# ISOLATION, CHEMICAL AND PHYSICAL INVESTIGATION OF OIL FROM AVOCADO FRUIT GROWN IN KENYA

ALLAN MWANGI MUTURI



A thesis submitted in partial fulfilment for the Master of Science in Chemistry of the University of Nairobi

# D E C L A R A T I O N

I declare that this thesis is my original work and has not been presented in any other University. The research was carried out in the Chemistry Department at the University of Nairobi.

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ALLAN MWANGI MUTURI

This thesis has been submitted for examination with our approval as University supervisors

.....

Dr. G.N. Kamau
Chemistry Department
University of Nairobi

.....

Prof. A.H.S. El-Busaidy
Chemistry Department
University of Nairobi.

# D E D I C A T I O N

Dedicated to Mam and Dad, Mary Wairimu Muturi and Allan Muturi Mukubu and the other members of my family.

A C K N O W L E D G E M E N T

It is my pleasure to thank the members of Chemistry department, University of Nairobi for the conducive atmosphere they offered during the course of this project.

I am very grateful to Dr. G. N. Kamau and Prof. A.H.S. El-Busaidy whose supervision and advice helped the work described in this thesis to reach its end. I wish to acknowledge valuable suggestions extended to me by Prof. R.M. Munavu on oils and fats. Special thanks are due to Dr. N. Olembo and Mr. B. Murigi for the immense work of availing avocado fruits. My thanks also go to Misters C. Mirikau, Z.P. Kithinji, M. Njau and K. Said for their technical assistance.

It is gratifying, at long last, to have one year's work within the covers of a single volume. To all who helped in any way, I thank you.

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# A B S T R A C T

Solvent extraction is one of the methods used for extracting avocado oil. The present study examined cold solvent extraction of avocado oil using 8 different solvents. The avocado fruits, fuerte variety, were obtained from Kiambu, Kitale and Murang'a. The edible portion of the undried fruit was ground to a homogeneous paste, mixed with about 200 ml of solvent, and then allowed to stand at room temperature for 24 hours. The oil content from fruits of average maturity was as follows: water 2.67-3.12 %, acetone 0 %, methanol 0 %, n-hexane 8.24 %, cyclohexane 11.21 %, petroleum ether (PE) 13.55 %, ethanol 12.14 % and carbon tetrachloride 6.20 %. The most effective solvent was evaluated on basis of price, availability, oil percentage, recovery and nature of oil. Using petroleum ether (80 % recovery), avocado fruits of varying maturity, yielded 1.83-31.85 % of oil. The various parameters of oil determined include $d_{25}$ 0.85389 $\pm$ 0.00117, $n_D^{25}$ 1.46475 $\pm$ 0.00063, $\eta_{25}$ 19.8957 centipoises, acid number 2.354 $\pm$ 0.264, saponification number 186.761 $\pm$ 3.523, saponification equivalent 300.491 $\pm$ 5.704, unsaponifiable matter 1.49 %, iodine number 87.809 $\pm$ 3.891, boiling point without decomposition 223.574

$^{o}C$, food value 9.8967 $\pm$ 0.1496 kcal/g, enthalpy of vapourization 5.7 kcal $mol^{-1}K^{-1}$ and moisture content 67.306-78.858 %. The Gas chromatography of fatty acid methyl esters gave the following composition: Palmitic 12.257 %, Stearic 3.865 %, Oleic 74.683 % and Linoleic 9.254 %. This study indicates that water decreases and the oil increases with increasing maturity of avocado fruits. Oil content, of fruits picked from the three areas, was not significantly different as shown by t-test at 95 % confidence level. The two G.C. instruments, Perkin Elmer and Gow-Mac packed with either Apiezon-L or Silar 10c, gave similar fatty acid methyl esters. Preliminary work, using avocado oil extracted with petroleum ether in the present work, revealed possible applications, particularly for, skin-related defects, minor cuts, athlete's foot, mild backache and hair recovery on a bald head. Moreover, appropriate period for picking local avocado fruits can be determined by measuring oil content.

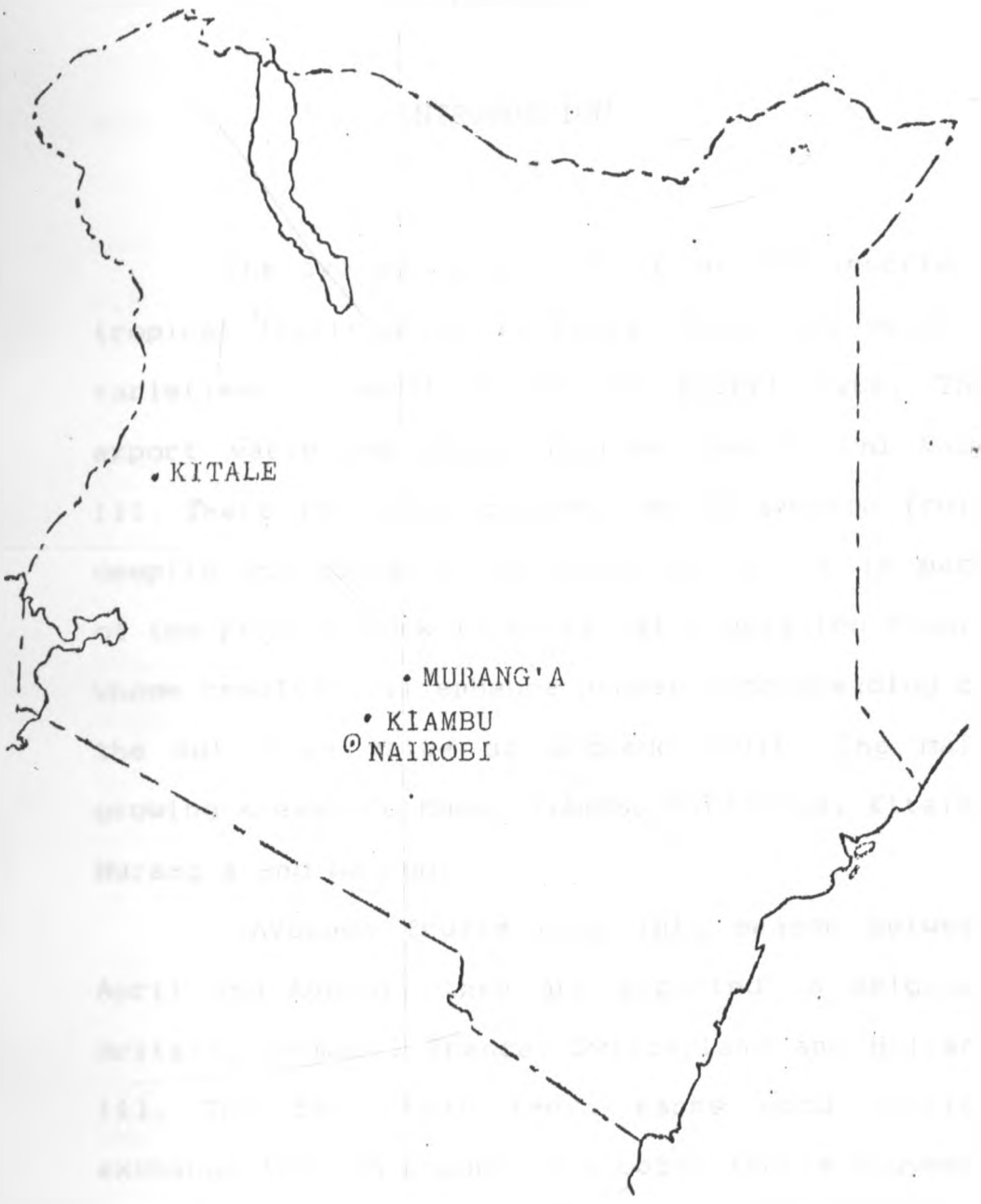



Figure 1. Map of Kenya showing the areas where Avocado Fruits were picked.

# CHAPTER 1

## INTRODUCTION

The avocado fruit is one of the important tropical fruits grown in Kenya. There are several varieties in addition to the export type. The export varieties grown include Fuerte and Hass [1]. There is little consumption of avocado fruit despite its superior nutritive value. It is part of the present work to carry out a detailed study, whose results will enhance proper understanding of the nutritive value of avocado fruit. The main growing areas are Embu, Kiambu, Kirinyaga, Kitale, Murang'a and Nairobi.

Avocado fruits come into season between April and August, they are exported to Belgium, Britain, Germany, France, Switzerland and Holland [1]. The fact that Kenya earns good foreign exchange through export of avocado fruits suggests that a wide ranging study is not only necessary, to monitor the maturity stages, but also to bring about the possibility of local application. Generally, it is difficult to determine the period of harvesting especially for the green-skinned

variety, such as fuerte. Oil content studies will throw more light on this aspect. Recent results show that mature fruits will not only ripen fast but will also have maximum yield of oil [2]. It has been reported that water content decreases with increase in maturity [3].

There is a continuing interest in avocado oil content on the basis of industrial application, such as manufacture of soap, cosmetics [4], medicine [4,5], food content, vitamins, proteins and carbohydrates [6-8]. Preliminary work and application of avocado oil on some individuals locally suggests commercial value, particularly useful for hair setting, treatment of minor cuts and skin-related defects [8-10]. This research project is also aimed at determining the most effective solvent for cold solvent extraction of avocado oil on the basis of price, availability, recovery and oil percentage. The types of single solvents to be tried include water, methanol, n-hexane, cyclohexane, acetone and petroleum ether. Once the most suitable solvent has been identified, it will be used for determination of various parameters that have not been reported previously in this country. These parameters include density, viscosity, refractive

index, iodine value, acid value, saponification value, boiling point and fatty acids composition. The average values of these parameters will be compared with data obtained from other countries such as United states, South America and Phillipines. Any significance attributed to these properties, particularly, with reference to commercial value, medicine, cosmetics and food content will be explored.

## 1:1 LITERATURE SURVEY

There has been several avocado analyses in other parts of the world. Jaffa and Albro found that avocado contains 69.16 % water, 20.10 % oil, 2.08 % protein, 7.40 % carbohydrates and 1.26 % ash. The authors also suggested that the digestibility of avocado oil is equal to that of the other oils [6]. Determination of fat content in new varieties of avocado showed 22 % of fat. However, the percentage of protein yielded by the new varieties of avocado were comparatively low [11]. Several methods of extracting oil gave a colourless oil in nearly every case. This was objectionable in that the oil possessed a bitter taste. The authors suggested that this taste developed during the process of extraction,

because avocado itself does not carry this feature [12]. Avocado oil extracted by digestion in $H_2SO_4$ was too dark for edible purposes. The constants evaluated for this type of oil included: $d_{25}$ 0.9132, n 1.470, acid number 2.8, saponification value 192.6 and iodine value 94.4. The composition of oil with regard to fatty acids was as follows: Oleic 77.3 %, linoleic 10.8 %, myristic trace, palmitic 6.9 % and stearic 0.6 %. The authors suggested that this oil could be used for soap [12].

From Peru, the avocado fruit had 13-20 % oil [7]. Density of this oil at 15 $^{o}$C was from 0.9124 - 0.9139, the solidification point from 7-9 $^{o}$C, the acidity index from 0.412-0.867 %, iodine index from 70.6-76.4, saponification index from 185.1-197.7 and n from 1.4654-1.4662. Presence of vitamin A in avocado oil was demonstrated by chemical and biological assays and presence of vitamin D and E by biological assays. Oil extracted from Phillipine-grown fruits was optically inactive and had $d_{31}$ 0.9181-0.9298, n 1.4682-1.4687, acid number 4.43-5.82, saponification number 193-194, iodine number 95.4 and unsaponifiable matter 1.0-1.1 % [13]. Shannon [14], reported comparison of fat content in

avocado fruit as measured by ether extracts and by the change in refractive index, n, of monochloronaphthalene as increasing amounts of oil were dissolved in it.

Bertoni and Co-workers used Gas-Liquid Chromatography [15] to obtain the following ranges of total fixed fatty acids as methyl esters: Myristic 0.5, palmitic 18.7-30.80, palmitoleic 7.7-20.7, linoleic 9.5-17.7, linolenic 0.4-3.3, stearic 0.2-0.6 and oleic acid 39.9-58.5 %. Gas Chromatography will be used to analyse for total fatty acids in avocado oil found in Kenya. Oreal [16], used avocado oil as one of the components in preparing a cosmetic skin oil, which would not leave a sticky and greasy touch. Attempts will be made, in Kenya, to apply avocado oil to both skin and hair.

## 1:2 OBJECTIVES

The main objectives of the current work include:

i) To provide an up-to-date analytical data of oil content of avocado fruits grown in Kenya.

ii) To report for the first time in this country, a detailed study of avocado oil (extracted with cold solvents), moisture content, composition of

fatty acids and physical parameters (refractive index, heat of vapourization, density, viscosity and boiling point).

iii) To determine the oil content as a function of maturity. This will assist farmers in selecting the right type of fruits for particular needs. For example, an industry interested in extraction of oil would require a fruit with most oil, on the other hand a person on diet would go for a fruit with less oil (less mature fruit).

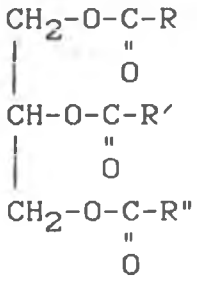
iv) To evaluate the composition of fatty acids, as determined by Gas Chromatography. This will shine more light to local applications as in cosmetic, pharmaceutical industries and in individual use of avocado oil.

v) To determine calorific value of the oil, which would be compared with other fuel values of some common foods; and determine unsaponifiable matter which has been found to contain vitamins and phytosterols. These will be of great importance to all consumers of avocado particularly as a dietary supplement; and

vi) To determine the amount of oil that a person consumes when He/She eats an avocado fruit.

## 1:3 GENERAL PROPERTIES OF FATS AND OILS

Fats are the main constituents of storage in fat cells of animals and plants, and are important food reserves of the organism. Liquid fats are often referred to as oils. By extracting these oils we get substances like corn oil, coconut oil, cottonseed oil, palm oil, tallow, bacon grease, and butter. Fats are carboxylic esters derived from the alcohol, glycerol, $HOCH_2CHOHCH_2OH$, and are known as glycerides. Specifically, they are triglycerides.



A triglyceride.

Where R, R′ and R" are alkyl groups, on this fat. Due to different permutations and combinations possible in the arrangement of R, R′ and R" we get very complex chemical structures of fats and oils. Only acids combining on even number of carbon are present in substantial amounts, the fatty acids are all straight-chain compounds ranging from three to eighteen carbons, with a few

exceptions of $C_3$ and $C_5$ compounds. The molecules are built up two carbons at a time from acetate units, in steps that resemble malonic ester synthesis of an organic chemist, so the even numbers are as a result of biosynthesis.

1:4 MALONIC ESTER SYNTHESIS

$CH_2(COOC_2H_5)_2 + Na^+ {}^-OC_2H_5 \rightarrow CH(COOC_2H_5)_2{}^-Na^+ + HOC_2H_5$

stronger acid — Ethylmalonate (Malonic ester)

Sodiomalonic ester

weaker acid

$$CH(COOC_2H_5)_2{}^-Na^+ + RX \longrightarrow RCH(COOC_2H_5)_2 + Na^+X^-$$

ethyl alkylmalonate (alkylmalonic ester)

$$RCH(COOC_2H_5)_2 \xrightarrow[\text{heat}]{H_2O,\ OH^-} RCH(COO^-)_2 \xrightarrow{2H^+} RCH(COOH)_2$$

$$RCH(COOH)_2 \xrightarrow{\text{heat, } 140\ ^oC} RCH_2COOH + CO_2$$

A mono substituted acetic acid

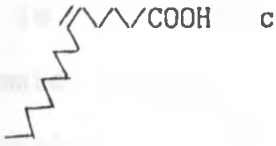
($CO_2$ is readily released)

Configuration around the double bonds is almost invariably cis, rather than trans [17]. This stereochemistry seems trivial but is actually of vital biological significance because it lowers the melting point. The closer the molecules of fat fit the stronger the intermolecular forces, and the higher the melting point. As shown below straight saturated chains and trans-unsaturated acid chains would fit together rather well, but

cis-unsaturated acid chains have a bend at the double bond, and fit each other badly. The effect of cis-unsaturation is lowering of melting point.

/\/\/\/\/\/\/\/\/COOH saturated fatty acid.

\/\/\/\//\/\/\/\/COOH trans-unsaturated fatty acid.



cis-unsaturated fatty acid.

Different fatty acid structures in nature must be due to enzymatic reactions resulting in accummulation of a particular molecular species. Early hypothetical fatty acid biosythesis pathways have been disproved by experiment. Like direct condensation of three hexose units to give a $C_{18}$ acid during condensation of sugar to fat does not occur, nor do aldol condensations involving acetaldehyde or pyruvic acid [18].

## 1:5 OILS AND FATS IN PLANTS

In production of oilseeds, no ecological area takes precedence over others. Production of dry matter in plant population is, of course, correlated to temperature, sunshine and other climatic conditions, however fat, the most highly condensed form of energy, is no more frequently stored in seeds of tropical plants than in those from temperate regions. Deposition of oils and fats is universal, not limited to specific taxonomic groups in plant tissues. Table 1 illustrates the distribution of oil crops on the basis of taxonomy and climate [19].

Table 1. Distribution of some main oil crops on a taxonomic and climatic basis.

| SPECIES | FAMILY | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| Soyabean | Leguminosae | + | - | - | - | + | - | + | - |
| Sunflower | Compositae | - | - | - | - | + | (+) | + | + |
| Groundnut | Leguminosae | - | + | + | - | + | + | + | - |
| Cotton | Malvaceae | - | - | + | +I | + | + | - | - |
| Maize | Gramineae | (+) | - | (+) | + | + | +I | + | - |
| Safflower | Compositae | - | - | + | - | - | + | - | + |
| Coconut | Palmae | + | + | (+) | - | - | - | - | - |
| Castor Oil plant | Euphorbiaceae | - | - | + | - | (+) | - | + | - |

() Limited cultivation possible, I Irrigation necessary, 1. Tropical rain forest, 2. Tropical monsoon, 3. Tropical savanna, 4. Dry tropical i.e. steppe and desert, 5. Humid subtropical, 6. Dry subtropical i.e. mediterranian, 7. Humid temperate, 8. Dry temperate.

## 1:6 ECONOMIC VALUE OF OILS AND FATS

Vegetable fats and oils have long been used for various purposes. Between 1938 and 1972 the world fat production more than doubled, major increases occurring with the edible fats [20]. Inspite of rapid growth in world population the annual per capita improved by about 2 % each year. Annual consumption had a world average in the order of 8.8 kg per capita within which we had actual defficient countries like India (with a mean of 5 kg) through regions of surplus consumption. Western Europe is an example of regions of surplus consumption with a mean of about 25 kg [20].

Relative share of vegetable oil from total edible fats and oils increased from 55.5 % before World war II to about 64 % in 1972. This trend originated from rising demands for polyenoic fatty acids (especially Vitamin F) in the food of populations with higher income and more sophisticated tastes. Otherwise industrial use of fats and oils was stable at an average of 15 % of the world production. Soaps no longer dominate this area, new uses created for fats and fatty acids include production of resins and plastics,

paints and lubricants, rubbers and coatings, cosmetics and pharmaceuticals [20]. As shown in table 2, the consumption of most oils increased from 1938-1972.

