oils and fats. A method to determine the anisidine value by means of infrared spectroscopic data and calibrations with standards has also been published (Dubois *et al.,* 1996).

Fourier transform infrared (FTIR) spectroscopy gives information about the different functional groups present in a sample, not only about a kind of compound. The technique is therefore very suitable and useful in order to study the oxidation process of edible oils.

CHAPTER THREE

MATERIAL AND METHOD

3.1 SAMPLE

5kg of pork was obtained from a major local slaughter house in Samaru, Zaria. The pork's tissue was cut into pieces. The pork oil was prepared by cooking the fats containing tissue for 30 minutes. It is allowed to cool then fried. The pork oil thus obtain was transferred into a 2 litre plastic container and stored at 2^0C until needed. It was used without further purification.

3.2 PHYSICOCHEMICAL PROPERTIES

3.2.1 Saponification Value

This is a measure of mean weight of the oil and it denotes the number of milligram of potassium hydroxide which is required to saponify 1 gram of oil that is to neutralise the free fatty acid and the fatty acids combined as glycerides.

Principle

The saponification value was determined by completely saponifying the oil with a known amount of potassium hydroxide the excess of which was determined by titration.

Analytical Importance

The saponification value is an index of mean molecular weight of the fatty acids of glyceride comprising a fat. The lower the saponification value the larger the molecular weight of fatty acids in the glycerides and vice versa.

Reagent

i. 0.5MAlcoholic potassium hydroxide: refluxe 1.2litre isopropyl alchohol for 1 hour with 10g KOH and by aluminium foil. It was distill and collected after discarding first

50cm^3. 40g KOH dissolved in the distilled alchohol keeping temperature below 15^{0}C allow standing for 3days before use.

ii. Phenolphthalein indicator 1%; dissolve 1.0g of phenolphthalein in 100cm3 propylalchohol

iii. Standard hydrochloric acid 0.5M

Procedures

2g of oil was weighed; 25 cm^{3}of 0.5M alcoholic potassium hydroxide was accurately measured to the cold oil and was heated under refluxe for one hour. The flask was swirled from time to time. The flask was then allowed to cool a little and the condenser was washed down with a little distilled water. The excess KOH was titrated with 0.50M HCL acid, phenolphthalein was used as indicator. Blank determination was carried out under the same condition without the oil (A.O.A.C, 2000)

Calculation

Saponification value = $\frac{56.1M\ (V_2-V_1)}{w}$

Where w = weight in gram of oil

M = molarity of HCl

V_1= volume in cm^3 of HCl used

V_2= volume in cm^3 of HCl used in blank

3.2.2 Iodine Value

The iodine value of oil denotes the weight in grams of halogen, calculated as iodine capable of being absorbed by 100g of sample under specified condition. It is an approximate measure of the number of olefinic and acetylenic bonds in the substances. It measures precisely the amount of unsaturation in the fatty acid radical of the glycerides.

Principle

The oil sample taken in carbon tetrachloride is treated with a known excess of iodine monochloride solution in glacial (wijs solution). The excess of iodine monochloride is treated with potassium iodide and the liberated iodine estimated by titration with sodium thiosulphate.

Analytical Importance

The iodine value is a measure of the amount of unsaturation (number of double bonds) in a fat/oil.

Reagent

i. Carbon tetrachloride

ii. Potassium iodine 10% aqueous solution.

iii. Standard sodium thiosulphate dissolve approximately 24.8g of ($Na_2S_2O_3$. SH_2O) in distilled water and make up to 1000cm^3 standardised by the following procedure.

5.0g of freshly powdered potassium dichromate which has been previously dried at 105^{0}C $\pm$ 2^{0}C for 1 hour, was dissolved it in distilled water and make up to I litre for standardisation of sodium thiosulphate, 25cm^3 of the solution was pipette into 250cm^3 conical flask. 15cm^3 of a 10% KI solution were added. It was allow standing in the dark for 5 minutes and titrated with sodium thiosulphate solution using starch as indicator. The end point is change of blue colour to green.

Wijs Solution

This was prepared by dissolving about 9g of iodinetrichloride in a mixture of 700cm^3 glacial acetic acid and 300cm^3 carbon tetrachloride.

Procedure

2g of the oil weighed was put into the conical flask; $10cm^3$ of carbon tetrachloride was added to dissolve oil. $25cm^3$ of the wijs solution was added. The content was mixed thoroughly and it was allowed to stand in the dark for 1hour at 27^0C.

After standing for one hour $15cm^3$ of potassium iodide and $100cm^3$ of water were added, this was titrated with sodium thiosulphate solution with proper shaking. Starch solution was added when yellow colour appeared sodium thiosulphate was added to the blue colour in the conical flask drop by drop until it disappeared completely. Blank determination was carried out under the same condition (A.O.A.C. 2000)

Calculation

$$\text{Iodine value} = \frac{12.59M\,(V_2-V_1)}{w}$$

Where w = weight in gram of oil

M = molarity of sodium thiosulphate

V_1= volume in cm^3 of sodium thiosulphate used

V_2= volume in cm^3 of sodium thiosulphate used in blank

3.2.3 Acid Value

Define as the number of mg of KOH required to neutralize the free fatty acid present in one gram of fat. It is the relative measure of rancidity as free fatty acids are normally found during decomposition of oil glyceride the value is also repressed as percent of free fatty acids calculated as oleic acid.

Principle

The acid value is determined by directly titrating the oil in an alchoholic medium against standard KOH solution.

Analytical Importance

The value is a measure of the amount of fatty acid which has been liberated by hydrolysis from the glyceride due to the action of moisture, temperature and/or lypolytic enzymes lipase.

Reagent

i. Solvent: Equal volume of toluene and isopropyl alcohol were mixed. It was neutralised with 0.1M KOH solution before used

ii. Phenolphthalein indicator 1% solution

iii. KOH 0.1M

Prepared by boiling of KOH in 1 litre of distilled water for 1 hour. 2.0g of $Ba(OH)_2$ was added and boiled for 5 minutes. It was cooled and was allowed to stand for 2 hours. It was standardized with potassium hydrogen phthalate.

Procedure

5g of the oil was weighed and 330cm3 of the mixed solvent already neutralised was added. The sample dissolved in the solvent and was titrated against KOH solution. The end point was pink (IUPAC 1987)

Calculation

$$\text{Acid value} = \frac{56.1M\,(V)}{w}$$

Where w = weight in gram of oil

M = molarity of KOH solution

V= average volume in cm^3 of KOH used

$$\text{Percentage free fatty acid} = \frac{\text{Acid value}}{1.99}$$

3.2.4 Peroxide Value

This is an indication of the extent of oxidation suffered by oil. It is expressed in milli equivalent of peroxide oxygen per kg oil. The peroxide value is usually used as an indicator of deterioration of oil. As oxidation take place the double bonds in the unsaturated fatty acid are attacked. This breakdown to produce secondary oxidation products which indicates a condition known as rancidity.

Principle

The peroxide value is determined by subjecting KI at room temperature to the oxidation effect of peroxide. The iodine thus liberated was titrated with standardised sodium thiosulphate.

Reagent

i. Acetic acid: Chloroform solvent mixture (3:2), 3 volume of glacial acetic acid with 2 volume chloroform

ii. Freshly prepared saturated iodine solution 1%

iii. Sodium thiosulphate 0.1M

Procedure

5g of the oil was weighed into conical flask, and dissolved in 30cm^3 of the solvent. 0.5cm^3 of saturated KI was added. The flask was properly shaken and allowed to stand for 1 minute, 30cm^3 water was added and the solution was added and the mixture was titrated with 0.1M sodium thiosulphate solution. About 0.5cm^3 starch solution was introduced immediately and the reagent drop- wise until the blue colour disappeared. The blank titration was carried out (A.O.A.C. 2000).

Calculation

Peroxide value = $\frac{100M(V_2-V_1)}{w}$

Where w = weight in gram of oil

m = molarity of sodium thiosulphate

V_1= volume in cm^3 of sodium thiosulphate used

V_2= volume in cm^3 of sodium thiosulphate used in blank

3.2.5 Hydroxyl Value (Acetyl Value)

The hydroxyl value is the number of mg of KOH required to neutralise the amount of acetic acid capable of combining by acetylation with 1g of sample. The method of determination of OH^- content does not differentiate between OH^-group in the fatty acid chains and those present in mono or diglyceride.