Table 2. World production of oils and fats (1000 metric tons in oil equivalent).

| | 1938 | 1958 | 1968 | 1972 |
|---|---|---|---|---|
| Liquid oil | 7787 | 11750 | 18975 | 22315 |
| Palm type oil | 2659 | 3295 | 4115 | 4955 |
| Industrial type oil | 1559 | 1550 | 1810 | 1780 |
| Animal fats | 8752 | 11125 | 14225 | 14890 |
| Marine oil | 830 | 840 | 1180 | 1305 |
| World total | 21587 | 28560 | 40305 | 45245 |

Exclusively for Industrial purposes, except for a limited amount of oils, most of the necessary raw material is from unpalatable or spoiled portions of edible plant fats or as by-product of their processing (e.g. de-acidification of salad oils). There are three types of fatty acids namely (i) Major, which forms the major constituents of natural lipids, (ii) Unusual, these are fatty acids which occur as the end product of metabolism and (iii) Minor fatty

acids, which are of related structures and occur as minor constituents.

In higher plants fatty acids accummulate mainly as triglycerides in seed or the fleshy part of the fruit, which acts as food stores. For the rest of the plant (leaf, stem and roots) we have fatty acids but as glycolipids and phospholipids. Table 3 illustrates various types of fatty acids.

Table 3. Names of some fatty acids.

| Common Name | Systematic Name |
|---|---|
| | Minor (saturated) |
| Myristic | Tetradecanoic |
| Palmitic | Hexadecanoic |
| Lauric | Dodecanoic |
| Stearic | Octadecanoic |
| | Major (unsaturated) |
| Oleic | Cis-9-Octadecanoic |
| Linoleic | Cis-9,Cis-12-octadecanoic |
| Linolenic | Cis-9,Cis-12,Cis-15-Octadecanoic |

These seven fatty acids accounted for 94 % of those in the worlds commercial vegetable fats by 1969. Frequently, oleate, linoleate and palmitate predominated [17].

Table 4. Structures and percentage world distribution of fatty acids.

| World distribution % | common name | symbol | stucture |
|---|---|---|---|
| 4 | Lauric | 12:0 | $CH_3[CH_2]_{10}.CO_2H$ |
| 2 | Myristic | 14:0 | $CH_3[CH_2]_{12}.CO_2H$ |
| 11 | Palmitic | 16:0 | $CH_3[CH_2]_{14}.CO_2H$ |
| 4 | Stearic | 18:0 | $CH_3[CH_2]_{16}.CO_2H$ |
| 34 | Oleic | 18:1(9c) | $CH_3[CH_2]_7.CH{:}CH.[CH_2]_7.CO_2H$ |
| 34 | Linoleic | 18:2(9c12c) | $CH_3[CH_2]_3.[CH_2.CH{:}CH]_2.[CH_2]_7.CO_2H$ |
| 5 | Linolenic | 18:3(9c12c15c) | $CH_3[CH_2.CH{:}CH]_3.[CH_2]_7.CO_2H$ |

The remaining 6 % is mainly composed of minor fatty acids.

United States, United Kingdom and Argentina are examples of countries with highest per capita consumption of oils and fats as depicted in table 5.

Table 5. Per capita consumption of oils and fats for 1972-74.

| Country/Region | Per capita oil consumption (g/day) | | |
|---|---|---|---|
| | Vegetable | Animal | Total |
| World | 26.9 | 34.1 | 61.0 |
| Africa | 29.5 | 10.9 | 40.4 |
| Angola | 25.1 | 9.0 | 34.1 |
| Chad | 28.1 | 8.4 | 36.5 |
| Congo | 22.1 | 4.6 | 26.7 |
| Kenya | 20.3 | 11.4 | 31.7 |
| Mozambique | 26.1 | 5.2 | 31.3 |
| South America | 27.0 | 30.2 | 57.2 |
| Argentina | 43.5 | 66.2 | 109.7 |
| Guatemala | 22.7 | 13.7 | 36.4 |
| Peru | 22.8 | 26.5 | 49.3 |
| Asia | 19.9 | 13.4 | 33.3 |
| Indonesia | 15.3 | 14.5 | 29.9 |
| India | 21.6 | 7.5 | 29.1 |
| Phillipines | 23.7 | 2.8 | 26.5 |
| Europe | 44.8 | 86.6 | 131.4 |
| United States | 47.3 | 119.1 | 166.4 |
| Canada | 28.7 | 124.5 | 153.2 |
| United Kingdom | 39.5 | 105.5 | 145.0 |
| Less developed countries | 24.3 | 12.2 | 36.5 |
| More developed countries | 45.5 | 88.4 | 133.9 |

F.A.O. (1977) [21].

## 1:7 AVOCADO FRUIT

Fuerte and Hass [1] are the export varieties grown in Kenya. Fuerte is extremely rich in oil. Some cultivars contain as much as 30 % [22]. However, the oil content varies with location, for example, Fuerte contains about 25 % in California but only 13-15 % in Florida. Many cultivars are hybrids. Fuerte, for example, is considered to be a natural Guatemalan-Mexican hybrid [22]. Avocado or percea americana is a member of the laureceae family, a tropical evergreen tree of about 20 m tall [23]. It yields fruits the size of plums, weighing upto 250 g , while some giant types yield fruits over 1 kg in weight. The fruit varies in colour from dark green to yellow or purplish when ripe. Avocado is prized for its high oil content and rich nutty taste. It is mainly eaten raw and may be served as half fruits sprinkled with lemon juice, sugar, salt or vinaigrette dressing. The cavity left after removal of the seed is often fitted, for example, with pawn mayonnaise [23]. Although avocado has been given the name "poor mans butter", a name that is obviously only

appropriate in tropical countries, in Europe as an imported vegetable it is very expensive. Kenya earns good foreign exchange through export of avocado fruits.

In April 1989 the prices were as high as Ksh.55 per box or Ksh.13,750 per tonne, which has 250 boxes. However, due to some farmers neglecting their trees by failing to apply required fertilizers, some fruits did not develop the quality deep green colour. Also due to fungus infection that intensifies when avocados are subjected to wet and refrigerated conditions during transportation, by the end of July, the volume of avocado exports had fallen by about 700 tonnes compared with the same period a year before. This reflected a loss of nearly Ksh.10 million where the export volume had dropped to 2721 tonnes compared to 3383 tonnes in 1988. This is according to a report by the Managing Director of Horticultural Crops Development Authority (HCDA), Mr. Martin Mulandi [24].

It is generally difficult to determine the period for havesting avocados especially the green-skinned varieties like fuerte. The season of maturity varies slightly from year to year for each variety, according to time of bloom, locality

and seasonal conditions during growth. To create a market and maintain the demand necessary for the profitable growing of avocados, it is imperative that the consumer is offered a fruit which has attained maturity sufficient to be palatable upon ripening. In order to obtain best returns from avocados, the farmer should know when to pick, how to pick and of course how to market them.

More sophisticated countries like Israel, America [California] and Australia maturity indices are determined by chemical analysis of the oil content of the fruit which should range from 8-15 % depending on the variety [1]. However, this method is not practised here in Kenya but there is scope for the future. It is the purpose of the current research to establish similar indices. In Kenya, there has not been complete characterization of avocado fruits in terms of physical and chemical properties of the oil. This work is aimed at exploring these properties for locally grown varieties and comparing them with those from other countries like Phillipines, Latin America and U.S.A. As a result of the present research project, detailed analytical data pertaining to avocado oil, from fruits grown in Kenya, will be reported for the first time.

Comparison of oil content in avocado from different parts of the country will be done. The avocado pears grown locally for edible purposes are not rich in oil, which constitute about 5 % of the whole fruit, while certain central American varieties grown experimentally by the Horticultural Research Station at Thika, have a content of 11-12 % [25].

## 1:8 OIL EXTRACTION PROCESS

Different methods are employed for extraction of avocado oil. These include:

i) Hydraulic power and considerable pressure.

ii) Solvent-extraction, in which, the meal or pulp is subjected to the action of hot solvent, or in presence of water, until liquid fat escapes from the enclosing cells and floats to the top of the mass. The choice of a method is determined by various considerations with regard to destined use of the resulting fat [26].

iii) Macerating followed by centrifuging. Oil can be liberated only if the fruit's cell structure is broken up, this is done by pounding in a ball mill. A solid-bowl centrifuge has proved to be the only effective way of separating the pulp's solid matter from the other two constituents, water and

oil. Once the solid has been removed, water and oil can be separated in a centrifugal separator. In certain more complicated types of centrifuge, it is possible to separate the solid, water and oil in one operation.

iv) Drying followed by pressing. Drying in an oven or a drum drier breaks up the structure and turns the sloppy pulp into a firm solid. In this form it is suitable for squeezing out the oil in a hydraulic press. This is mild drying to remove water which has low boiling point [27]. Less oil is obtained by this method compared with the others, because about a quarter of it is retained in the mash. Nevertheless, the oil percentage on dry weight is higher than that of the solvent extraction.

v) Drying followed by solvent extraction. Oil in the dried pulp can also readily be extracted with an appropriate solvent. This process requires greater care than the others, not only are some of the solvents volatile and highly inflammable, but also they give the oil a disagreeable odour which has to be removed [25].

Avocado oils of different colours were obtained when the fresh mash was ground to a homogeneous paste and mixed with 0.5-3 % unslaked

CaO. The mixture was allowed to stand for 15-30 minutes if green oil was desired and for an hour or more if yellow oil was desired [28]. The oil was separated with a filter press, floatation with $H_2O$, centrifugation or solvent extraction. Oil produced by any of these processes is dark and impure; it has to be refined and bleached. Attempts had been made to obtain the oil by applying an electric field to a suspension of avocado pear pulp diluted with water. A wide variety of methods have been tested, with a view to producing clear, odourless and free from fatty acid impurities with a minimum of oil loss in the process. Refining with caustic soda and subsequent bleaching with activated earth is both effective and economical; provided that the oil has been prepared from fruit pulp which is in good condition. If the fruit or pulp has been allowed to deteriorate in the first stages of whatever extraction procedure, the resulting oil can only be refined to an attractive product by a very wasteful process [25]. The manner in which the fruit deteriorates needs to be investigated. It is the aim of the current work to investigate various solvents on the basis of purity, economy and attractiveness/neatness of the oil. The best

solvent system obtained should go a long way in determining the precise oil content one attains after eating a whole single fruit.

Out of the eight solvents utilized in cold extraction method (current work), Petroleum ether gave the highest oil yield (see section 3:0). The oil produced by this method was gold-like in colour and had its own natural smell. The method that met the above requirements, involved petroleum ether cold solvent extraction. It had been tailored in persuit of a simple and low cost method.

## 1:9 COMPARISON OF OIL CONTENT WITH THE FRUIT MATURITY

In order to obtain the highest amount of oil from a fruit it is important to know the degree of maturity as suggested by several researchers, this method is being used by farmers in Israel, America (California) and Australia [1]. This would serve as a guide to farmers with regard to the right time for harvesting avocados. Analysis indicate that the amount of water decreases with increasing maturity of the fruit [3]. It is the objective of the present research project to study this phenomenon for avocado fruits grown in Kenya.

Avocado oil extracted from different varieties of the fruit in different provinces of Argentina ranged between 3.9-27.4 % [15]. This type of work has not been done in Kenya. At this point of time it is imperative that similar data be reported for avocado fruits grown in Kenya. This work will investigate differences observed in different regions and compare with data from other countries. Stoneback and Calvert found that the proportion of chemical constituents changed according to the degree of maturity for each variety of fruit [29]. Variation was particularly noticeable with regard to the fat content. Traub and co-workers reported extraction of avocado oil by digesting the ground sample in $H_2SO_4$ for 30 minutes at 55 $^{o}C$. The amount of oil was determined in a bobcock bottle [30]. Avocado oil (80 %) extracted from dried fruits was too dark for edible purposes [12]. The refined and bleached oil was still dark. The authors suggested soap making as a possible use, implying a possible limitation on the use of avocado oil, particularly for pharmaceutical purposes. Franzke and co-worker [8] investigated the moisture content in avocado fruit. According to work by Montano et al. [31], the amount of oil extracted increased with

decrease in moisture content of avocado flesh. Agricultural product constituents, usually depend on place of origin. Recent results show that mature fruits will not only ripen fast but will also have maximum yield of oil [2]. This observation will be explored in the current work.

## 1:10 FREE FATTY ACIDS

Fatty acids in oils and fats occur either as free fatty acids or as esters, where they are bound to the triglycerol molecule. Acid value, therefore, is an indication of the amount of free fatty acids present in a sample. Results of this determination are often expressed as free fatty acids, as percent oleic, percent palmitic or percent lauric acid in the sample, depending on the kind of oil or fat being handled. Expressing results as acid value is preferrable, since it involves no assumptions concerning composition of the acids present. Free fatty acids as oleic acid is numerically approximately half the acid value. Percentage of free fatty acids, calculated as such implies a knowledge or assumption of the free fatty acids molecular weight (Mw). If this is assumed (Mw), then the percentage of free fatty acid present is: Acid value x Mw/561. Mw, may be

taken as that of the fatty acid present in greatest amount or may be deduced from the saponification equivalent. In most cases [26] it suffices to calculate the free acidity for technical purposes in terms of oleic acid (equivalent 282), except in special cases such as the nut oils (where lauric acid, equivalent 200, or a mean equivalent of 220 may be substituted), palm oil (free acidity usually calculated to palmitic acid, equivalent 256) and castor oil (ricinoleic acid, equivalent 298). Two methods are commonly used for free fatty acids determination:

(i) If considerable amount of free fatty acids is present, this determination can be carried out at the same time as that of saponification value, by warming the neutral alcohol suspension of the fat until all is liquid. Adding alcoholic potash by titration in the presence of phenolphthalein until an almost permanent pink colour is produced, then proceeding to add the remainder of 25 ml of alkali from the burette (see section 2:7).

(ii) If the proportion of free fatty acids is small, the volume of alkali required in the foregoing method is too small to afford accurate results. The procedure is described in section 2:3.

## 1:11 DENSITY MEASUREMENTS

The density of fats and oils at temperatures which they will be stored, handled and processed need to be known for design of tanks, piping and processing equipment for these materials. Besides their obvious utility in chemical engineering, data on density or specific gravities of fatty oils has been used in connection with analysis and identification of oils [32]. Differences in specific gravity among various natural fatty oils are not great enough to make this property a sensitive identifying characteristic. Densities of fats, in their solid forms and changes in density with change in temperature of pure glycerides, are very useful in the study of phases in which these materials exist and of transitions from one phase to another. The most common density determination method consists of finding the weight occupying a known volume of a vessel. It is obviously impracticable to determine the volume from the geometry of a vessel. Instead, the vessel is calibrated in terms of the weight of pure water which it will hold [33, 34].

Pycnometers are preferably made of some resistance glass, having a low coefficient of

expansion, like, Pyrex, Vycor or fused quartz. In this determination the type known as Gaylussac pycnometer or the "Weld specific gravity bottle" will be used. It is a convenient and versatile model capable of an accuracy of about $1x10^{-5}$. It can easily be used for solids as well as liquids because of its large neck opening [33]. Volume depends somewhat on amount of pressure exerted on the ground joint.

1:12 CONTENT OF UNSAPONIFIABLE MATTER

The description of unsaponifiable matter is a misnomer, since the method really determines the amount of neutral (non acidic) material, substances that are insoluble in water and present either free or combined with fatty acids in the original material. In situations where large amounts of higher fatty alcohols or sterols are present as esters (e.g. sperm oil, beeswax, carnauba and other waxes or wool greases), then combined alcohols in question are registered as unsaponifiable matter in this estimation. This property in addition gives a measure of any free sterols or other water-insoluble alcohols, hydrocarbons or other neutral organic compounds

being examined. Vitamins A, D and E constitute part of the unsaponifiable matter.

## 1:13 DETERMINATION OF IODINE VALUE (I.V.)

The iodine value is a reflection of the degree of unsaturation in a sample. Many methods for determining I.V. have been deviced. The results are commonly expressed as percent iodine absorbed, i.e., grams of iodine per 100 grams of sample, whether or not the halogen used is actually iodine. Mostly used in United States are the Wijs and Hanus methods. The difference is very small with fats of low I.V., but often it is 2 % or more with oils having I.V. of 100 or more, with conjugated oils, on the other hand, Hanus method gives considerably higher values, but more variable ones, than Wijs method [32]. Wijs values obtained with non-conjugated oils are believed to be very close to those corresponding to actual unsaturation. Pure oleic, linoleic and linolenic acids and their esters give the theoretical values when examined by Wijs method [35, 36].

1:13:1 PREPARATION OF STARCH SOLUTION

Starch is required for the determination of iodine value. Starch as ordinarily supplied consists of "grains" composed of starch "granulose" enclosed in an envelope of starch "cellulose". It is accordingly necessary to use boiling water in making "starch paste" in order to rapture the cellulose envelope and set free the granulose. Starch paste is therefore prepared by rubbing starch with cold water to make a cream, and then pouring this cream into boiling water so that it thickens as the colloidal granulose becomes diffused through the water. For the present purpose the starch paste, if made in this way, should be dilute. Objection to this paste, however, as a reagent for iodine, is that fragments of cellulose remain in the paste, and that the granulose attached to them parts with its iodine less readily than free granulose in the colloidal solution. Consequently the starch losses in sensitiveness, and is less satisfactory for use than solution starch. The solution may, however, be filtered when dilute.

Solution starch is made by filtering a dilute solution of starch so as to remove cellulose fragments, then precipitating the starch

solution by alcohol. The white powder so obtained is collected and dried, and when boiled with water readily dissolves, yielding a liquid which gives a clear deep blue colour with a drop of iodine solution. On account of this superior sensitiveness, soluble starch should always be employed in preference to the ordinary kind. The solution should be dilute, since a very small quantity of starch is required to react with iodine [34].

1:13:2 PREPARATION OF SODIUM THIOSULPHATE SOLUTION

Iodine and sodium thiosulphate interact in solution in the following manner:

$$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

The reaction produces sodium iodide and sodium tetrathionate; and amount of iodide present in any solution, whether liberated from an iodide by an oxidizing agent or not, can be estimated accurately by this reaction because of the delicate test for free iodine with starch, a deep blue colour being produced by combination of very small amounts of these two substances in the cold. Iodine is much more soluble in a solution of potassium iodide than in water, on the account of

formation of polyiodide, eg., $KI_3$, this compound dissociates, however, when the solution is much diluted, so that iodine is precipitated. The equation given above shows that 2 atoms of iodine react with 2 molecules of sodium thiosulphate; from this it follows that a decinormal solution of the latter is decimolecular. Crystallized sodium thiosulphate is $Na_2S_2O_3.5H_2O$ with molecular weight of 248.2; therefore a decinormal solution of this reagent contains 24.82 g per litre. The salt can be obtained chemically pure, but its solution is decomposed by carbon dioxide dissolved in water, liberating thiosulphuric acid from which sulphur is slowly decomposed. It is well, therefore, to prepare a solution of strength slightly more than decinormal, and to allow it to stand for a few days before standardization to give time for its interaction with dissolved carbon dioxide.

Decinormal potassium dichromate solution liberates its equivalent of iodine from acidified potassium iodide, and sodium thiosulphate solution can be standardized by a titration against liberated iodine. This is expressed by the following equations, in which sodium thiosulphate is brought into relation with dichromate through the medium of iodine.

$$K_2Cr_2O_7 + 14HCl + 6KI \rightarrow 8KCl + 2CrCl_3 + 7H_2O + 3I_2$$

$$3I_2 + 6Na_2S_2O_3 \rightarrow 6NaI + 3Na_2S_4O_6$$

1:13:3 PREPARATION OF DECINORMAL POTASSIUM DICHROMATE SOLUTION

In Iodine Value (I.V.) determination it is required that sodium thiosulphate standard solution be used. This necessitates standardization of the solution using standard potassium dichromate. Ability of potassium dichromate as an oxidizing agent renders it a useful reagent in volumetric analysis. Oxidation of the dichromate involves a reduction of chromium from the state represented by $CrO_3$ to that represented by $Cr_2O_3$. Thus the reversible reaction: $2CrO_3 \rightleftharpoons Cr_2O_3 + 3/2O_2$. Further, a salt of the acidic oxide $CrO_3$, a dichromate, is formed when a base is present to promote its formation, and a salt of the basic oxide $Cr_2O_3$ i.e., a chromic salt, when an acid is present. $K_2Cr_2O_7$ contains 3 atoms of available oxygen, which are equivalent to 6 atoms of hydrogen, the equivalent weight of potassium dichromate is one-sixth of its molecular weight.

Therefore, 1 litre of a decinormal solution of this reagent contains one-sixtieth of a gram-molecule. This is one-sixtieth molar (4.903 g). The salt should be recrystallized, and regarded as pure enough for analysis [34].

## 1:14 REFRACTIVE INDEX (R.I.)

Refractive power of fats and waxes varies somewhat widely and is chiefly governed by the proportion and degree of unsaturated matter present. In fat industries it provides a most rapid means of following the progress of hydrogenation of a fat except actual continuous measurement of the hydrogen absorbed are not practicable as a rule on large scale. Hydrogenation of oils makes them hard, in fact the process is called hardening. The defination given by some textbooks and handbooks, which state that refractive index is a ratio of the velocity of light in vacuum to the velocity of light in the medium being measured, need to be modified or supplemented. Values of refractive index n of liquids are given on the basis of air rather than vacuum as the reference medium [32]. The difference in the two values is hardly negligible,

since for ordinary fatty oils it would be about 4 in the fourth decimal place, or the change produced by a temperature change of 1 $^{o}C$.

Temperature and pressure of air need not be specified except for determinations of extremely high precision since changes in air surrounding a refractometer have a negligible effect on the determination of refractive index of a liquid.

Important variables that need to be specified are temperature and wavelength of light used, for example $n_{D}^{25}$. If wavelength is not specified it is assumed to have been that of the D-line of sodium. 25 here stands for temperature of the water jacket, it could be higher say 40 or 60 $^{o}C$ for solid fats. Both the R.I. and the density values are useful for checking on the purity of a compound as well as identifying an unknown. Impurities have significant effect on these physical constants [37].

1:15 SAPONIFICATION VALUE

This is the number of milligrams of KOH required to hydrolyse 1 g of a fatty material. Data expressed in this form approach to simple numerical indices which are extremely useful to analysts who are largely occupied with the

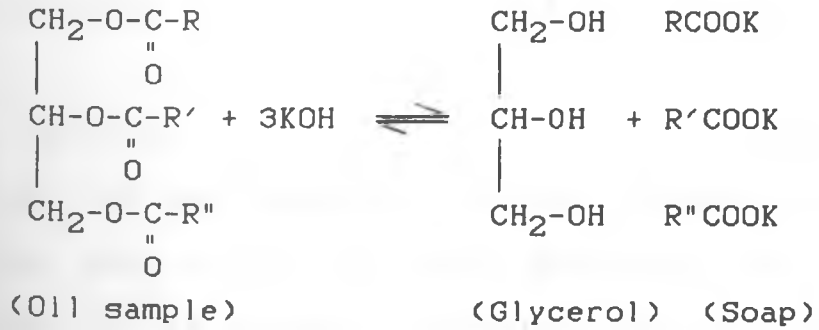
assessment of fats, in foods or other goods, and who by long practice are able to recognize the implication of any variation from normal saponification and acid value indices. Those engaged in chemical processing of fats, however, sometimes find it convenient to adopt terms of reference which convey, although perforce somewhat approximately, a more delicate idea of the actual molecular components which are being dealt with in bulk, saponification value is replaced by saponification equivalent. Saponification equivalent is the amount of fat or wax saponified by one gram-equivalent of KOH, and is therefore a mean gram-equivalent of the mixture of glycerides or wax esters present in a material being examined, assuming that the substance consists exclusively of neutral esters of these types. For many fats and some waxes used in technical practice, containing not more than 2 or 3 % of free fatty acids and less than 1 % of "unsaponiflable matter", the latter proviso is approximately satisfied, and saponification equivalent which is related to the saponification value by the formula:

$$\text{saponification equivalent} = \frac{56100}{\text{saponification value}}$$

affords a fairly clear indication of the mean molecular magnitude of an ester (glyceride or otherwise) which is present. Practical people, more accustomed to thinking about composition of mixtures in terms of weight fraction or weight percent, rather than in terms of mole fraction or mole percent find saponification value a more convenient and straight forward method of expression. Composition of a mixture of two components such as methyl palmitate and methyl stearate, or in general a mixture of A and B may be calculated from the direct proportionality

100(sap.V. of sample) = (wt.% of A)(sap.V. of A) + (wt.% of B)(sap.V. of B).

The same composition of course can be calculated from saponification equivalent, but the awkward reciprical relationship must be used. The reaction is:




(Oil sample) (Glycerol) (Soap)

## 1:16 CALORIFIC VALUE

The energy released when food is combusted is known as its fuel value, which is measured in calories. In the laboratory a Bomb calorimeter is used. The reaction in a Bomb takes place between a compound, usually an organic compound, and oxygen gas at high pressure. This reaction is supposed to give well defined products, in most cases $CO_2(g)$ and $H_2O(l)$. Heat absorbed by the reaction, q, which is negative for combustion reactions, is given by a product of measured temperature increase, DT, of the calorimeter and its heat capacity or water equivalent, W.

$$-q = W.\ DT\ cal. \qquad (1\text{-}1)$$

(1 Calorie is defined as heat needed to raise the temperature of 1 g of water from 14.5 $^{o}C$ to 15.5 $^{o}C$). For a reaction taking place at constant volume (Isochoric), as in the present case; we have according to the First Law of Thermodynamics that:

$$DE' = q. \qquad (1\text{-}2)$$

Where DE' is the change in internal energy by a reaction and q is the heat absorbed, it is negative in the present reaction. We will let these quantities refer to one mole of the compound

reacting with oxygen. DE′ is thus obtained at high pressure in the calorimeter, about 25 atm. To obtain the change in internal energy, ΔE, at a pressure of 1 atm., which is of more interest, one has to know how much energy of reactants and products changes with pressure. We have at the high presure:

$$DE' = E' \text{ products} - E' \text{ reactants}, \qquad (1\text{-}3)$$

and at 1 atm.,

$$DE = E \text{ products} - E \text{ reactants}. \qquad (1\text{-}4)$$

Change in energy for any system is given by the equation (from the First and Second Law of Thermodynamics).

$$dE = TdS - PdV. \qquad (1\text{-}5)$$

T, P, V and S are temperature, pressure, volume and entropy respectively. Now, we are interested in the change in E when pressure is changed by an amount dP and temperature is kept constant. We may divide both sides of the equation by dP and obtain:

$$dE/dP = TdS/dP - P\,dV/dP \qquad (1\text{-}6)$$

at constant T [38]. The calorie being an expression of heat or energy producing value of food. In this project it will be used to give a rough indication of the energy gained through consumption of avocado oil.

## 1:17 BOILING POINT

Equations that describe equilibrium conditions between two phases of the same substance are derived from the first two Laws of Thermodynamics with the aid of free energy functions that have been defined. Let us represent the equation in a closed system at any given temperature and pressure by

$$\text{Phase A} = \text{Phase B}. \qquad (1\text{-}7)$$

Since the system is at equilibrium, under constant temperature and pressure, any infinitesimal transfer of matter between Phase A and Phase B occurs with a free energy change of zero. That is

$$dG = G_A dn_A + G_B dn_B = 0. \qquad (1\text{-}8)$$

Where $G_A$ and $G_B$ are the molar free energies of A and B and $dn_A$ and $dn_B$ are the infinitesimal changes in the number of moles of A and B. Since the system is closed, then, $dn_B = - dn_A$.

$$(G_A - G_B)\, dn_A = 0 \qquad (1\text{-}9)$$

Since equation 1-9 holds for any infinitesimal transfer $dn_A$ whatsoever, quantities in parentheses must be equal to zero, therefore,

$$G_A = G_B. \qquad (1\text{-}10)$$

If temperature and pressure are changed by amounts dT and dP such that the system reaches a new state

of equilibrium, then the molar free energies of A and B change by amounts $dG_A$ and $dG_B$ such that

$$G_A + dG_A = G_B + dG_B$$

$$dG_A = dG_B. \qquad (1\text{-}11)$$

If we apply the equation ($dG = VdP - SdT$) for the total differential to the phases A and B the result is

$$dG_A = V_A dP - S_A dT. \qquad (1\text{-}12)$$

Where G, V, P, S and T are the free energy, volume, pressure, entropy and temperature respectively.

$$dG_B = V_B dP - S_B dT \qquad (1\text{-}13)$$

in which $V_A$ and $V_B$ are the molar volumes of A and B while $S_A$ and $S_B$ are their respective molar entropies. Substituting equation 1-12 and 1-13 into equation 1-11 we obtain

$V_B dP - S_B dT = V_A dP - S_A dT$ which we can rearrange to give

$$(V_B - V_A)dP = (S_B - S_A)dT$$

consequently,

$$dP/dT = (S_B - S_A)/(V_B - V_A) = DS/DV. \qquad (1\text{-}14)$$

From equation 1-14 we can conclude that dT and dP cannot be varied independently and still have a system at equilibrium. Once a value of dT

or dP is chosen the value of the other is fixed by equation 1-14.

We are interested in the value of the derivative dP/dT at a specified temperature and pressure such as is indicated by point "a" in Figure 2. For an isothermal, reversible condition (equilibrium condition) at constant pressure, the change in entropy is given by

$$S_B - S_A = DS = \int DQ_P/T = 1/T \int DQ_P = DH/T.$$

Where DQp and DH represent change in Q and H. Q and H are the heat evolved and the enthalpy (at constant pressure) respectively. Therefore equation 1-14 can be converted to

$$dP/dT = DH/TDV. \qquad (1\text{-}15)$$

This is generally known as Clapeyron equation. So far no special assumption as to the nature of phase A and B have been made in deriving equation 1-15. Evidently, the Clapeyron equation is applicable to equilibrium between any two phases of one component at the same temperature and pressure. Figure 2 shows equilibrium vapour-pressure curve for water. The broken line gives the slope at a specified pressure and temperature.

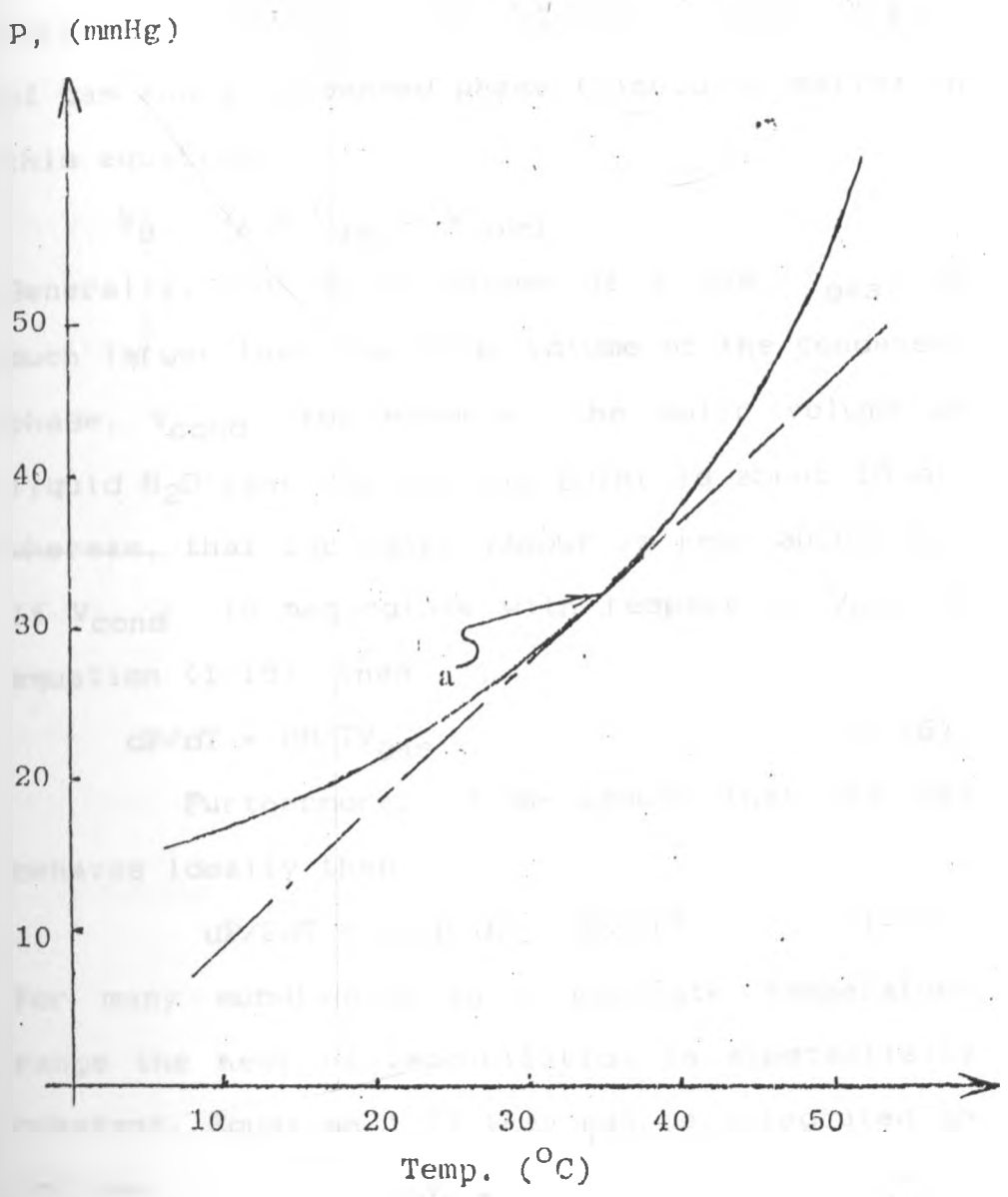



Figure 2. Variation of temperature with pressure. Point "a" specifies a particular temperature and pressure.

Clapeyron equation can be reduced to a convenient form when the equilibrium between A and B is that of gas and a condensed phase (liquid or solid) in this equation.

$$V_B - V_A = V_{gas} - V_{cond.}$$

Generally, the molar volume of a gas, $V_{gas}$, is much larger than the molar volume of the condensed phase, $V_{cond}$. For example, the molar volume of liquid $H_2O$ near the boiling point is about 18 ml, whereas, that for water vapour is near 30,000 ml. If $V_{cond}$ is negleglble with respect to $V_{gas}$ in equation (1-15), then

$$dP/dT = DH/TV_{gas}. \qquad (1-16)$$

Furthermore, if we assume that the gas behaves ideally then

$$dP/PdT = dlnP/dT = DH/RT^2. \qquad (1-17)$$

For many substances in a moderate temperature range the heat of vapourization is substantially constant. Equation 1-17 then can be integrated as follows

$$dlnP = DH/R\ dT/T^2 = -DH/R\ d(1/T)$$

and

$$lnP_2/P_1 = -DH/R\ (1/T_2 - 1/T_1) \qquad (1-18)$$

or, written as the indefinite integral

$$log\ P = -DH/2.303RT + constant. \qquad (1-19)$$

Any one of the Equations 1-17, 1-18, 1-19 is known as Clausius Clapeyron equation, and can be used either to obtain DH from known values of the vapour pressure as a function of temperature, or to predict vapour pressures of a liquid (or a solid) when the heat of vapourization (or sublimation) and one vapour-pressure is known. The equation also represents the variation in boiling point of a liquid with pressure [38]. In this project, the normal boiling point determination method could not be used satisfactorily due to decomposition of the avocado oil, however, the above equation provided a value for the oil's boiling point at 1 atmosphere.

## 1:18 DETERMINATION OF VISCOSITY

Viscosity is a property not much used by analytical chemists for establishing identity or detecting adulteration in fatty oils because most commonly used oils, except castor oil, have viscosities that do not differ much from each other. Viscosity, nevertheless, is an important property of the fatty oils and products made from them. In drying oil Industries, the heat-bodying, blowing and other methods for modifying oils,

cause large changes in viscosity. Viscosity is hereby used as a test for process control.

In design of pumps, piping, stills and other equipment used for oil processing; to the formulator of products containing fat, its viscosity may be a very important consideration. Methods of measuring and expressing results are generally the same for both fatty oils and other materials. Viscosity may be regarded as a type of internal friction against flow. Its unit of measurement, coefficient of viscosity, $\eta$, is defined as the force per unit area required to maintain a unit difference of velocity between two parallel layers that are a unit distance apart. When c.g.s. (centimeter, gram, second) units are used, then force per unit area is in dynes per square centimeter, velocity in centimeters per second and distance in centimeters, coefficient of viscosity $\eta$ is expressed in POISES. KINEMATIC VISCOSITY, v, which is conveniently measured by instruments in which weight of the liquid furnishes the force causing it to flow, is the ratio of viscosity, $\eta$, to the density, D, of the liquid. It is expressed in STOKES.

$$v = \eta/D.$$

where $\eta$ is in poises and D in grams per cubic centimeter. If $\eta$, t, and d are the absolute viscosity, time of flow and specific gravity of the fat, etc., $\eta_1$, $t_1$ and $d_1$ the absolute viscosity, time of flow, and specific gravity of water then the absolute viscosity of the fat is

$$\eta = \frac{td}{t1_1 d_1} \times \eta_1 .$$

Disregarding $\eta_1$, the expression gives viscosity of oil relative to that of water. The current work will address to the chemical and physical properties of avocado oil. It will be the first time such a detailed treatment of these properties is given to avocado fruits grown in Kenyan soil, or more specifically to the avocado oil.

## 1:19 GAS CHROMATOGRAPHY

Chromatography is a technique for separating a mixture into its individual components. The method described in its simplest form by M. Tswett in 1903 involved the separation of pigments from green plant leaves. The name "chromatography" arose from Greeks, and means "writing with colours". The technique is now used also for the separation of colourless compounds, and so, the

term "chromatography" is really inappropriate though still retained.

Chromatographic separations are carried out by ingenious mechanical manupulations involving a few of the general physical properties of molecules. The major properties are tendency for a molecule to (i) dissolve in a liquid (solubility), (ii) attach itself to a finely divided solid (adsorption) and (iii) enter the vapour state or evaporate (volatility). Mixtures of substances to be separated are placed in a dynamic or moving enviromental situation where they can exhibit two of these properties [39, 40]. This may involve using the same property twice, such as solubility in two different liquids, or it may involve two different properties entirely. In gas chromatography, separation depends upon the partition (or distribution) of components between a moving phase (the carrier gas) and the stationary phase (a non-volatile liquid adsorbed on an inert support such as kieselguhr). As the moving phase flows through the column packed with stationary phase, the sample also travels along and is separated into its components by virtue of differences, in the first place, of solubility of

these components in the stationary phase and secondly, of volatility.