Principle

The OH^- were acetylated with a known amount of acetic anhydride and 2g of sodium acetate the excess acetic anhydride was decomposed with water and the acetic acid formed was titrated with ethanolic KOH.

Reagent

i. Sodium acetate

ii. Acetylating agent – acetic anhydride

iii. Phenolphthalein indicator 1% solution in 96% ethanol

Procedure

10cm3 of oil sample was measured and introduce into a round bottom flask. $10cm^3$ of acetic anhydride was added. Condenser was attached in a reflux position; the content was boiled gently for 1 hour. The flask was allowed to cool for 15 minutes and $50cm^3$ of distilled water was added

through the top of the condenser. The flask was heated on a steam bath for 15minutes with frequent shaking to destroy the excess acetic anhydride. The contents were transferred into a separatory funnel and the flask was rinsed with 10cm3s of water two times. These were added to the content in the separatory funnel and the flask was rinsed with 10cm^3 of water two times. This was added to the content in the separatory funnel and was thoroughly shaken to make sure that the aqueous layer was in contact with the oil layer. After this, the aqueous layer was rejected and the oil layer was washed with 50cm^3 saturated NaCl. The washing was done until the washing was neutral to litmus. The oil was dried with anhydrous sodium sulphate and was filtered. 2g of acetylated oil was weighed and introduced into a round bottom flask. 25cm^3 of 0.5M ethanolic KOH solution was introduced to the acetylated oil and was refluxed for thirty minutes. Two drops of 1% phenolphthalein indicator was added and this was titrated with 0.5M hydrochloric acid. The blank determination was carried out (Cocks and Van Rede, 1960).

CALCULATION

$$\text{Hydroxyl value} = \frac{56.1M\,(V_2-V_1)}{w} + \text{Acid value}$$

Where w = weight in gram of oil

M = molarity of HCl

V_1= volume in cm^3 of HCl used

V_2= volume in cm^3 of HCl used in blank

3.2.6 Ash Content

The ash content is decribed as the percentage of inorganic residue remaining after ignition of oil.

Procedure

10g of oil was weighed into the crucible dish which had been weighed. The dish was heated carefully to the ignition point. When the burning was finished the dish was heated in the furnace at 700^0C. The dish was cooled and weighed again.

$$\text{Ash content} = \frac{100(W_3 - W_2)}{W_2 - W_1}$$

Where W_1= weight of crucible

W_2=weight of crucible + oil

W_3= weight of crucible + ash

3.2.7 Unsaponifiable Matter

This is a measurement of the water insoluble component produced after heating the fat with KOH. The amount of unsaponifiable matter found in edible oils is usually very small and high value may indicate contamination or adulteration.

Principle

The fatty matter was converted into soap by alcoholic potassium hydroxide solution. The solution was diluted with water and the unsaponifiable matter was extracted with diethyl ether.

Reagent

i. Alcoholic KOH solution (reference to note under saponification value for preparation)

ii. Diethyl ether

iii. KOH solution 0.5M

iv. Acetone

Procedure

2g of oil was accurately weighed into round bottom flask. 25cm^3 of alcoholic potassium hydroxide solution was added and the mixture was heated under reflux for one and half hour when saponification was completed the content of the flask was transferred into a separating funnel and the round bottom flask was washed with 50cm^3 distilled water. The flask was rinsed with 50cm^3 of diethyl ether which was then carefully poured into the separating funnel. The

separating funnel was shaken and the mixture was allowed to stand and the two layers separated. 10cm^3 of 1M HCl was added because of the emulsion formed. The aqueous layer was drawn off when the layer had separated. The ether layer was washed two times with 20cm^3 of water and was shaken vigorously. 20cm^3 of 0.5M aqueous KOH was added to the ether layer, it was thoroughly shaken. 5cm^3 of 0.1M HCl was added because of the emulsion formed. The aqueous layer was discarded and 20cm^3 of distilled water was added shaken and the water layer was discarded. The same process was repeated twice with 20cm^3 aqueous KOH and 20cm3s water. The ether layer was washed with 20cm^3 distilled water until the wash water was no longer alkaline to drops of 1% alcoholic phenolphthalein. The ether solution was transferred to a 250cm^3 conical flask and the ether was evaporated to a small volume. The remaining solvent was transferred to a small conical flask which has been weighed. Few cm^3 of acetone was added when the solvent was evaporated the evaporation was continued until all the solvent was evaporated. The residue was heated to a constant weight at 80^0C (A.O.A.C. 2000)

Calculation

$$\text{Unsaponifiable matter} = \frac{\text{residue} \times 100}{w}$$

Where w = weight in gram of oil

3.2.8 Determination of moisture content (Air oven method)

Definition

Moisture content of oil and fat is loss in mass of the sample on heating at 105^0C$\pm1^0$C under operating condition specified.

Procedure

Thoroughly mixed 5g of the oil was weighed into a previously dried crucible which had been weighed. The crucible containing oil was then heated in an oven at 105^0C for an hour. It was then removed from the oven and cooled in a dessicator and weighed. The crucible containing oil was further heated in the oven for a period of one hour cooled and weighed. This was repeated until change in weight between two successive observations does not exceed 1mg (I.S.I. 1984).

Calculation

Moisture content = $\frac{W_1 \times 100}{w}$

Where w = weight in gram of oil

W_1 = lost in weight on drying

3.2.9 Determination of Melting Point (Open Tube Capillary Slip Method)

Oil and fat are chiefly mixtures of glycerides. They do not exhibit either a definite or sharp melting point. Therefore the melting point does not imply the same characteristic that it does pure crystalline substances. Fats pass through a stage of gradual softening, before they become completely liquid. The melting point is therefore defined by the specific conditions of the method by which it is determined.

Principle

The temperature at which the oil or fat soften or becomes sufficiently fluid to slip or run as determine by open tube capillary slip method

Procedure

The sample was melted and filtered using filter paper to remove any impurities and last traces of moisture. A capillary tube was then introduced into the molten sample where a column of the sample about 10mm long was sucked into the tube. The tube was then placed in a small beaker containing ice until it solidifies. It was then sealed and placed into a refrigerator for an hour. The

tube was then removed and attached with a rubber band to a thermometer so that the lower end of the capillary tube and the thermometer bulb are at the same level. The thermometer with the capillary tube containing the sample was immersed in thiele tube containing water at about 10^0C. This was gradually and gently heated until the sample in tube start flowing (I.S.I. 1984).

3.2.10 Determination of Refractive Index

The ratio of velocity of light in vacuum to the velocity of light in the oil more generally, it expresses the ratio between the sine of angle of incidence to the sine of angle of refraction when a ray of light of known wavelength (usually 589.3mλ, the mean of 0 lines of sodium) passes from air into the oil. Refractive index varies with temperature and wavelength.

Procedure

The space between the prisms was filled with oil. Ample time was allowed for the oil and the prism to attain steady temperature. After this, the prism was closed and properly tightened. The instrument was adjusted so that the field duration time was coincidental with the cross wire (A.O.A.C. 921.08)

Significance

Refractive index of oil increases with increase in unsaturation and also chain of fatty acid.

3.3 FATTY ACID COMPOSITION BY GAS CHROMATOGRAPHY – MASS SPETROMETER (GCMS)

Principle

The methyl ethers are formed (methylation) using methanol and alkali and separated by Gas chromatography using flame ionisation detector, mass spectrometer gives the mass spectrum of individual fatty acids then identify by in-built library software.

Reagent

Carrier gas, methanolic sodium hydroxide, hexane conc HCl, $CaCl_2$

Procedures

i. Preparation of Methanolic Sodium Hydroxide

2kg of sodium hydroxide was weighed and dissolve into 100cm^3 of methanol. The mixture was stirred for about 2 minutes till a clear solution was obtained i.e. the sodium hydroxide dissolve completely in 100cm^3 of methanol forming methanolic hydroxide.

ii. Methylation Process

0.2g of oil was weighed into a quick fit conical flask and 6cm^3 of methanolic sodium hydroxide was added. It was refluxed for 10minutes on a steam bath. 30cm3 concentration HCl and 20cm^3 methanol was added and refluxed for another 10minutes on the steam bath and allowed to cool. Finally, 10cm3 of distilled water was added and the mixture poured into a separating funnel. The organic layer was collected and dried with calcium chloride (Nikolon and Geige, 1983).

iii. Fatty Acid Determination

Fatty acid methyl ethers (FAMEs) were analysed using SHIMADZU – QP2010 plus Gas chromatography mass spectrometer enquipped with flame ionisation detector (FID). With column temperature at 70^0C and injection temperature at 250^0C.the flow rate of the carrier gas (N_2) was set at 1.8cm^3/min. Identification of the FAMEs was based on retention time of reference compounds. Fatty acid composition was expressed as percentage of total FAMEs. The mean spectrometer operated in the election impact mode with electron impact energy of 70eV and a multiplied voltage of 1650V and collected data at a rate of 1scan s^{-1} over a range of m/z 30 – 350

3.4 THERMAL DEGRADATION STUDY USING FOURIER TRANSFORM INFRARED SPECTROMETRY FTIR

Samples

Five different oil samples were used for the FTIR analysis which include

i. Normal oil without heating (control)

ii. Oil heated at 50^0C for 1 hour duration

iii. Oil heated at 100^0C for 1 hour duration

iv. Oil heated at 150^0C for 1 hour duration

v. Oil heated at 200^0C for 1 hour duration

Spectral Acquisition

A film of a small amount of each sample (≈2µl) was deposited on NaCl disk avoiding the presence of air. The spectra were recorded from 4500cm^{-1} to 400cm^{-1} with a resolution of 4cm^{-1}. For each spectrum 32 interferogram were co-added before Fourier transformation and zero filled to give a data point spacing of ≈1.9cm^{-1}.

Study of Spectra

The height (intensity) of each band was measured automatically by means of a macro program taking baseline 3600 – 2472cm^{-1} and 1900 – 530cm^{-1}. The same procedure was used to determine the band arrears.

3.5 ELEMENTAL ANALYSIS USING NEUTRON ACTIVATION ANALYSIS (NAA)

Principle

The ash of the oil is generated at about 500^0C using a furnace. Ash obtained was subjected to NAA Analysis.

Procedure

NIRR1 which is a low power nuclear reactor with highly enriched uranium as fuel, light water as moderator and berrylium as reflector was used for the analysis. The reactor associated facilities for radio activity measurement are a gamma ray data acquisition system. It consist of a horizontal dip-stick High purity Germanium (HPGs) detector with a relative efficiency of 10% at 1332.5 keV gamma ray line, MAESTRO emulation software compatible with the ADCAM multichannel Analyzer (MCA) card, associated electronic modules all made by EG and GORTEC and a personal computer. The efficiency curve of the detector system at near and far source detector geometries have been determined by a standard gamma ray source in the energy range of 59.5-2254 keV and extended to 400keV for data processing gamma ray spectrum analysis, software WINSPAN 2004 (Iyu, 2004) which is a software developed at CIAE, Beijing, China was used. On the basis of the well-known activation equation, the software requires that calibration factors be predetermined by a multi- element standard reference material for elements of interest using adopted irradiation and counting required. The WINSPAN 2004 in addition to NAA calculations, perform peak analysis, remote control of MCA and other auxiliary functions such as efficiency calibration and nuclear data generation.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 PHYSICAL AND CHEMICAL PROPERTIES

Table 4.1 shows some of the physicochemical properties of the pork oil. The quality of the oil was assessed using parameters such as acid value, peroxide value, iodine value, saponification value, percentage free fatty acid, hydroxyl value, unsaponifiable matter, and moisture content where the values were compared with that of some edible oils.

Acid value is an important index of physicochemical property of oil which is used to indicate the quality, age, edibility and suitability of oil for use in industries such as paint (Akubugwo *et al.,* 2008). According to Demian (1990), acid value are used to measure the extent to which glycerides, in the oil has been decomposed by lipase and other physical factors such as light and heat. Thus, the high acid value of the pork oil when compared with that of soybean oil 0.09mg/g suggest that the pork oil is more susceptible to lipase action. This value (2.78mg/g) (Table 4.1) for the pork oil is higher than the 0.6mg/g proposed by Usoro et al. (1982), for edible oil.

Peroxide value is used as a measure of the extent to which rancidity reactions occur during storage. The low peroxide value of the pork oil 3.26 meq/g (Table 4.1) also corroborated the fact that the pork oil has resistance to lipolytic hydrolysis and oxidative deterioration compare with *arachis hypogea* with a peroxide value of 5.99meq/g (Atasie *et al.,* 2009). Again, the peroxide value of the pork oil falls within the range of 0 -10meq/g stipulated for freshly prepared oil (Cocks and Reds, 1966). Therefore, it is likely that storage for a long time may not lead to rancidity of the oil.

The saponification value helps determine the quantity of potassium (in mg) needed to neutralized the acids and saponify the esters contained in 1g of lipid (Roger *et al.,* 2010). The saponification value (209mg/g) (Table 4.1) determined for the pork oil is higher than

Table 4.1 Physiochemical Properties of Pork Oil

S/N	Properties	Value
1.	Saponification value (mg/g)	209
2.	Iodine value (cg/g)	62
3.	Acid value (mg/g)	2.78
4.	% free fatty acid	1.4 %
5.	Peroxide value (meq/g)	3.26
6.	Hydroxyl value (mg/g)	182.14
7.	Ash content	0.8%
8.	Unsaponifiable matter	0.6%
9.	Refractive index	1.560 at 25 ºC
10.	Moisture content	0.03 %
11	Melting point	41-42 ºC

that of some common edible oils such as soya bean (189-195meg/g), Peanut (187 – 196mg/g) and cotton seed oil (189-198mg/g) (Codex, 1993).

Saponification value of above 200mg/g give good quality soaps: while those below 200mg/g form soft soaps that is soft to touch (Khan *et al.,* 1972). Hence the relatively high saponification value of the pork oil will enhance its use in the soap industry.

Furthermore, the iodine value measures the degree of unsaturation in a fat or oil (i.e. the number of double bond) (Daintith, 2008). It determines the stability of oil to oxidation as well as allows the overall unsaturation of the fat to be determined quantitatively. Knowledge of the iodine value enables the combustion temperature of the oil to be evaluated (Roger *et al.,* 2010). The iodine value of the pork oil (62cg/g) (Table 4.1) classified the oil as non-drying oil with iodine values less than (100cg/g) (Asuquo, 2008). The iodine value of pork oil is lower than that of sunflower oil (110-143cg/g), castor oil (83.75cg/g), soya beans (120-143cg/g) and rubber seed oil (134.51cg/g) (Abayeh *et al.,* 1999). The low iodine value for the pork oil indicates that the oil is rich in saturated fatty acids, which ensures stability against oxidation and rancidification of foods prepare with the oil.

The quality of oil in most cases is determine by the free acid content the higher the value of the free acid content the lower the quality of the oil. The percent free fatty acid of the pork oil (1.4%) (Table 4.1) is greater than that of some edible oils; such as sunflower oil (0.056%), corn oil (0.127 – 0.166%) and palm oil (0.176 – 0.254%) (Othman et al, 2010), Generally for edible oil percent free fatty acids of 2.5% and below are acceptable and values above 2.5% are unacceptable (Pritchard, 1978).

The unsaponifiable matter of pork oil is 0.6% (Table 4.1). The unsaponifiable matter which is a measure of the water insoluble components produced after heating the oil with potassium

hydroxide. The value for the pork oil is lower than that of avocado pear (2.8%), dacryodes edulis (2.3%), red egusi seed (1.6%), sesame oil (1.2%), white melon (1.1%) and corn oil (0.92%), however, lower than that of palm kernel oil (0.22%), coco kernel oil (0.09%) and castor seed oil (0.5%) (Kapseu *et al.,* 2001; Asuguo, 2008). The amount of unsaponifiable matter in edible oil is usually low, high values may indicate contamination or adulteration. Also, in most edible oil the content of unsaponifiable matter are largely sterols (Cocks and Van Red, 1996).