Recent developments in preparing highly inert solid supports have permitted smaller amounts of the stationary phase to be used. Earlier, enough stationary phase had to be coated on the solid support to cover all the sites so that no absorption could occur. Few columns now use the once common 30 % stationary phase (by weight on the support), most are now in the 5-20 % range. The lower percentages will give a better resolution of the components of the sample when a small amount of the sample is used. However, a large amount of sample is required because of a low detector sensitivity, or for a preparative separation; the 20-30 % liquid coating should be used to give adequate resolution. The choice of oven temperature is influenced by the following facts: (a) Columns separate less effectively at higher temperatures. An increase of about 30 $^{o}C$ usually halves the solubility of a component of a mixture in the stationary phase, thus decreasing its retention time by half. Shorter retention times yield peaks that are closer together and poorly resolved, at even higher temperatures only a single peak is observed. (b) Columns operate

more efficiently at higher temperatures. There is usually much less tailing and fewer poorly shaped peaks, mainly because of the increased volatilities of the components. (c) The stationary phase could be eluted at very high temperatures. This bleeding will not only give spurious peaks, but will also destroy the column. With these facts, a temperature is usually chosen by experimentation which will give the required separation in a satisfactory amount of time. A good initial temperature is one a few degrees lower than the boiling point of the major component or components of a mixture, if the average boiling point is known. If no boiling point is known, then it is important to obtain a boiling point. If the materials have the same or nearly the same boiling point, the separation will be completely dependent on the nature of the stationary phase in the column [39]. The above aspects are optimized in the current research project in order to not only give well-resolved peaks but also peaks that are well defined so as to enable quantitative work to be done on avocado oil from the chromatograms.

# CHAPTER 2

# EXPERIMENTAL SECTION

## 2:0 REAGENTS AND CHEMICALS

Gas Chromatography standards, Linoleic acid, Linolenic acid, Arachidic acid methyl ester, Capric acid methyl ester and Lauric acid methyl esters were pure samples, they were purchased from Sigma Chemical Company Limited, their structures were known and their purity was better than 99%. Oleic acid and Stearic acid were standard laboratory reagents commercially obtained from local agents of BDH. The solvents carbon tetrachloride, n-hexane, methanol, petroleum spirit (40-60 $^{o}$C), ethanol (95%), acetone, toluene and cyclohexane were of analytical grade and were distilled before use. Pure sodium thiosulphate, potassium iodide, iodine, and potassium hydroxide pellets were obtained from the BDH agents in Nairobi (Howse and McGeorge). Potassium dichromate was further purified by recrystallization from a minimum amount of distilled water. Iodide trichloride was of analytical grade from BDH Chemicals Limited, Poole England. Concentrated

sulphuric acid, concentrated hydrochloric acid and glacial acetic acid were standard laboratory reagents, they were bought from Alpha Chemicals Limited. Apiezon-L used as the stationary phase was made in West Germany.

## 2:1 INSTRUMENTATION

Gas liquid chromatography analyses were performed on two different machines, Gow-Mac series 750 with an F.I.D. detector and Perkin Elmer 8500 Gas chromatograph also with an F.I.D. detector. The detector temperature was maintained at 225 $^{o}$C while the oven temperature was maintained at 200 $^{o}$C. The avocado oil samples were dissolved in carbon tetrachloride. Ultra Violet (U.V.) spectra was run on an SP8-150 UV/Vis Pye Unicam Spectrophotometer. The samples for U.V. were dissolved in carbon tetrachloride. Infra Red (I.R.) was done on SP3-300 Pye Unicam spectrophotometer. The oil sample was run neat. Nuclear magnetic resonance (N.M.R.) for the proton and carbon-13 were performed on Perkin Elmer R12 60 MHz instrument (University of Nairobi) and a Bruker instrument (University of Connecticut, U.S.A.) respectively. Solvent distillation was done using a hot water-bath GLF D 3006 Burgwedel

type 1002 made in the Federal Republic of Germany. Calorific value was estimated using a Baird and Tatlock Ltd. Bomb calorimeter made in England. For calorific value determination the oil samples were put in special plastic capsules with the specifications c5/2000/9, 4419 cal/g, 14 % moisture. This capsule was held in a silicon crucible. Refractive index was measured using ABBE's refractometer made by ZEISS of West Germany.

## 2:2 DETERMINATION OF THE BEST SOLVENT

Initial part of this work dwelt on a search for the best solvent with regard to avocado oil extraction. This involved trials with various solvents as listed in table 6. Several factors were used as guides in this determination. The factors included: (i) Quantity of oil extracted, (ii) appearance of oil, (iii) cost of solvent, (iv) odour of oil, (v) availability of solvent, (vi) ease of solvent application, (vii) solvent recovery and (viii) overall economy of the entire operation.

For each experiment, ten avocado fruits of average maturity were used. The outer-coat (pericarp) and the seed were removed. The edible

portion was then carefully ground into a fine homogeneous paste (mash). Part of the mash was transferred to a clean flask and accurately weighed, by difference. 200 ml of appropriate solvent was added and agitated vigorously for 3-5 minutes, then left for 24 hours. The flask was tightly covered to prevent evaporation of the solvent. After 24 hours, the solution was decanted into a clean 500 ml quickfit round bottomed flask, ready for distillation into a clean recovery flask. This process was repeated at least two times until the bright yellow-green mash turned to dirty yellow-green, with a tint of brown.

2:3 DETERMINATION OF FREE FATTY ACIDS

5-10 grams of the oil sample was accurately weighed into a 250 ml flask. 25 ml of neutral methyl alcohol and 25 ml of toluene were added. The solution was freely boiled for not more than two minutes. Two drops of phenolphthalein solution were added and titrated, with vigorous shaking, against a solution of approximately decinormal alcoholic potash (the exact strength of which was concurrently determined against standard 0.1 N HCl acid) until a semi-permanent pink colour

(persisting for at least 15 seconds) appeared [26].

## 2:4 DENSITY OF AVOCADO OIL

In order to clean the Pycnometer's interior, a cleaning solution was left inside for 24 hours. The cleaning solution was a mixture of potassium dichromate and concentrated sulphuric acid. The cleaning solution was decanted and the Pycnometer filled with distilled water for another 24 hours, to remove added acid. The water was poured out and the pycnometer rinsed several times with fresh distilled water. Drying was accomplished in about 20 minutes by inverting the pycnometer in a large dessicator and applying sunction. The vacuum was broken from time to time if much water was initially present. The drying process was repeated until a constant weight was obtained. The pycnometer was filled with the oil sample using a pipette. The sample was thermostated at 25 $^{o}$C. During this operation it was necessary to ensure that no air bubbles were present when inserting the stopper. After 24 hours in the thermostated bath, the pycnometer was removed then thoroughly wiped dry with an adsorbent paper and finally weighed. Density was calculated from the equation

d = W/V. Where W = weight of sample and V = Volume of the pycnometer, 2 ml.

## 2:5 DETERMINATION OF IODINE VALUE

The following solutions were required: decinormal standard sodium thiosulphite, potassium iodide solution (10 %), starch solution, carbon tetrachloride (pure and dry) and Wijs solution. The Wijs solution was prepared by dissolving iodide trichloride (7.8 g) and iodine (8.5 g) in glacial acetic acid. This solution was dilluted to 1000 ml with cold glacial acetic acid.

0.12-0.17 g of oil was accurately weighed. This was put into a wide-necked bottle (fitted with a well-ground stopper) and dissolved in 10 ml of pure carbon tetrachloride. Then 20 ml Wijs solution was added and the bottle's content agitated, for 15-30 seconds. The bottle and its contents were set aside in the dark for addition process to proceed to completion. This required 30 minutes. The solution was mixed with 15 ml of distilled water and titrated with standard thiosulphate solution, using starch as the final indicator. A blank determination was carried out with each batch of absorption test, in all respect as in the actual analysis, except that no oil was

present. Iodine value = $(v_b - v_w)/w \times 12.792 \times I$, where 127.92 is the atomic weight of iodine, w the weight of oil. $v_b$ and $v_w$ were the volumes, in millilitres, of sodium thiosulphate solution (reckoned as decinormal) used in the blank and in the actual analysis respectively. I, was the actual concentration of sodium thiosulphate solution [26].

Iodine value is the number of grams of iodine that combine with 100 g of oil or fat, it gives the degree of unsaturation of a substance.

## 2:6 DETERMINATION OF REFRACTIVE INDEX

The instrument used consisted, essentially, of two highly polished prisms hinged together and surrounded by a jacket through which water was passed. A determination was made by opening the hinged prisms very cautiously and thoroughly cleaning the prism faces with a little acetone applied on cotton wool. Three drops of filtered oil were placed on the horizontal face. The instrument was then closed and left for three minutes to ensure that the oil reached the temperature of the jacket. Reading was made directly, by turning the focusing telescope until

a line of total reflection passed through the intersection of two hair-lines fixed in the field of view. The readings were taken from above and below the hair-line. An average of the two was reported as the Refractive Index [33].

## 2:7 SAPONIFICATION VALUE OF OIL SAMPLE

The required materials were (i) Oil, (ii) 0.5 N alcoholic potash, where 8 g of KOH pellets were dissolved in 250 ml of 95 % ethanol (the stoppered flask was agitated for two minutes then filtered to remove undissolved carbonate and kept in a tightly closed flask) and (iii) standard acid 0.5 N HCl. 10.0038 g of fat was dissolved in 100 ml of petroleum ether (40-60 $^{o}$C). 10 ml of the petroleum ether solution was transferred into a weighed weighing bottle. The ether was allowed to evaporate and the weighing bottle heated in an oven at 110 $^{o}$C to a constant weight for determination of the oil's dry weight. Similarly, 10 ml of the ether solution was transferred into a 100 ml round-bottomed quickfit flask. This solution was allowed to evaporate, it was not heated in an oven. 25 ml of alcoholic KOH (accurately pipetted) was added to the evaporated

sample. Boiling chips were added and the flask attached to a reflux condenser. The contents were refluxed gently on a water-bath for two hours. This solution was then allowed to cool and 25 ml of distilled water added through the reflux condenser. Two drops of phenolphthalein indicator were added to the saponification mixture and titrated with standard 0.5 N acid ( HCl ) to a faint end-point. The contents were preserved for preparation of methyl esters. This titration measured the alkali remaining after saponification. 25 ml of the original alcoholic KOH was titrated with the standard acid accurately to determine the amount of alkali originally added. This second titration also standardized KOH solution. From the difference between the two titrations, amount of alkali used in saponification was calculated.

Saponification equivalent calculated is also an average equivalent weight of the fatty acids. If w is the weight of the fat taken, $v_b$ and $v_w$ the number of millilitres of normal acid required to neutralize the blank and the actual solution after refluxing, then, saponification equivalent = $1000\ w / (v_b - v_w)$ and the saponification value = $(v_b - v_w) \times 56.1/w$. Because it is the weight of sample

in grams divided by weight of KOH in milligrams and multiplied by 56,104, the saponification equivalent of a pure fatty acid or ester is therefore equal numerically to its equivalent weight [26].

## 2:7:1 PREPARATION OF SODIUM THIOSULPHATE SOLUTION

25 g of sodium thiosulphate was dissolved in distilled water and diluted to a litre (no trace of acid was allowed to come into contact with this solution). The solution was transferred to a suitable bottle and allowed to stand for three days before standardizing. Sodium thiosulphate solution was standardized as follows: 10 ml of concentrated hydrochloric acid was added to 20 ml of decinormal potassium dichromate solution in a 500 ml flask. The solution was dilluted to about 200 ml with distilled water. 0.5 g of crystallized potassium iodide was added to this solution. Iodine was quickly liberated and the solution became brown. The resulting solution was titrated with a nearly decinormal sodium thiosulphate, interrupting the process to add starch when the end of the reaction approached. Titration was continued until a bluish black colour, formed on addition of starch, changed to

pale green due to chromic chloride. This titration process was repeated until when concordant results were obtained [34].

## 2:7:2 PREPARATION OF DECINORMAL POTASSIUM DICHROMATE SOLUTION

10 g of pure potassium dichromate was ground to a fine powder and dissolved in the least quantity of water. The solution was filtered through a hot funnel. The hot funnel prevented crystallization of the salt before filtration. A large funnel 150 cm across was employed. The filtrate was received in a clean flask. The flask was cooled in running water to facilitate recrystallization of the salt in a finely granulated state. The salt was pumped dry and left for 24 hours on a filter paper. When the salt seemed crisp and dry, it was heated in a porcelain dish until it turned dark without melting. While heating, the salt was continuously stirred with a glass rod. The dry dichromate was transferred to a stoppered test-tube that was well cocked. 4.903 g of the dichromate was accurately weighed, dissolved in distilled water and diluted to 1000 ml. This solution was kept in a stoppered bottle [34].

## 2.8 INVESTIGATION OF UNSAPONIFIABLE MATTER.

About 4 g of avocado oil was accurately weighed into a 100 ml round bottomed flask. 50 ml of approximately 0.5 N alcoholic potash was added. The exact strength of the alkali was concurrently determined by titration against a standard 0.5 N HCl. The mixture of oil and alkali was refluxed for one hour and then transferred to a 250 ml separating funnel, washing out the flask with 100 ml of distilled water. A 100 ml of diethyl ether (b.pt. 34.6 $^{o}$C) was added to the funnel rinsing the flask with a little ether. Contents of the separating funnel were agitated vigorously and allowed to settle. The soap solution was ran off and the ether solution collected. The extraction process was repeated twice with 50 ml of ether, shaking vigorously each time. The soap solution was discarded and the ether extract transferred to the funnel. 40 ml of water was used in washing the ether extract without violent shaking. This treatment was repeated with 25 ml portions of distilled water until the washings were no longer alkaline to phenolphthalein. All traces of water were drained and a few grains of anhydrous sodium sulphate added. After addition of the drying agent

the funnel was agitated and the contents allowed to settle. 10 minutes later the ether solution was decanted into a weighed flask. The residual sulphate was washed with 25 ml of ether. The washings were also transferred to the weighed flask. Ether was then evaporated on a water-bath. 5 ml of acetone was added and then completely removed by immersing the flask into a water-bath and drawing air through the solution. The flask was then transferred to an oven and its contents dried at a temperature below 90 $^{o}$C to a constant weight. The difference between the final constant weight and the initial weight of the empty flask represented the quantity of the unsaponifiable matter. This was expressed as a percentage of the total weight of oil used as shown in section 3:6.

## 2:9 ESTIMATION OF CALORIFIC VALUE

0.5 g of the oil sample was poured into a special plastic capsule (c5/2000/9, 4419cal/g, 14.6 % moisture). Both the weight of capsule and oil were accurately determined. A length of about 5 cm platinum wire was connected to the terminals. A length of 6 cm (No. 60) sewing cotton thread was tied to the platinum-wire and its end fixed to the capsule. A lid was placed on the Bomb and the

locking nut properly screwed on. The cylinder valve was opened very carefully and oxygen slowly allowed into the Bomb to a pressure between 20-25 atm. The high pressure line was automatically released of pressure when a connection to the valve was opened. The Bomb was fixed in a container which had 1700 ml of tap water.

In order to minimize heat exchange, between calorimeter vessel and the water-jacket, water temperature was below that of the jacket before, and above after burning the sample. Temperature rise was about 3 $^{o}C$, so water in the calorimeter vessel, was at around 2 $^{o}C$ below that of the water-jacket before burning the sample.

The Bomb terminals were connected to a firing unit. The lid was positioned on calorimeter then the thermometer and stirrer were fixed. Finally the stirring motor was started. The apparatus was allowed to equilibrate for 5 minutes. Temperature was recorded for every half a minute until a steady state was reached. The Bomb was fired and time of firing noted. Temperature was recorded continuously for every half a minute until the temperature change was again uniform (about 30 minutes after firing).

The Bomb was removed from calorimeter and pressure released by an attached screw. The Bomb was not opened until the pressure was 1 atm. With the knowledge of the temperature change, DT, and the heat capacity of calorimeter, W, the heat of reaction was found by -q = W.DT. W, consists of the heat capacity of water in the calorimeter (1700 ml), which was taken as 1700 Cal/degree, plus the heat capacity of the container and the Bomb, $W_b$. The heat capacity of all these parts was well known from a combustion experiment carried out with benzoic acid which has heat of combustion as 6318 Cal/g. DT had been measured. Since -q and DT were known, W could be calculated. Such an experiment with benzoic acid gave for the present Bomb calorimeter W = 2335 Cal/degree. In this way the heat of combustion of the cotton thread and different sources of error were also partly compensated for [42, 43, 44].

## 2:10 DETERMINATION OF VAPOUR PRESSURE

Initially the apparatus (Figure 3) was tested for leaks by pumping air to a pressure of about 10 mmHg and closing the stopcock D. A significant leak indicated a noticeable pressure increase within one minute. The apparatus was

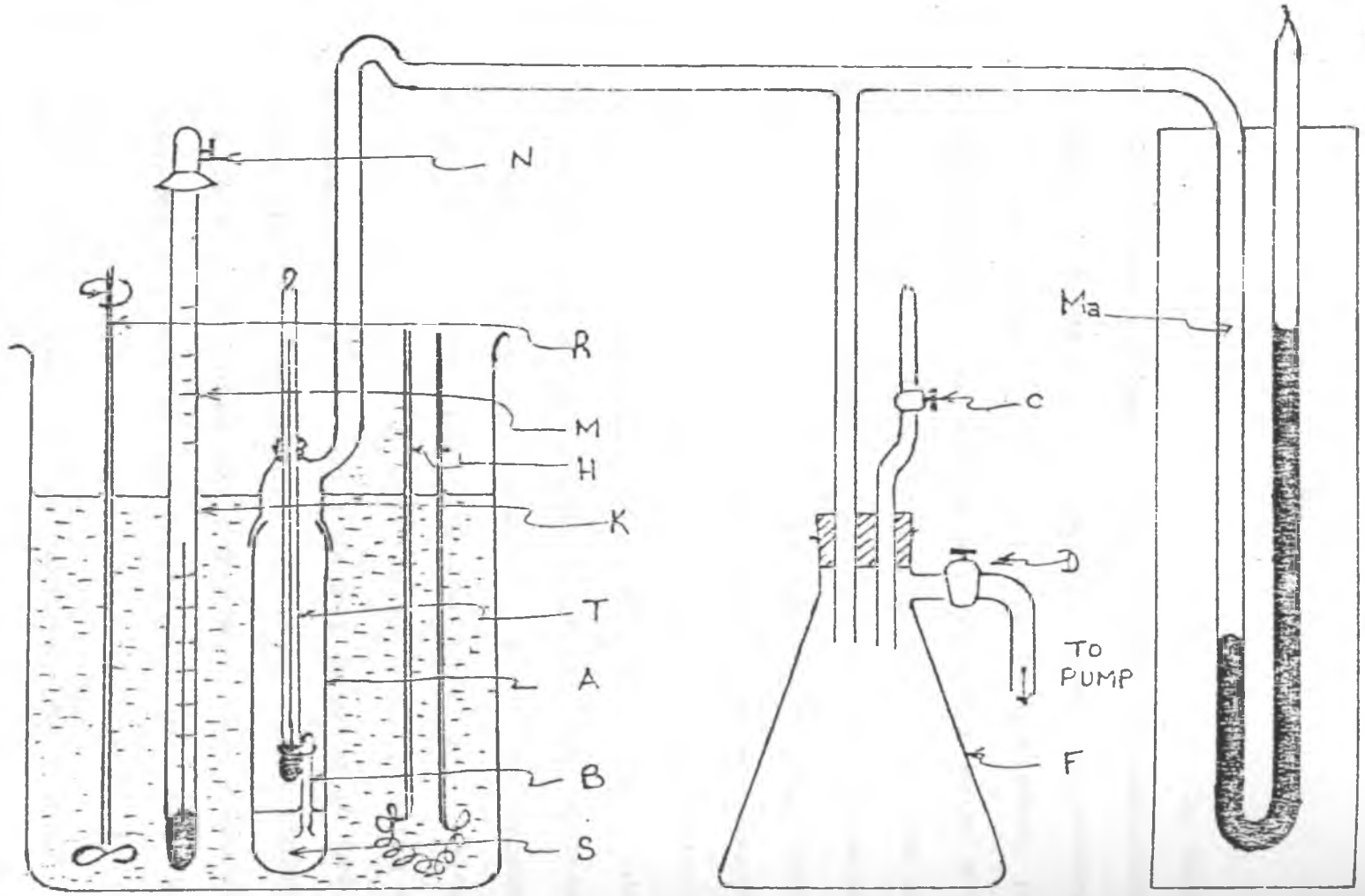



Figure 3. Apparatus for vapour pressure determination.

filled with air again by opening stopcock C. The water-bath was removed and tube A opened to introduce avocado oil for investigation. Standard joint A had stopcock-grease only on its lower part, so that grease would not contaminate the oil. Tube B was detached and a clean dropper used to fill it almost completely with the oil. B was then inserted and reattached to the thermometer. Stopcock C was closed and the vacuum pump started. Stopcock D was carefully closed until a continuous stream of bubbles issued from tube B. The pressure was held at this value with D closed for several minutes. If bubbles did not appear at this temperature and at pressures below 10 mmHg, the temperature was raised. When tube B had been well swept out, pressure was slowly increased by opening C for short intervals. The pressure was increased until the liquid levels in A and B were equal. The pressure was kept at this level for approximately one minute. Pressure inside B was now due to vapour of the oil only, which was equal to a value shown by the manometer. These pressure and temperature were recorded. The pressure and temperature were raised and vapour pressure measurements repeated for a temperature range of 25-50 $^{o}$C.