The refractive index (1.56) does not differ much from the refractive indices of castor oil (1.47), Rubber seed oil (1.46), *Blighia sapida* (1.449), and *Detarium microcarpum* (1.465) (Kyari, 2008), (Dhellot *et al.,* 2006) and (Anhwange *et al.,* 2004).

4.2 FATTY ACID COMPOSITION OF PORK OIL

Table 4.2 show the percentage fatty acid composition of the pork oil. This consist of odd chain, branched chain as well as the more common even-chain and cis-unsaturated monoenoic and polyenoic fatty acids but distinctively without trans-unsaturated fatty acids.

In the pork oil saturated chain constitute 49.85% of the total fatty acids (Table 4.2) and this is higher than the saturated acids found in oils (tallow) of true ruminants like sheep and cattle which are about 40.0% (Sinclair *et al.,* 1982). Among the saturated acids the most abundant is plamitic acid (28.19%) which is also the second most abundant acid in pork oil accounting for more than one quarter of the total fatty acids. Some stearic and myristic acids are present along with smaller amounts of normal and branched chain ranging from C_{12} to $C_{17.}$ Saturated fatty acids can increase the risk of heart diseases from atherosclerosis by raising blood cholesterol (Appel *et al.,* 1997). But not all saturated fatty acids have the same effect on cholesterols synthesis in the liver. Only those of chain length 12, 14 and16 (lauric, myristic and palmitic acid) have been

shown to elevate blood cholesterol. Of these however, myristic acid elevates cholesterol the most (Mensink, 1993). Of the branched chains only those with cyclopropane ring have been identified.

Table 4.2 Percentage Fatty Acid Composition of Pork Oil

Fatty Acid	Percentage (%)	Common Name	IUPAC Name
$C_{12:0}$	0.21	Lauric Acid	Dodecanoic Acid
$C_{14:0}$	1.84	Myristic Acid	Tetradecanoic Acid
$C_{15:0}$	0.13	Pentadecyclic Acid	Pentadecanioc Acid
$C_{16:1}$	2.01	Palmitoleate Acid	9-Hexadecenoic Acid
$C_{16:0}$	28.19	Palmitic Acid	Hexadecanioc Acid
$C_{17:0}\triangle$	1.46	Lactobacillic Acid	Cyclopropaneoctanoic Acid
$C_{17:0}$	0.03	Margaric Acid	Heptadecanioc Acid
$C_{18:1}$	46.49	Oleic Acid	9-Octadecenoic Acid
$C_{18:0}$	19.45	Stearic Acid	Octadecanioc Acid
$C_{22:6}$	0.20	DHA	4,7,10,13,16,19-Docosahexaenoic Acid

The monounsaturated chains in pork oil account for almost one-half of the total fatty acids (Table 4.2). This group of acids consist of oleic acid (46.49%) and palmitoleic acid (2.01%). In both ruminant and non ruminant species oleic acid is known to be the major monoenoic acid (Crawford et at., 1970; Sinclair et at., 1982 and USDA, 1989, 1991).

Only one polyunsaturated fatty acid (PUFA) has been identified in the pork oil (Table 4.2). DHA the only PUFA detected in the pork oil. The ratio of the polyunsaturated chain to the saturated ones is very low 0.0004 as compared with 0.22, 0.26, and 0.36 in cattle, sheep and goat oil, respectively (Sinclair *et al.,* 1982; USDA, 1989, 1990). The percentage of PUFA in pork oil (0.02%) falls below those reported for oil of domesticated ruminants such as cattle (8.8%) and sheep (10.3%) and the PUFA percentage reported for the oil of non-domesticated ruminant like buffalo (28.6%) and Samba deer 31.4% (Sinclair *et al.,* 1982).

The fatty acids of the pork oil are dominated by saturated and monounsaturated even chains. Small quantities of odd numbered fatty acids are also detected 0.16% (Table 4.2).

However, the major fatty acids constituent of the pork oil are: oleic, palmitic and stearic acids they make up more than 90% of the total fatty acids (Table 4.2) this generally show a similar profile to pork oil fatty acids as represented by Maria et al. (2004), Elena *et al.,* (2004), Maw *et al.,* (2003) and Anderson *et al.,* (1970).

Remarkably, PUFA like linoleic and linolenic acids which are absent in the pork oil (Table 4.2) were reported to be present in some pork oils as studied by Spencer *et al.,* (1976), Koch *et al.,* (1968); Marchello *et al.,* (1983). This is because the concentration of linoleic and linolenic acids in adipose tissue lipids is proportional to the quantity in the diet (Leat, 1962; Babatunde *et al.,* 1968). They cannot be synthesized by pigs.

4.3 ELEMENTS IN PORK OIL

Table 4.3 shows the concentration of metals in pork oil in part per million (ppm)

The entire elements present in the pork oil are in the following order.

Ca> Fe > Ba> Al> Na> K> Zn> Ti> Mn > Cr >V> Br > Tb> La> Sc>Ar. According to Ismail *et al.,* (2011) minerals can be divided into three groups: major elements, trace elements and ultra trace elements. Major elements include; Na, K, Ca, Cl, P, S are needed more than 50mg/day. Trace elements; Fe, I, Zn, Se, Cu, Mn, Cr, Mo, Co, Ni, are needed less than 50mg/day. Ultra traced elements are Al, As, Ba, Bi, Br, Cd, Cs, Ge, Hg, Li, Pb, Rb are not really essential.

Ca is found has the highest concentration more than any other element in the pork oil with a concentration of 4005ppm (Table 4.3). Ca is known in human nutrition for the development and growth of the skeleton, it also controls the membrane structure, membrane permeability and provides stability to the cell (Han *et al.,* (2003) The concentration of Ca in pork oil far exceed the concentration of Ca in some Nigerian edible oil with range of 30.0 – 88.o µg/g (Ahmed, 2000). This may be as a result of pigs feeding habit.

Fe is the second most abundant element in the pork oil with the concentration of 3762ppm (Table 4.3) Fe a necessary metal and is core component of the red blood cell, its deficiency can cause anaemia (Hamilton and Whitemey, 1982). The concentration of Fe in the pork oil is higher than that in some edible oil sunflower (0.27 – 1.31µg/g), corn oil (0.22 – 1.3µg/g), soya oil (0.30 -0.76 µg/g) and olive oil (0.49 – 2.20ug/g) (Garrido *et al.,* 1994).

Al is another element present in large concentration in the pork oil with 2136ppm (Table 4.3). Al associations with disease have been hypothesized but remain to be confirmed. Higher levels of Al may possible be related to Alzheimer disease. The concentration of Al

Table 4.3 Concentration of Elements in Pork Oil Using NIRR1

Element	Concentration (ppm)
Al	2136±175
Ca	4005± 62
Ti	155 ± 40
V	1.9 ± 0.3
Mn	87 ± 9
Na	2022 ± 113
K	1986 ± 201
As	0.15 ± 0.01
Br	1.8 ± 0.1
La	0.46 ± 0.05
Tb	0.5 ± 0.1
Sc	0.16 ± 0.02
Cr	7 ± 2
Fe	3762 ± 200
Zn	227 ± 15
Ba	3651 ± 340

in the pork oil surpasses that reported for some edible oil with concentration range of 19.4 – 44ug/g (Ahmed, 2000).

The concentration of Na in pork oil 2022ppm (Table 4.3) is also higher than that of some Nigerian edible oils with concentration range of 17.5 – 72.88μg/g (Ahmed, 2000). Moreover, the ration of Na/K is ≥ 1, which indicate, according to Prevot (1966), that the oil has undergone a refinement process. However, K concentration in the pork oil is within the range of some different Italian commercial oil as published by Solinas et al. (1985).

Zn with a daily intake limit of up to 15mg/day (ATSDR, 1994) Zn, concentration 227ppm falls within the range of some edible oil with a concentration range of (68.0 – 576.0 μg/kg (Nash *et al.,* (1983) and Martini-Polvillo et al. (1994). Zinc is an essential metal for the human body in minimal amount, while it is dangerous in higher concentration.

Mn, Cr and V with concentrations of 87ppm, 7ppm and 1.9ppm respectively (Table 4.3). The concentrations of these elements are relatively higher than those of some edible oils considered in Erol et al. (2008) and Garrido et al. (1994).

In general, there seems to be higher metal concentration in the pork oil compare to some edible oils identified above. This is probably associated with pig mode of feeding and the rearing method mostly employed (extensive).

4.4 Pork Oil Thermal Degradation Study Using Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of the lipids of pork adipose tissue is similar to that of other animal or vegetable fats and oil studied before (Guillen and Cabo, 1997a, 2000) because all of them consist mainly of triglycerides. However, it shows some differences either in exact frequency and

absorbance of the bands, due to differences in length and unsaturation degree of acyl groups of the glycerides.

Appendix A shows the FTIR spectrum of the melted pork oil (control) before been submitted to thermal degradation. Some of the most significant bands together with a tentative assignment (Guillen and Cabo, 1997b) are the following: the band at approximately 2938 cm^{-1} due to the asymmetric stretching vibrations of the methylene functional group, the band at about 1744cm^{-1} is due to the stretching vibration of the ester carbonyl group of the triglycerides, the band near 1450cm^{-1} is due to the bending vibrations of the CH_2 and CH_3 aliphatic groups, the band near 1364cm^{-1} due to symmetric bending vibrations of the CH_3 groups, the band near 1168cm^{-1} is associated with the stretching vibrations of the C=O ester groups and with the bending vibration of the CH_2 group (Guillen and Cabo, 1997b) and related to the proportion of saturation acyl groups in oil samples (Guillen and Cabo, 1997a), and finally the band near 720cm^{-1} due to the overlapping of the methylene rocking vibration of straight chain paraffins of the acyl groups and to the out-of-plane vibration of cis-disubstituted Olefins.

Table 4.4 shows the pronounced frequency value extracted from the FTIR spectra in Appendices A – E.

Changes in the Region between 3000cm^{-1} and 2900cm^{-1}

The bands at approximately 2938cm^{-1} (Appendices A-E) are due to the symmetric and asymmetric stretching vibration bands of the aliphatic CH_2 functional group (Bellamy, 1975). When the oil sample was subjected to thermal degradation there was a gradual decline of the frequency values as the heating temperature increases. Yet, the frequency values are within the acceptable range. Hence, the degradation has little or no effect on functional groups present in the oil samples, at this particular

Changes in the Region between 1800cm^{-1} and 1600cm^{-1}

The frequency and the absorbance of the band at approximately 1744cm^{-1}, due to the ester carbonyl functional group of the triglycerides also changed during the thermal

Table 4.4 Frequency (cm^{-1}) variation related to different Temperature

	Control	50^{0}C	100^{0}C	150^{0}C	200^{0}C
1.	460.04	453.29	461.00	456.18	463.90
2.	716.58	716.58	715.61	719.47	720.44
3.	1168.90	1166.97	1167.94	1170.47	1170.79
4.	1364.68	1363.72	1365.65	1367.58	1369.50
5.	1450.52	1451.48	1451.48	1452.52	1453.41
6.	1743.71	1744.67	1743.71	1741.78	1738.89
	2938.65	2928.04	2927.08	2928.04	2926.11

degradation process. The frequency values suffer a shift towards lower wave number around 1738cm^{-1} (Table 4.4) at increased temperature of 200^{0}C. This behaviour has been associated with the appearance of saturated aldehyde functional groups (Frankel *et al.,* 1980; Hamilton, 1989), causing an absorption at 1728cm^{-1} which overlaps with the band of the ester functional group. There are also changes in the absorbance value of the band near 1744cm^{-1} but as in preview papers (Guillen and Cabo, 2000) the changes in absorbance ratio in which this band is involved are not as good for monitoring the thermal degradation process as those mentioned.

Oil sample with frequency value lower than 1744cm^{-1} are involved in oxidation process. The more this process is advanced the smaller is the frequency value of this band. Value fewer than 1744cm^{-1} are due to the appreciable proportion of the aldehyde functional groups in the sample. The more quickly the frequency of band shift the lower the value, the faster the degradation process and lower will be the thermal stability of the oil sample.

As indicated in (Table 4.4) at the temperature of 150^{0}C and 200^{o}C there is a marked decreased in the frequency value which suggest the appearance of saturated aldehyde functional groups.

Changes in the Region between 1500cm^{-1} and 1400cm^{-1}

In oil samples there appear a band due to bending vibration of CH_2 and CH_3 aliphatic groups near 1450cm^{-1} (Guillen and Cabo, 1997b). During the degradation process this particular band shows very little deviation from the frequency value of the control 1450cm^{-1} – 1453cm^{-1} (Table 4.4). Therefore, the changes observed in the frequency of these bands during the degradation process are not significant.

Changes in the Region between 1400cm^{-1} and 1300cm^{-1}

The frequency at about 1364cm^{-1} which is due to symmetric bending vibration of the CH_3 groups suffer an increase in the frequency value as temperature increases (Table 4.4) . But the increase

does not suggest a formation or presence of any functional groups. However, changes in the bands are not significant for this region.

Changes in the Region between 1200cm^{-1} and 1100cm^{-1}

The band at about 1168cm^{-1} has been proved to be related to the proportion in the sample of saturated acyl group (Guillen and Cabo, 1997a). The frequency of the bands suffers slight changes during the degradation process (Table 4.4). in the oil samples the frequency relatively remain unchanged at 50^{0}C and 100^{0}C but increase near 1171cm^{-1} at i temperature of 150^{0}C and 200^{0}C; this is suggest possible oxidative process taking place in the sample at the higher temperature..

Changes in the Region between 1000cm^{-1} and 500cm^{-1}

Oil samples have a band at about 720cm^{-1}. This band has been associated with the overlapping of the methylene rocking vibration and the out of place bending vibration of cis-disubstituted olefine (Silverstain *et al.,* 1974; Gunzler and Bock, 1975) during the thermal degradation period the frequency value of this band near 720cm^{-1} (Table 4.4).

The degradation process of the samples shows a pattern corresponding to different stages of a degradation process. The five infrared spectra distinguish with clarity the most important spectral changes produced during the degradation of the pork oil. In this study changes observed in the frequency value of the bands are taken into account.

Deep-frying is one of the most commonly used means of preparing food, although it is also one of the complex and least understood processes. The oil, as well as the food undergoes severe changes due to the high temperature of the process. On the other hand, the changes are difficult to predict because of the high number of variables involved depending on the oil, on the food and on the process conditions. Therefore, the evaluation of the complex mixture of new compounds,

ingested as part of fried foods, and the understanding of its dependence on the main variables of the frying process as of great interest not only for processors and food technologist, but also for nutritionist and consumers.

Table 4.4 summarizes the main alteration that occur during frying resulting from the oil been at high temperature of 50^0C, 100^0C, 150^0C and 200^0C in the presence of air moisture. Hydrolysis occurs due to the presence of moisture in the food. This may involve breaking ester bonds, releasing free fatty acids and diacylglycerols. Additionally, due to the presence of air and exposure to high temperature, oxidation and thermal alteration can take place in the unsaturated fatty acids, leading to modified Triacylglycerols with at least one of the three fatty acyl chains altered. To increase the complexity, the different reactions are inter-related. For examples, free fatty acids originate by hydrolysis are oxidized more rapidly than in the original Triacylglycerols and most of the non-oxygenated new compounds are formed by radical interaction through auto oxidation reactions under low air availability (Velasco *et al.,* 2008).

The chemistry of lipid oxidation at the high temperature of food processes like baking and frying is high complex since both oxidative and thermal reactions are involved simultaneously as the temperature increases, the solubility of the oxygen decreases drastically although all the oxidation reactions are accelerated (Velasco *et al.,* 2008; Frankel, 2005).

CHAPTER FIVE

5.1 CONCLUSION

From the analytical results obtained, from the work done on the pork oil sample. It can be concluded that the pork oil is of good quality because most of the characteristics of the oil which make it edible falls within a responsible range and agrees favourable with values reported in the literature.

For example the unsaponifiable value which can indicate adulteration, contamination and unsuitability falls within good range, this further indicate that the oil sample is of good quality. The result also show that the peroxide value of the oil is very low indicating that the shelve life; the rate at which the oil go rancid is very low if properly kept. Again the saponification values indicate that the oil can be used to produce good quality soap.