## 2:11 DETERMINATION OF VISCOSITY

The Ubbelohde viscometer was aligned in a vertical position. This alignment was the same for consecutive measurements, to insure that the liquid's driving head was constant throughout. A flask containing the oil sample was first left in a thermostated water-bath so that no errors would be incurred due to volume changes, also the time for equilibration was considerably reduced. A constant volume of oil was introduced into the viscometer and left for 30 minutes to establish a temperature equilibrium. Air bubbles were eliminated by using a sunction pump. The oil at 25 $^{o}C$ was forced upto approximately a centimeter above the upper mark by means of gentle air pressure on the side arm. Pressure was then released. As the oil meniscus moved past the upper mark, the time interval for transit between the upper and the lower mark was measured using a stop watch.

The Ubbelohde Viscometer has a suspended level construction which eliminates the problem of constant loading volume, because a driving head depends not on the difference between two changing

levels, but is determined by a fixed point and the liquid's average level in the capillary bulb.

## 2:12 PREPARATION OF METHYL ESTERS FOR GAS CHROMATOGRAPHY

The saponification process previously done (section 2:7) had split the fat and yielded fatty acids as potassium salts. After titration with acid they were converted to free fatty acids, which would later be methylated and be subjected to gas chromatography. Methylation is necessary because free fatty acids are likely to react with the stationary phase, they would also dimerize and make interpretation of the chromatographs more difficult.

The titrated saponification mixture was about 60 ml in volume. 40 ml of 2 N $H_2SO_4$ was added and the fatty acids extracted with 50 ml of petroleum ether (40-60 $^oC$). The aqueous layer was re-extracted with another 50 ml of petroleum ether (40-60 $^oC$) and a combination of the ether extracts was washed with distilled water until only faintly acid. The ether solution was dried for 24 hours with anhydruos $Na_2SO_4$. The clean petroleum ether layer was decanted and the solvent distilled off on a water-bath. 30 ml of dry

methanol and 4 ml of concetrated sulphuric acid were added to the distillation flask and the mixture gently boiled on a water-bath, under reflux, for about 3 hours. A condenser for distillation was attached and about two-thirds of methanol removed. The residue was dilluted with 50 ml of distilled water and extracted twice with 25 ml portions of petroleum ether (40-60 $^{o}$C). The combined ether extracts were washed with distilled water until the washings were no longer acidic. This solution was dried over anhydrous sodium sulphate for 24 hours. Lastly, the petroleum ether was distilled off on a water-bath and the residue poured while hot into a clean specimen tube. The residue consisted of methyl esters of avocado oil.

## 2:13 STATIONARY PHASE PREPARATION AND CONSTRUCTION OF A G.C. COLUMN

5.2287 g of chromosorb (chrom. W AW), 80/100 mesh was well stirred in a solution of 0.7843 g Apiezon-L and 50 ml petroleum ether (40-60 $^{o}$C). The solvent was evaporated on a water-bath, stirring continuously to avoid formation of lumps. The dried powder was ready to be packed as stationary phase. Column assembly is a moderately simple operation when the coated support is

available. The column was fabricated by plugging one end of a copper tube, that had the desired dimensions (id. 1/8" and 1.5 m long), with glass wool. Then the tube was slowly filled with coated support. While filling, the tubing was constantly tapped and vibrated to insure a firm and even packing. The filling end was plugged with glass wool before bending the tubing to a cylindrical coil (solenoidal shape). A column works better when straight, however, efficient oven design requires that, at least, no sharp bends are present. Since every column will bleed some stationary phase when new, all columns should be conditioned before use. In this case, conditioning was done by heating at 20-30 $^{o}$C above 200 $^{o}$C, the temperature at which it was going to be used. During the conditioning process, the carrier gas was allowed to flow through the column for 10 hours [39]. The detector had been disconnected to prevent it from being fouled by any volatiles coming off. During the current research project, if the column was to be used at a specific temperature much lower (or higher) than the temperature at which it was conditioned, a short reconditioning for about two hours was done.

# CHAPTER 3

## RESULTS AND DISCUSSION

### 3:0 DETERMINATION OF THE BEST SOLVENT

The solvents used for this cold extraction process were water, acetone, methanol, n-hexane, cyclohexane, petroleum ether (40-60 $^{o}$C), ethanol and carbon tetrachloride. Fruits of nearly the same maturity were used. The age estimation was managed by the help of an experienced farmer from Gatanga in Murang'a [46]. This is the first reported detailed attempt on avocado oil extraction with various solvents in Kenya. Out of an accurately weighed portion of mash from ten avocado fruits, of average maturity, an extraction process was carried out in the manner outlined in section 2:2. The results are recorded in table 6. Looking for the best solvent required setting up guidelines that would help in eliminating some, as well as eventually coming up with the best choice among many possibilities.

Initially water was my automatic choice for avocado oil extraction, mainly, because of its availability and almost no cost when compared with

Table 6. Amount of avocado oil (%) extracted with 8 different solvents.

| Name and B.pt $^{o}C$ | % solvent recycled | Weight of mash (g) | Weight of oil extracted (g) | % oil extract |
|---|---|---|---|---|
| Water 100 | ---- | 218.8314 | 6.8314 | 3.122 |
| | ---- | 272.4002 | 7.2743 | 2.670 |
| Acetone 56 | 62 | 183.7276 | Not separated | |
| Methanol 64.5 | 41 | 194.4136 | Not separated | |
| n-hexane 69 | 69 | 201.3449 | 16.5945 | 8.2418 |
| Cyclo-hexane 80.7 | 50 | 172.9190 | 19.3819 | 11.2086 |
| Pet/ether 40-60 | 82 | 214.6446 | 29.0881 | 13.5517 |
| Ethanol 78 | 60 | 206.5096 | 25.0682 | 12.1389 |
| $CCl_4$ 76 | 53 | 227.6030 | 14.1038 | 6.1967 |

the other solvents. From two trials, water gave about 3 % of oil. Other than the oil having a bad smell that developed during extraction process, extraction with water also required prolonged heating at temperatures above those provided by a water-bath. This would introduce extra expenses in terms of fuel and time that would have to be incurred if water was chosen as the extraction solvent. Later it was necessary to use an oil-bath to separate oil and water. Another negative factor with water as a solvent was the difficulty in decanting the oil/water solution from the rest of the mash. This was because water tended to carry a fine suspension of the mash. A lot of time, again, was spent waiting for the solution to filter through the sieve. Acetone was the second solvent that was put to test. After 24 hours (at room temperature), the solution containing acetone and oil was separated from the mash. After a distillation of 62 % of the solvent, two layers (not characterized) were formed. It was difficult to separate the two layers. This in itself, would have been a negative contributing factor towards choosing acetone as a solvent. At this time, the contents of the distillation flask were giving out an awful smell. The third solvent was methanol.

Three fractions were collected during distillation. The first was collected between 63-67 $^{o}C$, it had a light yellow colour, the residue formed a yellowish froth that had the smell of avocado. Further distillation gave a second fraction between 67-70 $^{o}C$, it was also yellow in colour. Between 79-82 $^{o}C$ there was a third fraction of a clear solution which had the odour of methanol. The final residue was composed of an uncharacterized layer and small amounts of oil. These results eliminated methanol. Fourth, was n-hexane. n-Hexane extracted 16.5945 g of the oil, which constituted 8.2 % of the total edible mash. At Ksh.1,850.00 for 2.5 litres, the price of n-hexane was prohibitive. The final oil product had a smell of n-hexane even after evaporating the solvent for one hour. The fifth extraction was done using cyclohexane. Cyclohexane has a rather high boiling point, 80.7 $^{o}C$. The solvent recovery was low, 50 %. With this solvent 11.2086 % of avocado oil was obtained. This was a good quantity, however, the avocado oil had a sharp smell of the solvent. This implied that some of the solvent was left with the oil, possibly due to the high boiling point of cyclohexane. The extraction process went in three steps, one

distillate appeared at 74 $^{o}$C and the second between 76-77 $^{o}$C. The third portion was collected beyond 80 $^{o}$C. Petroleum ether turned out to be the best solvent with a boiling point range of 40-60 $^{o}$C. Distillation of this solvent on a water-bath was quite convenient. The oil yield was 13.5517 %, higher than any amount from the other solvents. Avocado oil extracted by petroleum ether had among other good features, a golden colour and avocado's natural fragrance. The price of petroleum ether (40-60 $^{o}$C) was Ksh.350.00 per 2.5 litres. It was cheaper than the other solvents save for water. Ethanol gave 12.1389 % oil that had the solvent's smell. With a boiling point of 78 $^{o}$C and its high solubility in water, ethanol was difficult to distill off completely. Lastly, carbon tetrachloride was tried. Carbon tetrachloride has a boiling point of 76 $^{o}$C, almost as high as that of ethanol(78 $^{o}$C). Its recovery was 53 % and the oil yield 6.1967 %. Besides being toxic when inhaled and when in contact with the skin, carbon tetrachloride retained its smell with the final product. Petroleum ether (40-60 $^{o}$C), clearly emerged with superior properties when compared with the other solvents. The oil extracted with petroleum ether had several attractive features

which enabled direct application to skin-related defects. This factor coupled with high yield, best recovery, highly impressive colour and ability to distil at a shorter time relative to other solvents lead to exclusive use of petroleum ether as the best solvent for extraction of all avocado oil used in this work.

## 3:1 EFFECT OF MATURITY ON OIL CONTENT

Cold solvent extraction of oil, using petroleum ether (40-60 $^{O}$C), from avocado fruits of four different levels of maturity was done. This was necessary in order to find out how the oil content in less mature fruits compared with that in more mature ones. If one is able to know whether a fruit is mature or not, then results obtained from this experiment will go a long way in establishing criterion for the right period to pick avocado fruits. Definately, those interested in avocado oil would go for fruits with high oil content; whereas, those on diet and those who are sensitive to cholesterol, would go for fruits with little oil. Thus, information derived from this work would be important to both individuals and the industries. The results are given in table 7

below. All the fruits were obtained from one farm at Gatanga in Murang'a District.

Table 7. Variation of oil content with fruit maturity.

| Run No. | Weight of mash (g) | oil extracted (%) |
|---|---|---|
| (a) | | |
| 1. | 192.5959 | 2.8322 |
| 2. | 205.3369 | 2.7195 |
| 3. | 142.6025 | 2.1986 |
| 4. | 228.4456 | 2.9261 |
| 5. | 153.0049 | 2.3939 |
| 6. | 193.6469 | 2.4190 |
| 7. | 204.4669 | 2.7900 |
| 8. | 229.6443 | 2.9088 |
| 9. | 152.0497 | 1.8307 |
| (b) | | |
| 1. | 180.0081 | 4.0195 |
| 2. | 204.8103 | 3.6749 |
| 3. | 146.6217 | 3.5618 |
| 4. | 203.2043 | 4.4543 |
| 5. | 160.5036 | 2.8077 |
| 6. | 176.1191 | 4.1024 |
| 7. | 202.4023 | 3.9953 |
| 8. | 185.4833 | 3.6931 |
| 9. | 134.7453 | 3.0068 |
| 10. | 259.0853 | 4.3884 |
| (c) | | |
| 1. | 109.2418 | 17.2468 |
| 2. | 188.3238 | 16.9076 |
| 3. | 154.4802 | 8.4042 |
| 4. | 140.9580 | 8.6877 |
| 5. | 77.1525 | 11.8821 |
| 6. | 96.2476 | 14.7959 |
| 7. | 112.8854 | 8.6248 |
| 8. | 127.8484 | 10.1438 |
| 9. | 345.1441 | 11.0678 |
| 10. | 235.7066 | 10.4124 |
| 11. | 220.6169 | 11.8754 |
| 12. | 258.5462 | 10.1820 |
| 13. | 348.6600 | 9.0970 |
| 14. | 305.7195 | 12.6020 |
| (d) | | |
| 1. | 151.0226 | 21.3335 |
| 2. | 394.4573 | 24.5361 |
| 3. | 176.4081 | 31.8507 |
| 4. | 176.5413 | 29.1304 |

* Starting with the least mature fruits, the four levels of maturity are (a) tender glossy green, (b) tender green, (c) light dark green and (d) dull or dark green.

This work was made possible by the help of Mr. Benson Murigi, an experienced farmer living in Gatanga (Murang'a). All fruits, at the successive levels of maturity, were continuously obtained from Mr. Murigi's farm. From table 7, one can make the following conclusions: (1) The colour of fruit can be used to estimate the amount of oil. The tender glossy green fruits have the least amount of oil. This is followed by the second class (b), tender green fruits. However, oil content between these two age groups is almost the same. In fact it was difficult to distinguish between the two classes in terms of colour. On the other hand there is significant difference when two extreme classes are compared (class (a) and class (d)). Fruits in class (d) have oil content about ten times that of fruits in class (a). This type of information is important to farmers who may not know the correlation between the colour and the amount of oil present in a particular fruit. (2) By comparing the weight of the edible portion of the fruit with the oil content, no conclusion can

be reached regarding the oil percentage and the total weight of mash (table 7). Moreover, table 7 suggests that when a person eats 100 g of the edible portion of a mature fruit about 20-30 g of it constitutes the avocado oil.

## 3:2 DETERMINATION OF MOISTURE CONTENT

This work was done on the edible part of the fruit. Weight changes were monitored to a constant value as the mash went through a drying process in the sun. Fruits at two different levels of maturity were used and results recorded in table 8. Moisture content results were as follows: 1A (72.8778 %), 2A (74.9472 %), 3A (78.8582 %), 4A (77.1406 %), 5B (67.3064 %) and 6B ( 69.9630 %). Note A, represents less mature fruits and B, represents more mature fruits.

The first four fruits were younger than the last two. Evidently, the first class of fruits (light dark green) had a much higher moisture content than the more mature ones (dull or dark green). This behaviour has been observed elsewhere in the world. M.E. Jaffa and F.W. Albra reported that during a nutritive value analysis of avocado fruits, grown in California, 69.16 % was water [6]. Still in California A.R.C. Haas, reported

Table 8. Determination of moisture content on the edible portion of avocado fruits at two different levels of maturity.

| Weight of wet mash (g) | Weight of drying mash (g) | Date weighed |
|---|---|---|
| 1A. 178.8240 | | 29/12/88 |
| | 51.5320 | 2/1/89 |
| | 48.6533 | 3/1/89 |
| | 48.6012 | 3/1/89 |
| | 48.5277 | 4/1/89 |
| | 48.5013 | 6/1/89 |
| | 48.5010 | 8/1/89 |
| 2A. 195.1560 | | 29/12/88 |
| | 55.1637 | 2/1/89 |
| | 49.9781 | 3/1/89 |
| | 49.7163 | 3/1/89 |
| | 48.9355 | 4/1/89 |
| | 48.8990 | 6/1/89 |
| | 48.8920 | 8/1/89 |
| 3A. 77.5240 | | 29/12/88 |
| | 18.8986 | 2/1/89 |
| | 16.8537 | 3/1/89 |
| | 16.7660 | 3/1/89 |
| | 16.5992 | 4/1/89 |
| | 16.3913 | 6/1/89 |
| | 16.3900 | 8/1/89 |
| 4A. 163.3650 | | 1/7/89 |
| | 43.2633 | 3/7/89 |
| | 39.3068 | 8/7/89 |
| | 37.3555 | 10/7/89 |
| | 37.3445 | 12/7/89 |
| | 37.3443 | 13/7/89 |
| 5B. 179.3469 | | 1/7/89 |
| | 66.2706 | 3/7/89 |
| | 62.1390 | 8/7/89 |
| | 58.8269 | 10/7/89 |
| | 58.6351 | 12/7/89 |
| | 58.6350 | 13/7/89 |
| 6B. 197.5659 | | 1/7/89 |
| | 65.2445 | 3/7/89 |
| | 62.3159 | 8/7/89 |
| | 60.0419 | 10/7/89 |
| | 60.0012 | 12/7/89 |
| | 60.0010 | 13/7/89 |

about the water content of avocado fruits and leaves. The report further indicates that water decreased with increasing maturity, and that water percentage in the seed (without seed coat) of Fuerte variety was usually greater than in seeds of the Pebble and Dorothea varieties. Water was comparatively uniform in the seeds of fully grown fruits at various stages of maturity [3].

C. Franzke and H.J. Henning reported in 1956 an average moisture content of 74.3 % [8]. Values of moisture content determined in this project are similar to those found in other regions of the world. This project also tentatively, supports the suggestion [3] put forward by A.R.C. Haas, that water content decreased with increasing maturity of avocado fruits.