The fatty acids of pork oil are dominated by even-numbered chains. Small quantities of odd-numbered fatty acids chain are also detected. Both the saturated and unsaturated fatty acids are present in almost equal proportion oleic acid is the major continent of the unsaturated fatty acids. The oil also shows total absence of linoleic and linoleic acids.

FTIR spectroscopy is a useful technique for monitoring the thermal degradation process of pork oil. The timing, either of the appearance and disappearance of certain infrared bands or of the shifting of the frequency value of some bands could be considered as an indicator of oxidative stability of the sample. All the changes observed in the FTIR spectrum of pork oil sample reflect oxidative stages.

Major elements, trace element and ultra-trace element alike were found in very good concentration which can serve as supplement in nutrition.

On the whole this piece of work seems to be one of the first attempts to determine the quality or to characterise the pork oil in Nigeria. The results obtained are likely to be useful to the generality of the people.

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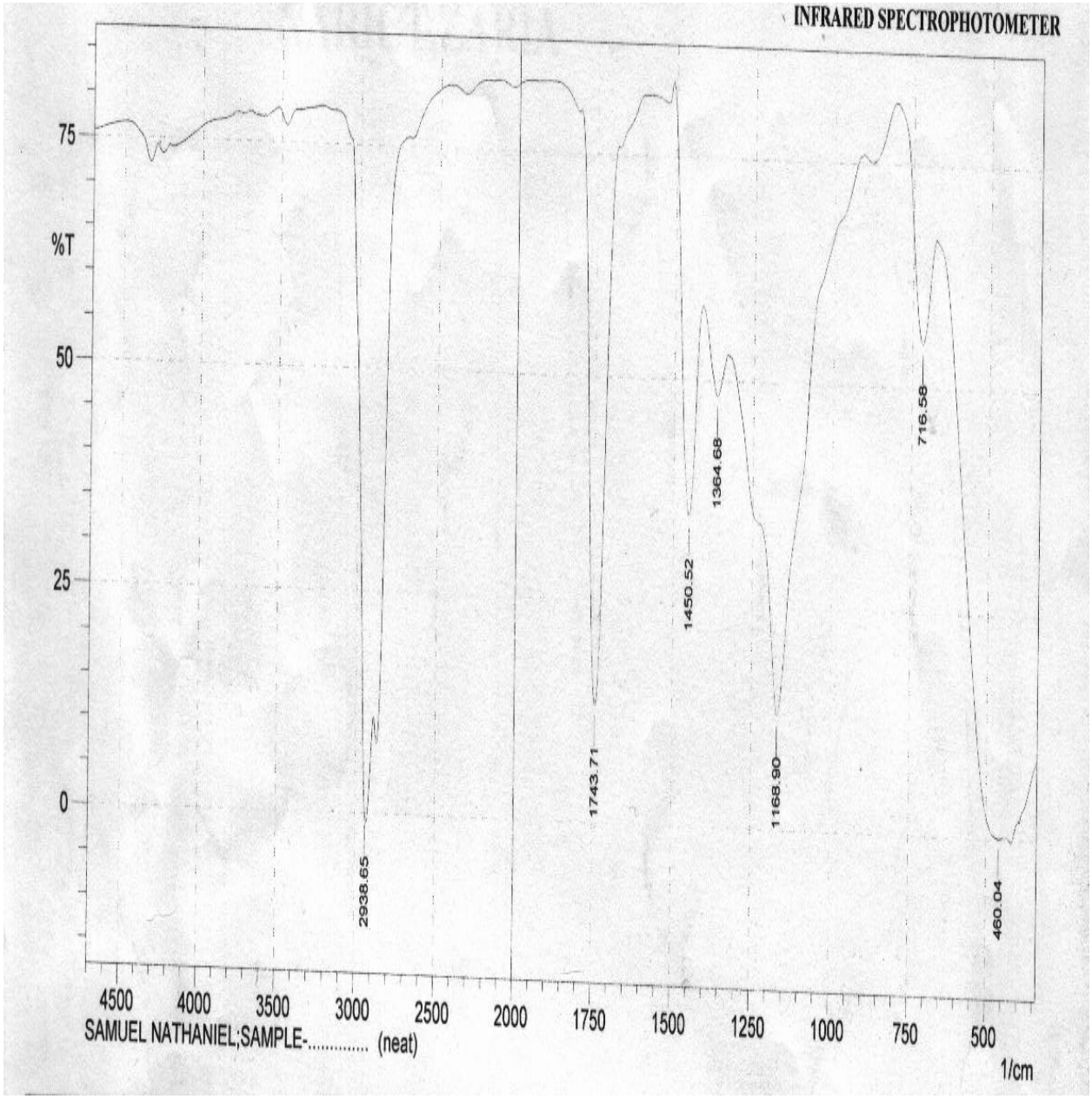
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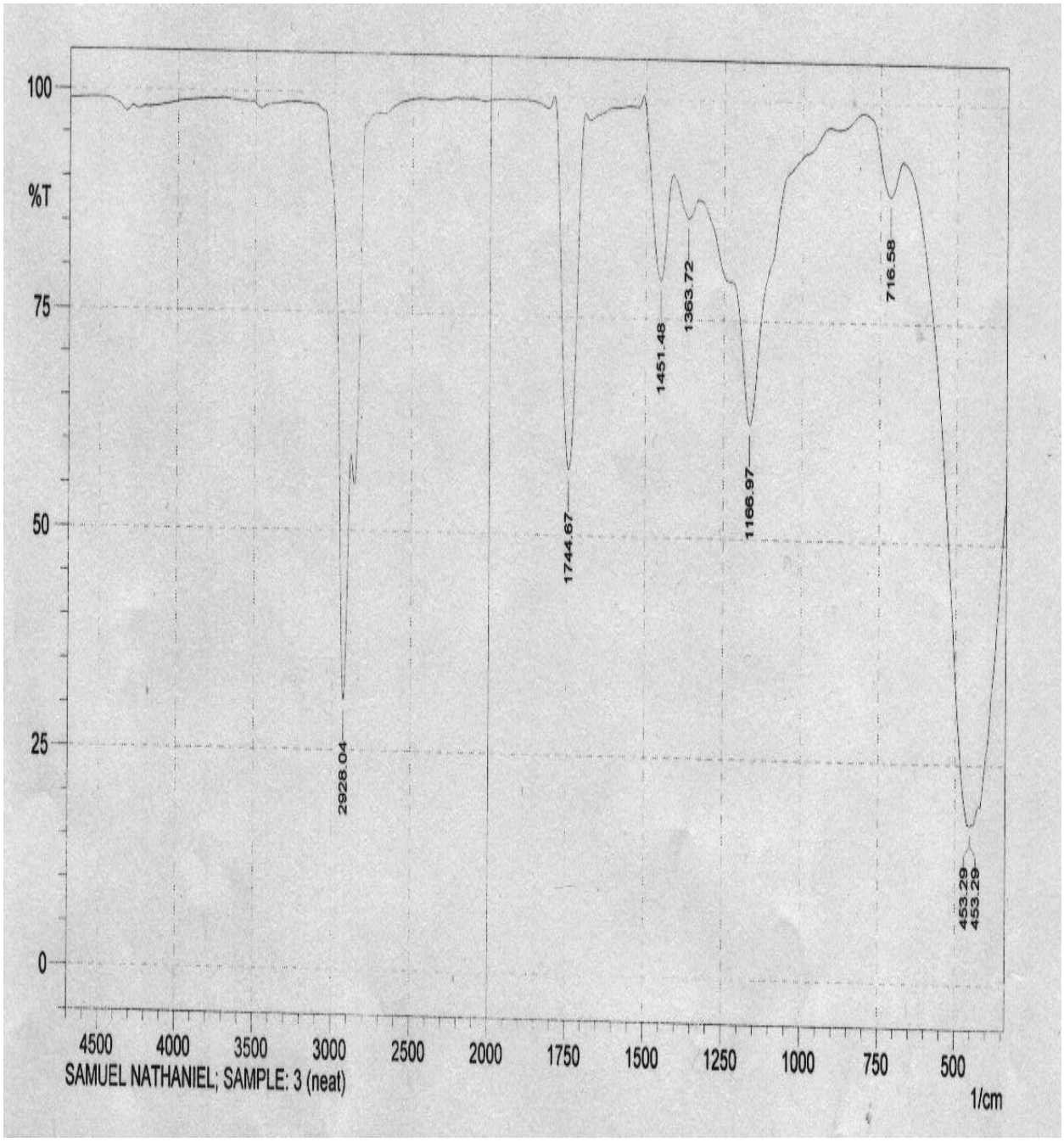
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APPENDIX A



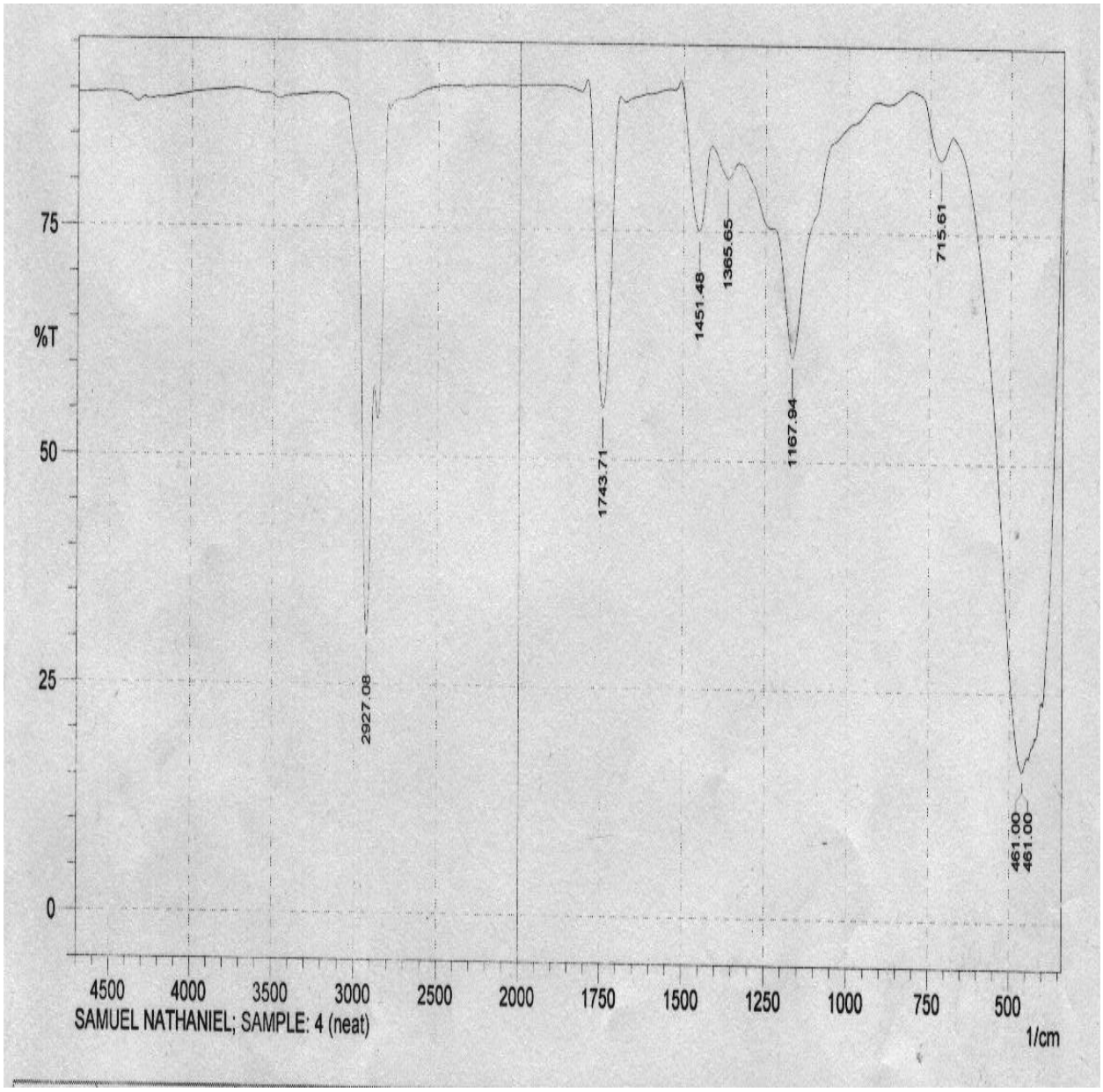
Spectra of Pork Oil (Control)

APPENDIX B



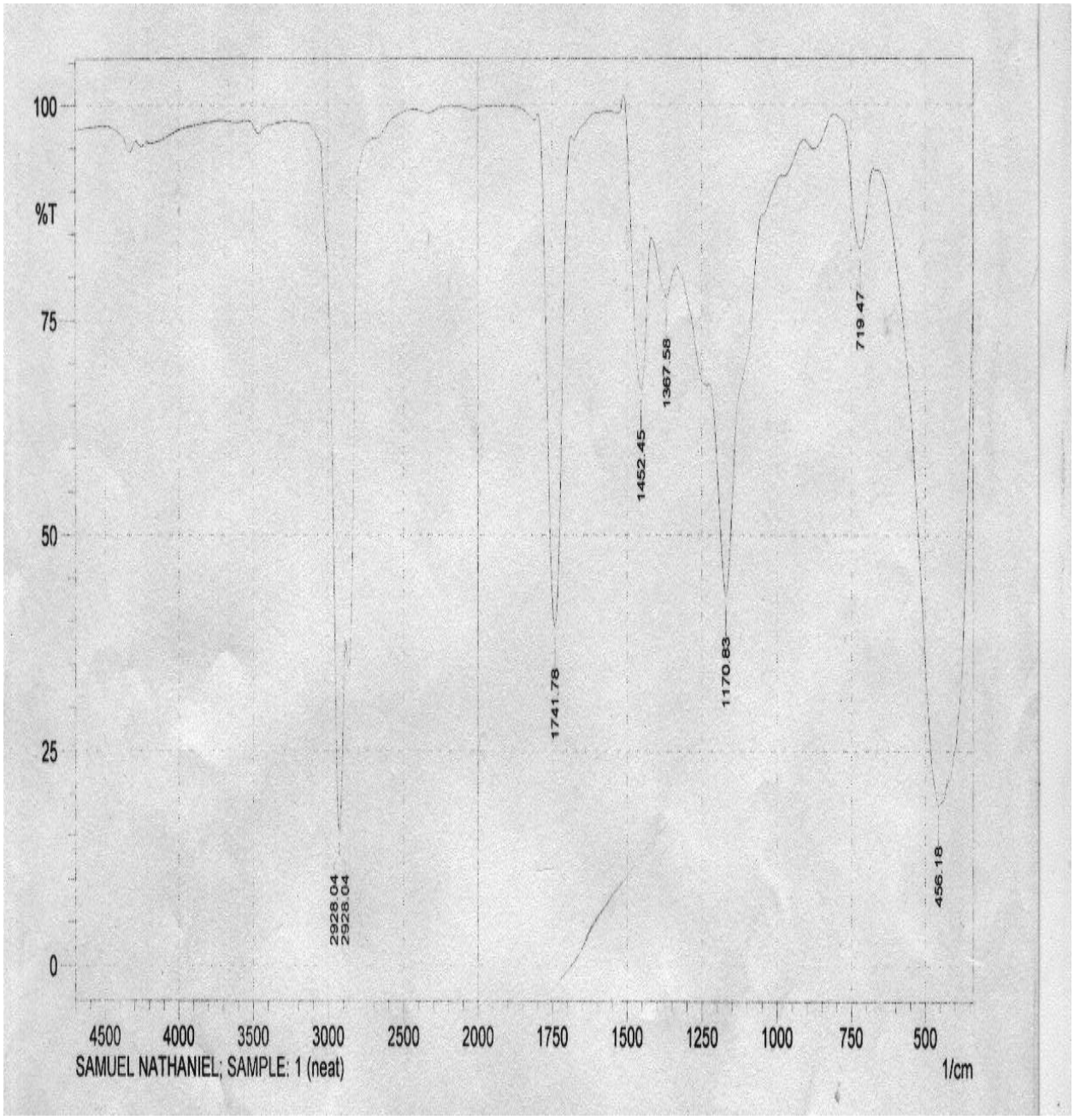
Spectra of Pork Oil Heated At 50^{0}C for 1 Hour