## 3:3 OIL CONTENT IN FRUITS FROM DIFFERENT PARTS OF KENYA

Three regions (Figure 1) were covered in this determination. The regions were Kitale, Kiambu and Murang'a . Fruits of average maturity (class C of table 7) were used and results recorded in table 9. Avocado fruits analysed from three different regions of Kenya do not show much disparity, they

Table 9. Oil content of avocado fruits from three regions.

| Run No. | Kitale | Kiambu | Murang'a |
|---|---|---|---|
| 1. | 11.3221 | 13.5518 | 17.2468 |
| 2. | 12.8803 | 7.0235 | 16.9076 |
| 3. | 9.4579 | 14.4022 | 8.6877 |
| 4. | 14.3537 | 9.0271 | 11.0678 |
| 5. | 11.8214 | 13.1516 | 10.4124 |
| 6. | 12.4501 | 11.3957 | 11.8754 |
| 7. | | | 10.1820 |
| 8. | | | 9.0970 |
| 9. | | | 12.6020 |
| 10. | | | 11.8821 |
| 11. | | | 14.7959 |
| 12. | | | 8.6248 |
| 13. | | | 10.1438 |
| 14. | | | 13.5517 |
| 15. | | | 12.7620 |
| 16. | | | 13.7657 |
| Range | 9.4579-14.3537 | 7.0235-14.4022 | 8.6248-17.2468 |
| Mean | 12.0476 | 11.4253 | 12.1003 |
| S.D. | 1.4976 | 2.6297 | 2.5824 |

S.D. represents standard deviation.

have almost the same mean of about 12 % (oil content), with Kiambu having a slightly lower value of 11.4 %. Results of fruits from Kiambu and Murang'a are more varied with a standard deviation of about 2.6 %. Fruits grown in Kitale had a standard deviation of about 1.5. Larger differences in standard deviation have been observed in other parts of the world. Jaffa and Mackay reported [11] that an immature fruit may be either cut in half or in much smaller divisions and will ripen in a few days without much

deterioration, if well covered and air is excluded. Determination of the fat content in 22 samples of new varieties of avocado showed in three varieties 22 %, 22.83 % and 23.71 % of fat respectively. In two specimens of another variety 29.78 % and 31.59 % were observed. W.J. Stoneback and Ralph Calvert reported an average of 20 % from avocado fruits [29]. The pulp of varieties grown in California and Florida contain at least 80 % of fat on dry weight basis, although the refined and bleached oil was still too dark for edible purposes [12]. In Peru, the avocado pear had 13-20 % oil [7]. C. Franzke and H.J. Henning reported a 19.4 % fat content [8]. From Argentinian avocado, report of the chemical composition by M.H. Bertoni, G. Karman de Sulton and G.P. Cataneo mentioned a yield of 3.9-27.4 % avocado oil [15]. Fruits grown in Kenya also have high oil yield. The experiment on the most mature fruits recorded a range of 21.33-31.85 % avocado oil (table 7). Using t-test at 95 % level of confidence, oil content in the fruits from Kitale, Kiambu and Murang'a was not significantly different.

## 3:4 ACID VALUE

This is the amount in milligrams of KOH required to neutralize free fatty acids in 1 g of sample. Fourty five fruits were used in extraction of sufficient oil for this analysis. These fruits were obtained from Murang'a. The small standard deviation, suggests good day-to-day reproducibility of the procedure (taking into account that the determinations were performed on different days). Acid value = V x 56.1 x A/W. Where: V = Volume of alkali used, W = Dry weight of oil and A = The alkali factor (the actual concentration of KOH). The actual KOH concentration was calculated as follows: The reaction that takes place is KOH + HCl $\longrightarrow$ KCl + $H_2O$. Since KOH and HCl react in a one to one ratio, the amount of KOH in a titre of 16.90 ml is equal to the amount of 0.1 N HCl in 2 ml, which is 2 x 0.1 = 0.2 moles. 2 ml of 0.1 N HCl had been accurately pipetted into a clean conical flask. So the concentration of KOH is 0.2/16.90 = 0.0118343 N (table 10). Similar calculations were done for the other two KOH solutions, before they were used for acid value determination. The calculated values are in table 10.

Table 10. Determination of actual concentration of KOH.

| Solution No. | Volume of KOH (ml) | Concentration of KOH or Alkali factor (A) |
|---|---|---|
| 1. | 30.10 | 0.006645 |
| 2. | 16.90 | 0.0118343 |
| 3. | 15.80 | 0.0126582 |

The above three solutions were used for determination of acid value. The acid values are tabulated below (table 11). Each determination had oil extracted from five avocado fruits.

Table 11. Calculated acid value for avocado oil samples.

| Run No. | Vol. of Alkali V(ml) | W (g) | Alkali factor A | Acid value (mg) |
|---|---|---|---|---|
| 1. | 32.75 | 5.6836 | 0.006645 | 2.1481 |
| 2. | 54.30 | 7.0940 | " | 2.8534 |
| 3. | 27.80 | 7.1256 | 0.0118343 | 2.5902 |
| 4. | 21.90 | 7.1133 | " | 2.0440 |
| 5. | 21.80 | 7.0824 | " | 2.0435 |
| 6. | 22.75 | 7.0425 | " | 2.1447 |
| 7. | 24.10 | 7.2772 | 0.0126582 | 2.3517 |
| 8. | 24.80 | 7.0441 | " | 2.5001 |
| 9. | 24.75 | 7.0059 | " | 2.5087 |

For all determinations a mean of 2.354 mg and a standard deviation of 0.264 over a range of 2.0435-2.8534 mg was calculated. The mean acid value obtained, compares favourably with that of avocado oils in California which had a value of 2.8 [12]. The slight difference expected in acid value between U.S. and Kenyan avocado fruits, can be attributed to differences in enviromental conditions.

## 3:5 SAPONIFICATION VALUE AND SAPONIFICATION EQUIVALENT

In the determination of alkali factor, S, for this analysis, 0.5 N HCl was put in the burette and titrated against 25 ml of alcoholic KOH which was accurately pipetted into a conical flask. KOH reacts with HCl in the following way: HCl + KOH $\longrightarrow$ KCl + $H_2O$. One mole of KOH reacts with one mole of HCl. The volumes obtained in three titrations were 23.80 ml, 23.55 ml and 23.65 ml, these had a mean of 23.65 ml and a standard deviation of 0.1080. The amount of HCl present in 23.65 ml was 0.5 x 23.65 moles. An equivalent amount of KOH in 25 ml was therefore used to neutralize HCl. Hence, the alkali factor, S, was 23.65/25 x 0.5 = 0.473 N.

In determining the oil's dry weight, a solution of oil in petroleum ether (40-60 $^{o}$C) was prepared. The actual amounts used were 10.0039 g of oil in 100 ml of petroleum ether. 10 ml of this solution was pipetted into a weighed bottle from which the petroleum ether was evaporated and dried in an oven (110 $^{o}$C) to a constant weight. The dry weights obtained are shown in the first column of table 12. Concurrently, for each dry weight determined, 10 ml of the solution was refluxed as described in section 2:7. The refluxed solution was titrated with standard 0.5 N HCl to give the amount of unreacted KOH. A similar titration against a blank (no oil) was performed. The difference between the acid volumes used against the oil solution and the blank was divided by the dry weight of the oil then multiplied by 56.1 (molecular weight of KOH) and by S, the alkali factor. Saponification values (S.V.) and values for saponification equivalent (S.E.) were calculated using the equations shown below. Results are shown in table 12. The saponification values obtained had a range of 179.9141-192.0887, a mean of 186.761 and a standard deviation of 3.523. This small standard deviation is an indicator of high precision of the experiments.

Table 12. Saponification and Saponification Equivalent values.

| Dry weight of oil(g) | Volume of 0.5 N HCl.(ml) | | Saponification Value | Saponification Equivalent |
|---|---|---|---|---|
| | Blank | Solution | | |
| 1. 0.9808 | 23.65 | 16.55 | 192.08873 | 292.05253 |
| 2. 0.9808 | 23.65 | 16.60 | 190.73599 | 294.12383 |
| 3. 0.9808 | 23.65 | 16.75 | 186.67778 | 300.51782 |
| 4. 0.9808 | 23.40 | 16.55 | 185.32504 | 302.71138 |
| 5. 0.9808 | 23.60 | 16.60 | 189.38325 | 296.22471 |
| 6. 0.9808 | 23.40 | 16.75 | 179.91409 | 311.81548 |
| 7. 0.9895 | 23.40 | 16.60 | 183.97230 | 304.93721 |
| 8. 0.9895 | 23.50 | 16.60 | 185.03645 | 303.18350 |
| 9. 0.9895 | 23.65 | 16.65 | 187.71814 | 298.85231 |

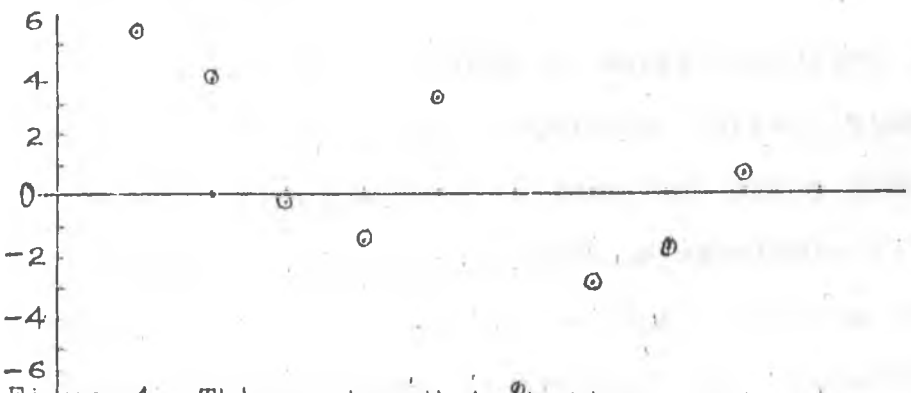


Figure 4. The random distribution about zero, for saponification value.

The calculated saponification values are close to those obtained in Peru (185.1-197.7) [7], Phillipines (193-194) [13] and California 192.6

[12]. The differences observed in different regions of the world suggest possible enviromental influences. Values on the last column of table 12 were obtained through dividing 56100 by the saponification value. S.E = 56100/S.V. These had a range of 292.05-311.815, a mean of 300.491 and a standard deviation of 5.704. Saponification value is given by the following equation. S.V. = $(v_b - v_w)/w \times 56.1 \times S$. Where, $v_b$ and $v_w$ are acid volumes required to neutralize KOH in the blank and in the saponification mixture respectively. w, is the dry weight of oil and S, the alkali factor. The random distribution about zero (Figure 4), suggests good reproducibility.

## 3:6 DETERMINATION OF UNSAPONIFIABLE MATTER

Avocado oil stands in marked contrast to the numerous commercial vegetable oils, since in cosmetic preparations it does not serve simply as a cosmetic base but is used as vegetable oil with the character of an active agent because of its special ingredients. Avocado oil possesses a relatively high spreading ability for a vegetable oil, this is due to its contents of phytosterols and vitamins, which form part of the unsaponifiable matter.

A 4.0100 g sample of oil extracted from 10 avocado fruits was carefully taken through the process described in section 2:8. The final product weighed 0.0600 g which is actually 1.4963 %. A 1.0-1.1 % range has been reported for oil extracted from Phillipine grown avocado fruits [13]. In 1986 a product specification by Wirkstoff CLR (a company in Germany) indicated that avocado oil contains 5 g of phytosterols, approximately 20,000 I.U. vitamin A, 40,000 I.U. vitamin D and 300 I.U. of vitamin E [47]. These quantities were from 20-40 g of unsaponifiable matter produced by 1 kg of avocado oil CLR. A special reference was made on the effect of avocado oil on dry and scaly skin. Avocado oil preparation was further recommended for treatment of parasitic skin damage and eczemas. In this connection it was reported that avocado oil accelerates the formation of crusts and cicatrization of wounds [47]. Preliminary work indicated slow healing of mild backache, minor cuts, skin-related defects and athlete's foot (dermatophytosis). Avocado oil from our laboratory is currently being applied to a bald head. For the last six months monitoring of hair growth show positive results [48]. More work needs to be done on this area.

## 3:7 DETERMINATION OF IODINE VALUE

In order to standardize sodium thiosulphate solution, approximately 0.1 N sodium thiosulphate solution was put in a burette and titrated against 20 ml of 0.1 N standard potassium dichromate solution in a 500 ml conical flask. These two were prepared as described in section 2:7:1 and 2:7:2 respectively. Solution starch was used as the final indicator. The colour changes were from, a clear brown solution to bluish black then finally to pale green solution, at the end point. Four titrations were carried out and they gave: (i) 19.60 ml, (ii) 19.60 ml, (iii) 19.80 ml and (iv) 19.65 ml titres. These had a mean of 19.66 ml. So the factor, I, or the concentration of sodium thiosulphate solution used was: I = 0.1 x 20/19.66 = 0.1017729 N. As a result of the procedure outlined in section 2:5, iodine values were calculated (table 13). The iodine values obtained had a range of 82.5129-95.4299, a mean of 87.8091 and a standard deviation of 3.8916. These values are comparable with those for Peruvian avocado pears, having a range of 70.6-76.4 [7]. Varieties grown in California had an iodine value of 94.4 [12]. Oil from avocado fruits grown in Phillipines

had an iodine value of 95.4 [13] and varieties harvested in Jujuy Tucuman and Mendoza provinces of Argentina an iodine value of 77.98 [15].

Table 13. Iodine value determined from eight oil samples.

| | Volume of sodium thiosulphate solution used against (ml) | | weight of oil (g) | Iodine value[a] (g/100g) |
|---|---|---|---|---|
| | Blank | Sample | | |
| 1. | 38.20 | 26.90 | 0.1700 | 86.499326 |
| 2. | 38.20 | 27.20 | 0.1570 | 91.175097 |
| 3. | 38.40 | 27.90 | 0.1608 | 84.974077 |
| 4. | 38.40 | 29.40 | 0.1381 | 84.807065 |
| 5. | 38.40 | 30.10 | 0.1309 | 82.512861 |
| 6. | 38.40 | 29.50 | 0.1329 | 87.146154 |
| 7. | 38.20 | 29.70 | 0.1230 | 89.928431 |
| 8. | 38.20 | 29.40 | 0.1200 | 95.429935 |

[a] see the experimental section for calculation of iodine value.

Thus, iodine values obtained in this work compare favourably with those obtained elsewhere. The slight differences in iodine values determined in separate regions of the World, may be attributed to variation of climate. Q-test was used for

retention or rejection of values given on the last column of table 13 at 96 % confidence level.

## 3:8 DENSITY MEASUREMENTS

In order to compare and contrast density data on avocado oil from other parts of the world with that obtained from Kenya, the experiment described in section 2:4 was carried out. The results of the experiment are shown in table 14. For each oil sample used in these measurements, an average of four fruits was used.

Table 14. Density measurements.

| | Weight of Pycn.(g) | Weight of Pycn/oil (g) | Weight of oil (g) | Density (g/ml) |
|---|---|---|---|---|
| 1. | 11.3585 | 13.0627 | 1.7042 | 0.8521 |
| 2. | 11.3560 | 13.0622 | 1.7062 | 0.8531 |
| 3. | 11.3565 | 13.0624 | 1.7059 | 0.85295 |
| 4. | 11.3493 | 13.0610 | 1.7115 | 0.85295 |
| 5. | 11.3487 | 13.0610 | 1.7123 | 0.85615 |
| 6. | 11.3508 | 13.0620 | 1.7112 | 0.8556 |
| 7. | 11.3535 | 13.0617 | 1.7082 | 0.85395 |
| 8. | 11.3537 | 13.0618 | 1.7081 | 0.85405 |
| 9. | 11.3530 | 13.0612 | 1.7082 | 0.8541 |
| 10. | 11.3534 | 13.0613 | 1.7079 | 0.85395 |

The volume of the pycnometer (pycn) used was 2 ml. A temperature of 25 $^{o}$C was maintained using a thermostat. This experiment gave a density range of 0.8521-0.85615 with a mean of 0.85389 and a standard deviation 0.00117. The density observed in this work can be compared with the density range of 0.9124-0.9139 obtained at 15 $^{o}$C in Peru [7]. Q-test was employed for retention or rejection of calculated density at 96 % level of confidence (table 14).

## 3:9 REFRACTIVE INDEX OF AVOCADO OIL

Refractive index is a measure of how much a ray of light bends when passing from one medium to another. Density is a determining factor in the refractive index of a medium. Addition of different components to oil, a common process in industries, will generally change density of the oil and in effect alter its refractive index. So, a rapid method for process control, is the measurement of refractive index. In this research project, results of refractive index were obtained using ABBE's refractometer at 25 $^{o}$C. The values are recorded in table 15. Each reading was for an oil sample obtained from ten avocado fruits.

Table 15. Average refractive index from four samples of avocado oil.

| | Refractive index reading | | |
|---|---|---|---|
| Run No. | From above | From below | Average |
| 1. | 1.4655 | 1.46575 | 1.4653 |
| 2. | 1.4660 | 1.4655 | 1.4658 |
| 3. | 1.4640 | 1.4640 | 1.4640 |
| 4. | 1.46475 | 1.4645 | 1.4646 |

Readings were taken from above and below an intersection of the refractometer's hair-line. They gave a range of 1.4640-1.46575, a mean of 1.46475 and a standard deviation of 0.000630872. The results observed differ only slightly from those measured elsewhere. A chemical study of avocado oil in Phillipines indicated $n_{30.5}$ of 1.4682-1.4687 [13]. Oil from avocado grown in California and Florida had an average refractive index $n_{20}$ of 1.4700 [12] and investigations in Peru revealed a range of n between 1.4654-1.4662 [7]. The difference in readings for these regions of the world, suggests a need to carry out similar work for different world zones that grow avocado fruits. The differences, possibly, indicate an influence of the enviroment.

## 3:10 VAPOUR PRESSURE DETERMINATION

Variations of vapour pressure and temperature were used in calculations of the enthalpy of vapourization and the boiling point of avocado oil. An oil sample from a homogeneous mixture of oil extracted from five avocado fruits was used for this experiment. For each temperature reading, a difference in the mercury heights between two arms of the manometer was calculated (table 16).

Table 16. Variation of vapour pressure with temperature.

| | Temp.t ($^{o}C$) | 1/T ($x10^{-3}K^{-1}$) | P(1) (cm) | P(2) (cm) | P(3)[a] (cm) | P atm. ($x10^{-3}$atm) | lnP |
|---|---|---|---|---|---|---|---|
| 1. | 25.4 | 3.35 | 40.3 | 38.5 | 1.8 | 23.7 | -3.74 |
| 2. | 30.2 | 3.30 | 40.4 | 38.4 | 2.0 | 26.3 | -3.64 |
| 3. | 35.2 | 3.24 | 40.4 | 38.4 | 2.0 | 26.3 | -3.64 |
| 4. | 40.2 | 3.19 | 40.6 | 38.1 | 2.5 | 32.9 | -3.41 |
| 5. | 45.2 | 3.14 | 41.0 | 37.9 | 3.1 | 40.8 | -3.20 |
| 6. | 49.7 | 3.10 | 41.3 | 37.6 | 3.7 | 48.7 | -3.02 |

[a] P(3) = P(1) - P(2) and P = P(3)/760

The results of table 16 were used to plot lnP versus 1/T (Figure 5). The slope from this figure

had a value of -2851.872. This slope is equal to -DH/R (see equation 1-12). Substituting for the value of R, gives the enthalpy of vapourization DH. $-DH = 8.314 \times -2851.872 = -23710.463$ $JK^{-1}mol^{-1}$. Extrapolation of the plot to 1 atmosphere gave the boiling point of avocado oil as 223.574 $^{o}C$ (boiling point without decomposition). At the time this work was done no literature was available for the boiling point and for the heat of vapourization, of avocado oil. Literature search for these two parameters continues in order to complement the values obtained in this work. A boiling point determination done through the normal process, in the open, gave a boiling point range of 232-244 $^{o}C$. Most of the oil distilled at over 238 $^{o}C$, however, decomposition of the oil took place and a repugnant smell replaced the avocado natural fragrance. This information could be very important especially with regard to the use of avocado oil as a substitute for the ordinary vegetable cooking oil. The sharp repulsive smell of avocado oil at high temperatures is a negative effect towards the use of this oil for domestic cooking.