APPENDIX C



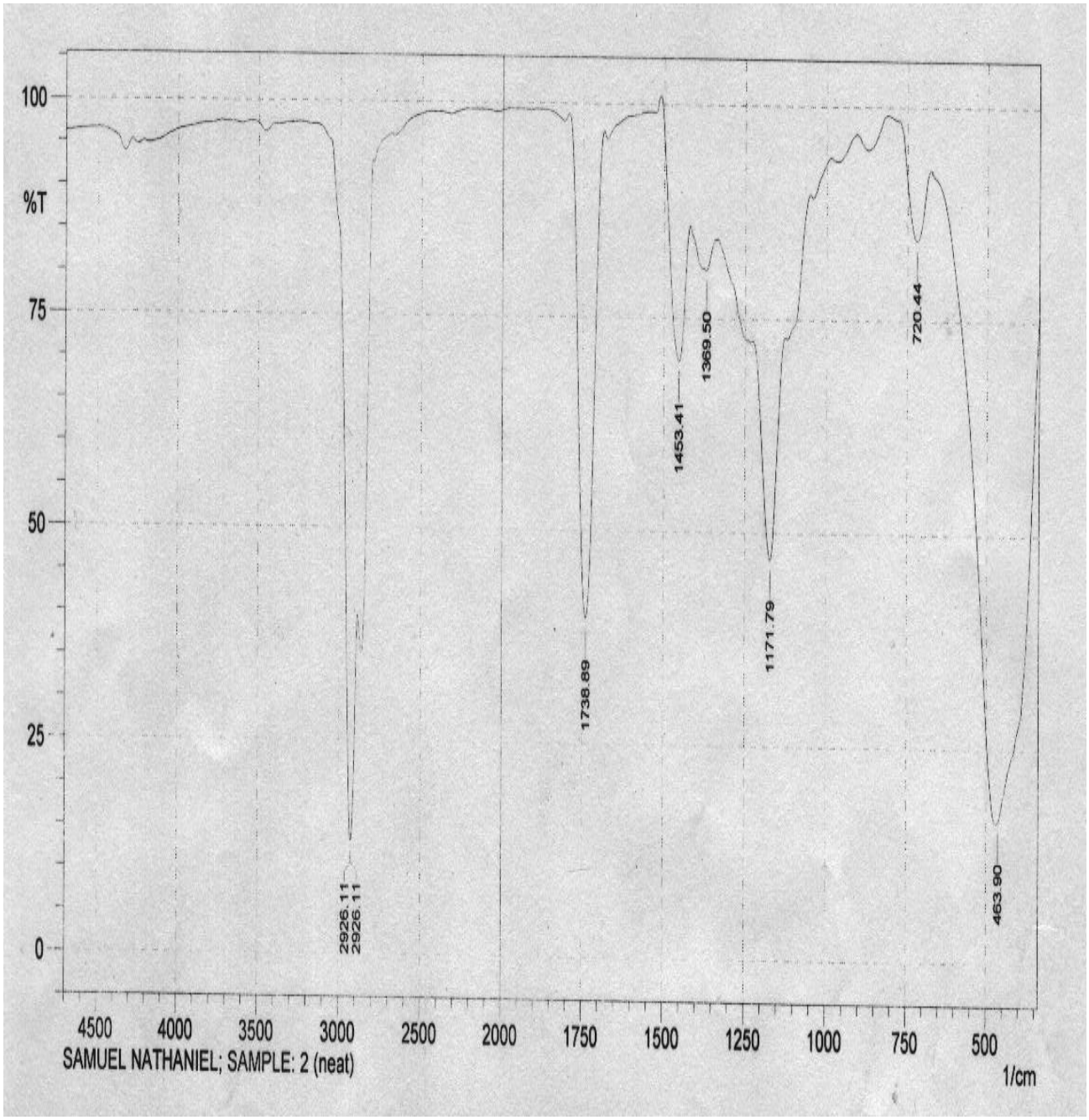
Spectra of Pork Oil Heated At 100^{0}C for 1 Hour

APPENDIX D



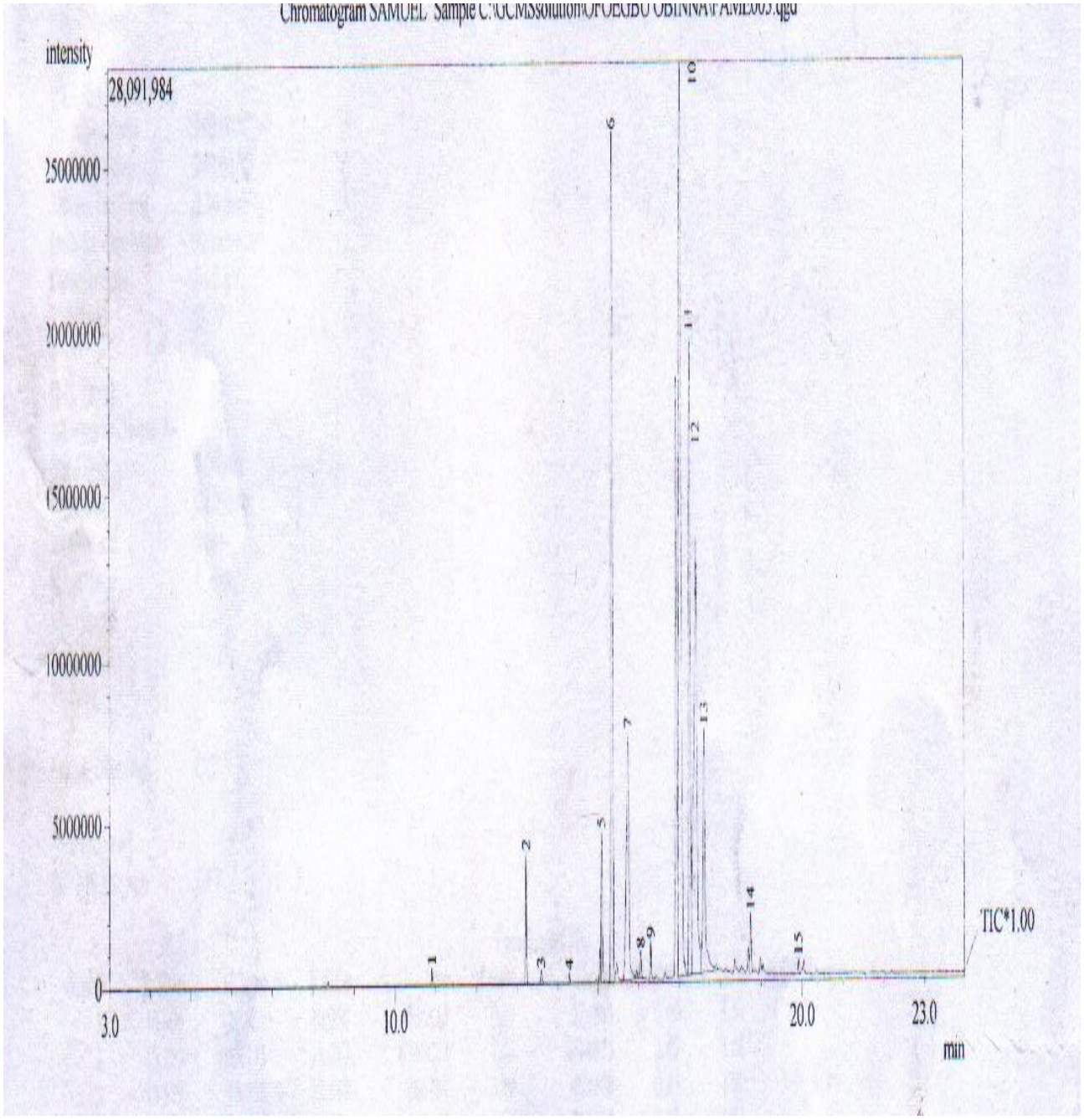
Spectra of Pork Oil Heated At 150^{0}C for 1 Hour

APPENDIX E



Spectra of Pork Oil Heated At 200^{0}C for 1 Hour

APPENDIX F



A Gas Chromatogram of The Pork Oil Showing Peaks of The Constituet Fatty Acids