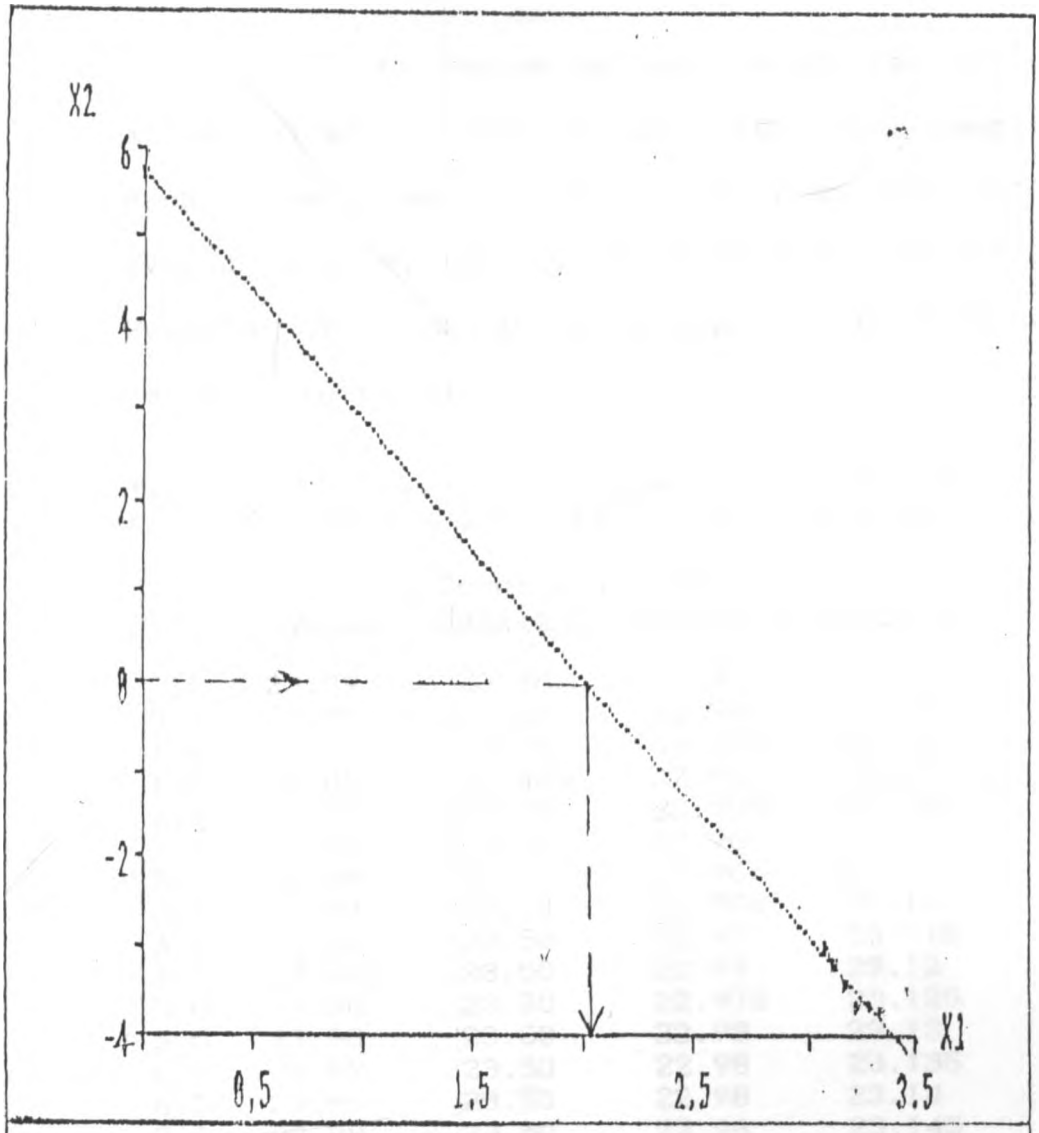



Figure 5. Plot of ln P (X2) versus $\frac{1}{T(Kx10^{3})}$ (X1).

## 3:11 CALORIFIC VALUE

Calorific value estimation was carried out as described in section 2:9. Four oil samples, each taken from oil extracted from ten mature avocado fruits, were used in this determination. Results of temperature changes with time are recorded in table 17.

Table 17. Variation of temperature with time before and after firing the Bomb.

| Time (min) | Temperature ($^{o}C$) Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| 0.0 | 23.89 | 23.48 | 22.935 | 23.04 |
| 0.5 | 23.89 | 23.48 | 22.94 | 23.05 |
| 1.0 | 23.89 | 23.48 | 22.945 | 23.06 |
| 1.5 | 23.89 | 23.485 | 22.95 | 23.07 |
| 2.0 | 23.89 | 23.50 | 22.955 | 23.08 |
| 2.5 | 23.89 | 23.50 | 22.96 | 23.09 |
| 3.0 | 23.89 | 23.50 | 22.96 | 23.10 |
| 3.5 | 23.89 | 23.50 | 22.965 | 23.11 |
| 4.0 | 23.89 | 23.50 | 22.97 | 23.115 |
| 4.5 | 23.885 | 23.50 | 22.97 | 23.12 |
| 5.0 | 23.88 | 23.50 | 22.975 | 23.125 |
| 5.5 | 23.88 | 23.50 | 22.98 | 23.13 |
| 6.0 | 23.88 | 23.50 | 22.98 | 23.135 |
| 6.5 | 23.88 | 23.50 | 22.98 | 23.14 |
| 7.0 | 23.88 | 23.50 | 22.98 | 23.145 |
| 7.5 | 23.88 | 23.50 | 22.985 | 23.145 |
| 8.0 | 23.875 | 23.50 | 22.985 | 23.15 |
| 8.5 | 23.875 | 23.50 | 22.985 | 23.155 |
| 9.0 | 23.87 | 23.50 | 22.99 | 23.155 |
| 9.5 | 23.87 | 23.50 | 22.99 | 23.16 |
| 10.0 | 23.87 | 23.50 | 22.99 | 23.16 |
| 10.5 | 24.13 | 23.55 | 23.005 | 23.17 |
| 11.0 | 24.55 | 24.13 | 23.095 | 23.23 |
| 11.5 | 25.15 | 24.75 | 23.29 | 23.32 |
| 12.0 | 25.55 | 25.15 | 23.46 | 23.48 |
| 12.5 | 25.75 | 25.38 | 23.64 | 23.68 |
| 13.0 | 25.92 | 25.53 | 23.80 | 23.76 |
| 13.5 | 26.03 | 25.62 | 23.91 | 23.875 |
| 14.0 | 26.11 | 25.68 | 24.03 | 23.99 |

Table 17 continued.

| Time | Temperature (°C) | | | |
|---|---|---|---|---|
| (min) | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
| 14.5 | 26.18 | 25.735 | 24.135 | 24.10 |
| 15.0 | 26.22 | 25.765 | 24.215 | 24.175 |
| 15.5 | 26.28 | 25.795 | 24.30 | 24.26 |
| 16.0 | 26.30 | 25.815 | 24.365 | 24.345 |
| 16.5 | 26.315 | 25.835 | 24.445 | 24.42 |
| 17.0 | 26.33 | 25.85 | 24.51 | 24.505 |
| 17.5 | 26.345 | 25.86 | 24.57 | 24.545 |
| 18.0 | 26.35 | 25.87 | 24.635 | 24.62 |
| 18.5 | 26.365 | 25.875 | 24.705 | 24.695 |
| 19.0 | 26.38 | 25.88 | 24.755 | 24.745 |
| 19.5 | 26.385 | 25.89 | 24.81 | 24.795 |
| 20.0 | 26.39 | 25.89 | 24.855 | 24.85 |
| 20.5 | 26.395 | 25.89 | 24.905 | 24.90 |
| 21.0 | 26.40 | 25.89 | 24.945 | 24.945 |
| 21.5 | 26.405 | 25.89 | 24.995 | 24.995 |
| 22.0 | 26.405 | 25.895 | 25.03 | 25.04 |
| 22.5 | 26.41 | 25.895 | 25.07 | 25.08 |
| 23.0 | 26.405 | 25.89 | 25.11 | 25.135 |
| 23.5 | 26.405 | 25.89 | 25.145 | 25.18 |
| 24.0 | 26.405 | 25.89 | 25.18 | 25.22 |
| 24.5 | 26.405 | 25.89 | 25.21 | 25.25 |
| 25.0 | 26.405 | 25.89 | 25.25 | 25.29 |
| 25.5 | 26.405 | 25.89 | 25.275 | 25.335 |
| 26.0 | | 25.89 | 25.29 | 25.365 |
| 26.5 | | | 25.325 | 25.395 |
| 27.0 | | | 25.34 | 25.43 |
| 27.5 | | | 25.36 | 25.46 |
| 28.0 | | | 25.38 | 25.48 |
| 28.5 | | | 25.39 | 25.52 |
| 29.0 | | | 25.40 | 25.54 |
| 29.5 | | | 25.42 | 25.57 |

Results in table 17 above, were used in a plot of temperature versus time which provided the temperature changes that were used for calculation of values shown in table 18. The calorific values which are recorded as the heat of combustion, were finally calculated from values in the first six columns of table 18.

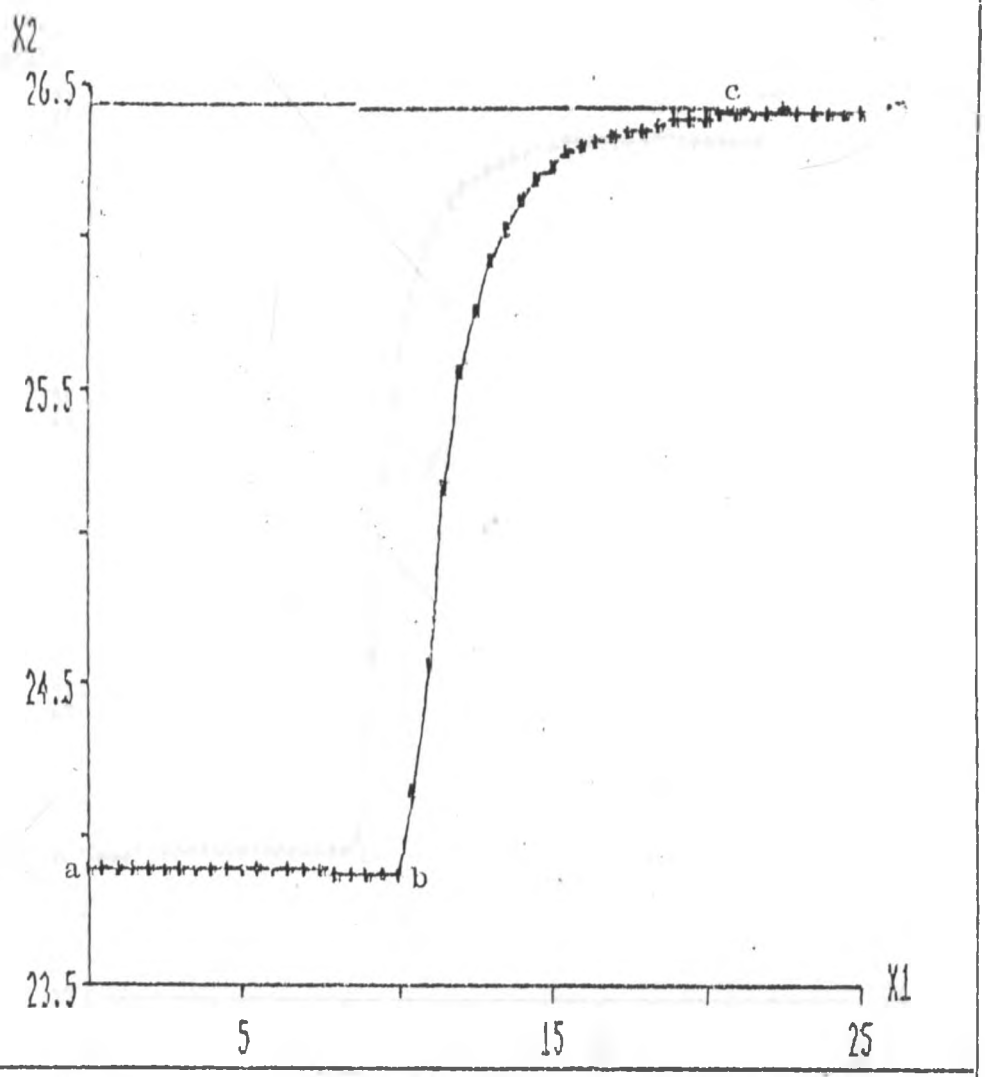



Figure 6. Variation of temperature with time (Sample 1).

Where X2 represents temperature (°C), X1 represent time (minutes). a-b represents time before firing. Point c shows constant temperature after firing.

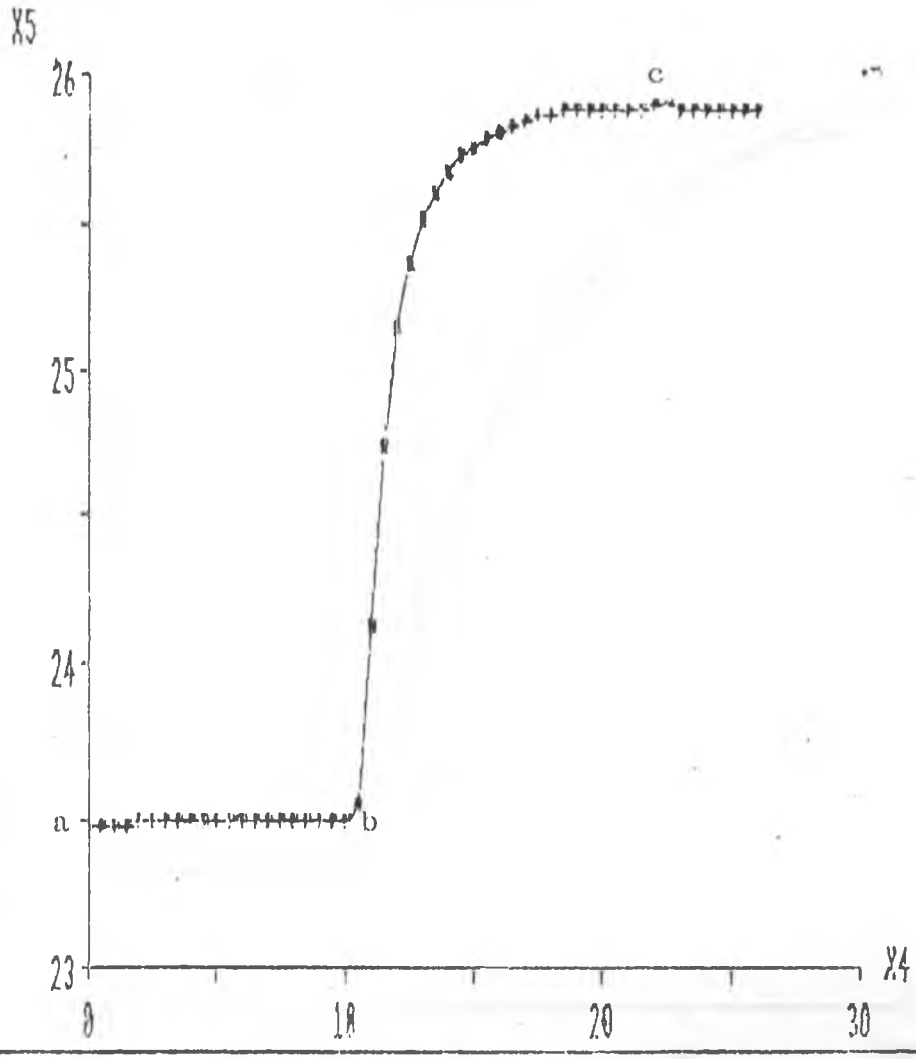



Figure 7. Variation of temperature (X5 in °C) with time (X4 in minutes) (Sample 2).

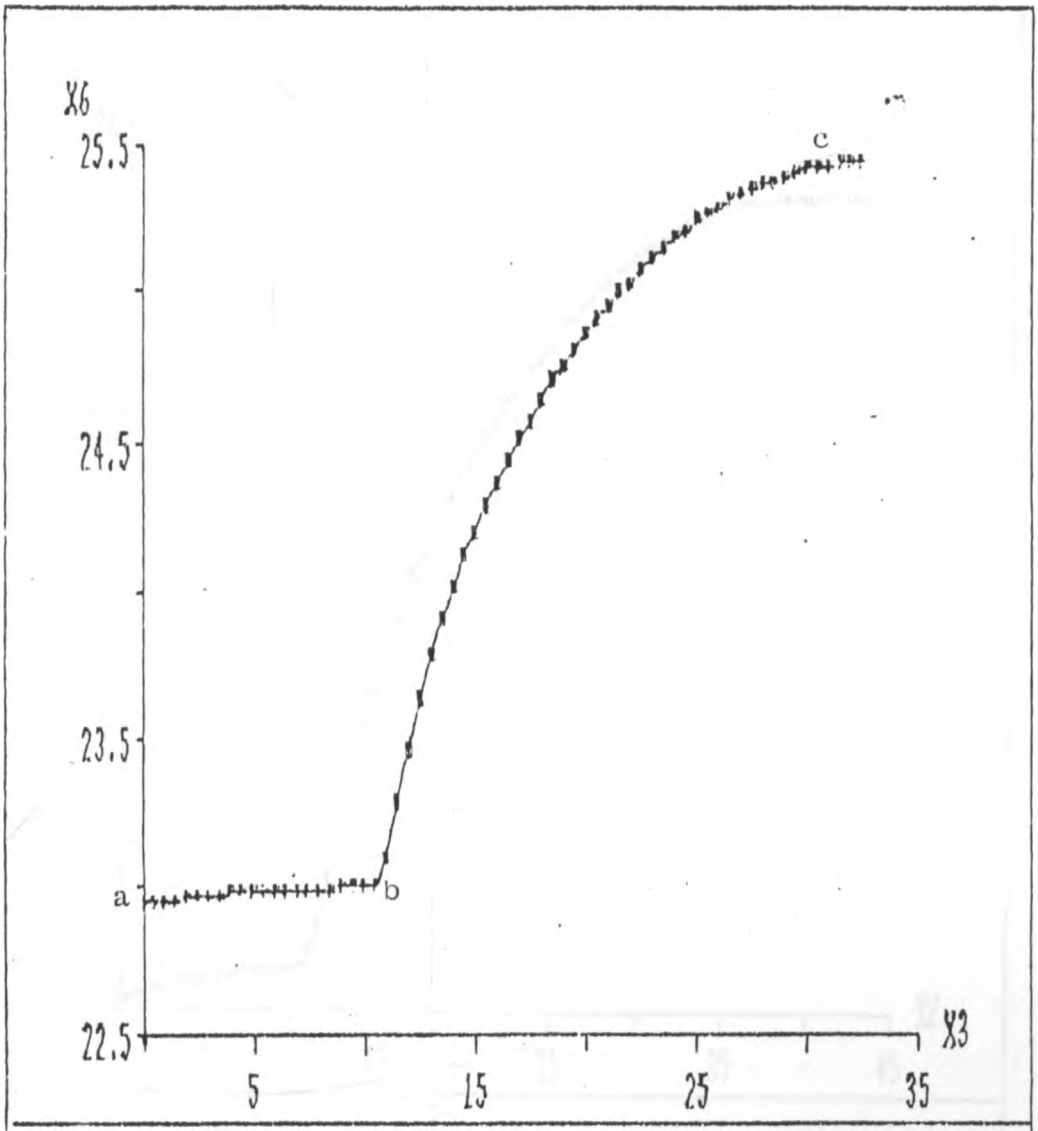



Figure 8. Variation of temperature (X6 in °C) with time (X3 in minutes) (Sample 3).

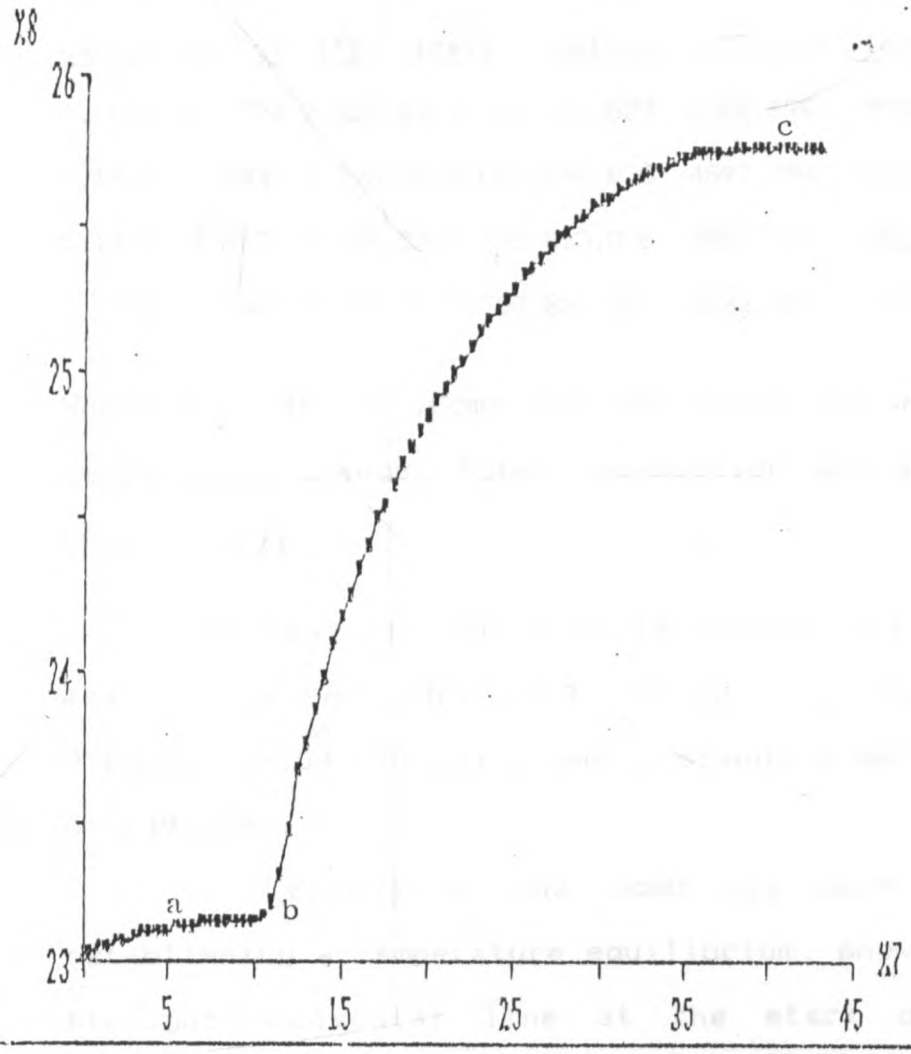



Figure 9. Variation of temperature (X8 in °C) with time (X7 in minutes) (Sample 4).

Table 18. Calculated calorific values.

| Wt. of cap(g) | Wt. of oil(g) | DT. (°C) | Tt. heat (cal) | Heat by cap(cal) | Heat by oil(cal) | Heat of Comb (cal/g) |
|---|---|---|---|---|---|---|
| 0.1622 | 0.5224 | 2.535 | 5919.225 | 718.529 | 5200.696 | 9955.390 |
| 0.1577 | 0.4847 | 2.396 | 5594.660 | 696.876 | 4897.784 | 10104.773 |
| 0.1840 | 0.5079 | 2.485 | 5802.475 | 813.096 | 4989.379 | 9823.546 |
| 0.1888 | 0.5445 | 2.620 | 6117.700 | 834.307 | 5283.392 | 9703.201 |

Where Wt., DT, Tt.,Comb and cap. stand for weight, temperature change, Total, combustion and capsule respectively.

The heat of combustion of avocado oil had a mean value of 9896.7273 cal/g, a range of 9703.201-10104.773 cal/g and a standard deviation of 149.6097.

Firing of the bomb was done after establishing a temperature equilibrium, shown as a straight horizontal line at the start of the experiment (Figure 6, 7, 8 and 9). The exact temperature change DT (column 3, table 18) was calculated from the difference between the initial constant temperature (before firing) and the final constant temperature (after firing) (Figure 6, 7, 8 and 9). Section 2:9 describes how the heat of combustion was calculated using the equation -q =

W x DT, where W is the heat capacity of the calorimeter, DT the temperature change and q the heat of combustion. The values in table 18 are related as shown, for one row. A capsule that weighs 0.1626 g (c1), when completely burnt to carbon dioxide and water gives out 0.1626(c1) x 4419 = 718.5294 cal (c5) (where c stands for column). The total heat evolved in this experiment was calculated using the equation -q = W x DT which gave:-q = 2335 x 2.535(c3) = 5919.225 cal (c4), the heat contributed by avocado oil alone was 5919.225 (c4) - 718.5294 (c5) = 5200.6956 cal (c6). Lastly, the heat of combustion of avocado oil was 5200.6956 (c6) / 0.5224 (c2) = 9955.3897 cal/g (c7). Values of the other rows were obtained in a similar way. The calorific value from this research project had a range of 9703.2007-10104.773 cal/g, a mean of 9896.7273 cal/g (9.9 k cal/g) and a standard deviation of 149.6097. This shows good reproducibility of the experiment. The calorific value obtained for the avocado oil from fruits grown in Kenya, is an important piece of information. At the time of compiling this thesis, literature on calorific value of avocado oil was not available. It was interesting to see how other foods compared with avocado oil in terms of their

calorific values. Glucose ($C_6H_{12}O_6$), a carbohydrate, has an average value of 3.72 kcal/g and glyceryl trimyristate ($C_{45}H_{86}O_6$), a fat, has a value of 9.20 kcal/g [41]. The average calorific values quoted for vegetable oils is about 44 kJ/g [49] (1 calorie = 4.1840 Joules). This indicates that oil from Kenyan avocado fruits has a calorific value close to the average value for fats. Cheese has a calorific value of 4.7 kcal/g, one gram of eggs contains 1.4 kcal. It has been estimated that a person of average weight, running or jogging, needs to consume about 100 cal/mile [41]. One gram of avocado oil, taken by this person, would therefore, supply Him/Her with enough energy for about 100 miles. Avocado oil has a calorific value twice that of cheese and about eight times that of eggs. Instead of an expensive breakfast of cheese and eggs, a table spoonfull of avocado oil would, more than adequately, supply the needed calories. Avocado fruits have a high percentage of oil, the digestibility of which has been found to be equal to that of other oils. In light of the high oil content and the oil's digestibility, Jaffa and Albro suggested, as a conservation measure, the use of the avocado pulp as a butter substitute [6].

## 3:12 VISCOSITY OF AVOCADO OIL

Viscosity is mostly expressed either as an absolute viscosity or as relative viscosity. Therefore, it was necessary to calculate both the absolute and the relative viscosities for the avocado oil samples. Time of flow for distilled water and avocado oil was recorded (table 19). Six oil samples (each from ten fruits) were used. Each oil sample took about 3 hours, at times it was necessary to repeat the whole exercise as a result of interruptions during the long wait. The density and viscosity values for distilled water were obtained from the Handbook of Chemistry and Physics [45].

Table 19. Measured time of flow for the oil samples and water.

| Run No. | Sample Time (seconds) | Distilled water Time (seconds) |
|---|---|---|
| 1. | 11730.0 | 451.8 |
| 2. | 11698.5 | 451.8 |
| 3. | 11680.0 | 451.7 |
| 4. | 11776.4 | 451.7 |
| 5. | 11772.0 | 451.8 |
| 6. | 11777.8 | 451.9 |

Information from table 19 is as follows:
Mean of 11739.116 (Std. dev. 39.149) for the sample and mean of 451.7833 (std. dev. 0.00695) for distilled water.

Viscosity of the oil samples was calculated from the expression $\eta = \eta_1 . td/t_1 d_1$ Where $\eta$, t and d are the absolute viscosity, time of flow and density of oil respectively. $\eta_1$, $t_1$ and $d_1$ are the absolute viscosity, time of flow and density of distilled water respectively. $d_{25}$ is the density of distilled water at 25 $^{o}C$, it is given as 0.99707g/ ml, $\eta_{25}$ is the viscosity of water at 25 $^{o}C$, it is given as 0.8937 centipoises [45].

$$\eta_{25} = \frac{11739.116 \times 0.854256 \times 0.8937}{451.7833 \times 0.99707}$$

$$= \underline{19.8957 \text{ centipoises}}.$$

19.8957 centipoises is the absolute viscosity of avocado oil at 25 $^{o}C$. Its viscosity relative to that of water is 22.2621, this is called the relative viscosity. It was obtained by omitting the absolute viscosity of water from the above calculation. The kinematic viscosity v is given by the absolute viscosity divided by the samples density, $v = \eta/D$. Substituting for $\eta$ and D gives $v = 19.8957 / 0.8542 = 23.2901$ stokes. By the time this work was performed, no literature

was available for avocado oil. However, viscosities of some fatty acids and oils are available, for example viscosity of Oleic acid $\eta_{30}$ = 25.6 centipoises, stearic acid $\eta_{70}$ = 11.6 centipoises, glycerine (glycerol) $\eta_{20}$ = 100 centipoises and Olive oil $\eta_{20}$ = 84 centipoises [44]. The moderate viscosity of avocado oil, renders it ideal for application in hair-setting, application on skin-related defects and incorporation in cosmetics and pharmaceutical products. Industries need to know viscosity parameters in order to design pipes with proper dimensions.

## 3:13 GAS CHROMATOGRAPHY OF FATTY ACID METHYL ESTERS FROM AVOCADO OIL

Two columns used for this work were (i) 15 % Apiezon-L stationary phase on chromosorb W AW 80/100 mesh and (ii) 6 % Silar 10c on chromosorb W HP 100/120 mesh. The Apiezon-L was initially fixed to Gow-Mac series 750 with an F.I.D. detector. Four peaks of methyl esters from the avocado oil were distinctively resolved (Figure 11). Their retention times were as shown in table 20.

Table 20. Fatty acid composition of avocado oil using Apiezon-L.

| Gow-Mac | | | Perkin Elmer | | |
|---|---|---|---|---|---|
| Retention time (Minutes) | | Area (%) | Retention time (Minutes) | Area (%) | Fatty Acid |
| 8.10 | 8.30 | 7.14 | 8.84 | 3.5672 | Stearic |
| 9.00 | 9.50 | 25.32 | 9.97 | 20.9445 | Palmitic |
| 19.60 | 19.90 | 64.94 | 20.45 | 75.3237 | Oleic |
| 20.90 | 21.00 | 2.60 | 22.78 | 0.1644 | Linoleic |

Using pure standard's retention times and the peak area enhancement (spiking), the four components of the fatty acids present in avocado oil were identified as shown in column six of table 20. The fifth column shows percentage peak areas as calculated by a G.C. computer (Figure 10).

In order to complement the Gow-Mac data, Perkin Elmer G.C. column packed with Silar 10c was used. This G.C. also separated the fatty acid methyl esters into four peaks (Figure 12). Perkin Elmer series 8500 Gas chromatograph was used, it was fitted with an F.I.D. detector. Results were as recorded in table 21. Similarly, using peak area enhancement method, the four components were identified as shown in the fifth column of table 21.

Table 21. Fatty acid composition of avocado oil using Perkin Elmer G.C. packed with Silar 10c.

| Peak No. | Retention time (min) and % Peak area in brackets [a]. | | | Fatty acid |
|---|---|---|---|---|
| | (i) | (ii) | (iii) | |
| 1. | 2.63(11.825) | 2.64(13.050) | 2.53(11.897) | Palmitic |
| 2. | 2.96(3.601) | 2.96(4.371) | 2.84(3.624) | Stearic |
| 3. | 4.21(76.026) | 4.18(72.847) | 4.04(75.075) | Oleic |
| 4. | 4.96(8.548) | 4.98(9.732) | 4.97(9.481) | Linoleic |

[a] (i), (ii) and (iii) represented number of replicates at different days.

Major differences between results from the two columns were (i) the retention times and (ii) the peak areas. For palmitic and stearic acid methyl esters, the former eluted faster than the latter in the Silar 10c column, but the retention times were reversed in the Apiezon-L column, where stearic eluted faster than palmitic acid methyl ester. The peak areas of oleic and stearic acid methyl esters were reproduced by the two columns: Apiezon-L gave 75.3237 % and 3.5672 % for the two fatty acid methyl esters respectively. Silar 10c gave mean peak areas of 74.6487 % and 3.8653 %

respectively for the two fatty acid methyl esters. Percentage areas of palmitic and linoleic acid methyl esters were different as seen from table 20 and 21. However, there is no doubt that palmitic had the larger peak area than linoleic acid methyl ester, and actually, palmitic was the second most abundant component after oleic acid methyl ester.

Gas Chromatography revealed presence of four fatty acids; palmitic, stearic, oleic and linoleic acids. Similar analysis done in other parts of the world, have come up with results that are not very different from those obtained in this research project. M.H. Bertoni and Co-workers, analysing different varieties of avocado fruits grown in Argentina, used Gas Chromatography of total fixed fatty acid methyl esters to obtain the following ranges: myristic trace 0.5 %, palmitic 18.7-30.80 %, palmitoleic 7.7-20.7 %, stearic 0.2-0.6 %, oleic 39.9-58.5 %, linoleic 9.5-17.7 % and linolenic acid 0.4-3.3 % [15]. The composition of fatty acids in oil from the pulp of avocado fruits grown in California and Florida, was as follows: oleic 77.3 %, linoleic 10.8 %, myristic trace, palmitic 6.9 %, stearic 0.6 % and arachidic acid trace. This was according to G.S. Jamieson et al [12]. A product specification from Bayer East

Africa Ltd., gave the fatty acid composition of avocado oil as follows: oleic 55-70 %, palmitic 14-20 %, palmitoleic 5-10 % and linoleic acid 8-15 % [27]; for avocado oil extracted by mechanical press (dried fruits). At the Israel Institute of Technology, Department of Food Engineering and Biotechnology, similar results were obtained by Gas chromatography (GLC) in a packed column. 1 % (w/v) of sulphuric acid in absolute methanol was used to prepare methyl esters. The fatty acid composition was as follows: palmitic (16:0) 11.80 %, palmitoleic (16:1) 2.18 %, Stearic (18:0) 0.68 %, oleic (18:1) 70.50 %, linoleic (18:2) 14.20 % and linolenic (18:3) 0.50 % [50]. Similar fatty acids have been found in other oil seeds [51].

METHOD 4 MODIFIED CALCULATION: %

| RT | AREA | BC | AREA % |
|---|---|---|---|
| 8.84 | 761.0575 | V | 3.5672 |
| 9.97 | 4468.4041 | V | 20.9445 |
| 20.45 | 16069.9328 | V | 75.3237 |
| 22.78 | 35.0809 | V | 0.1644 |

4 PEAKS > AREA/HT REJECT

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OD 4 MODIFIED

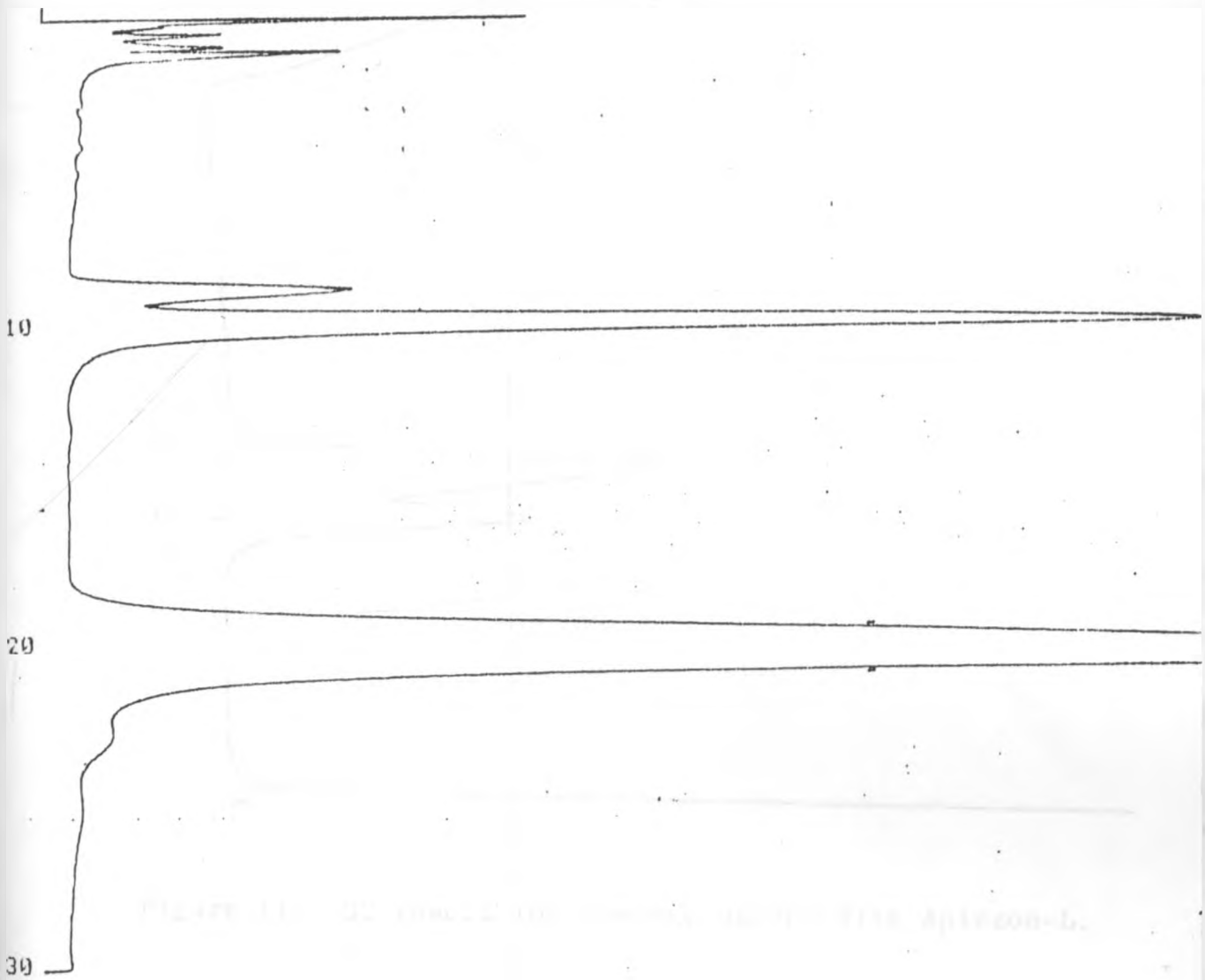


Figure 10. GC result for Perkin Elmer packed with Apiezon-L.

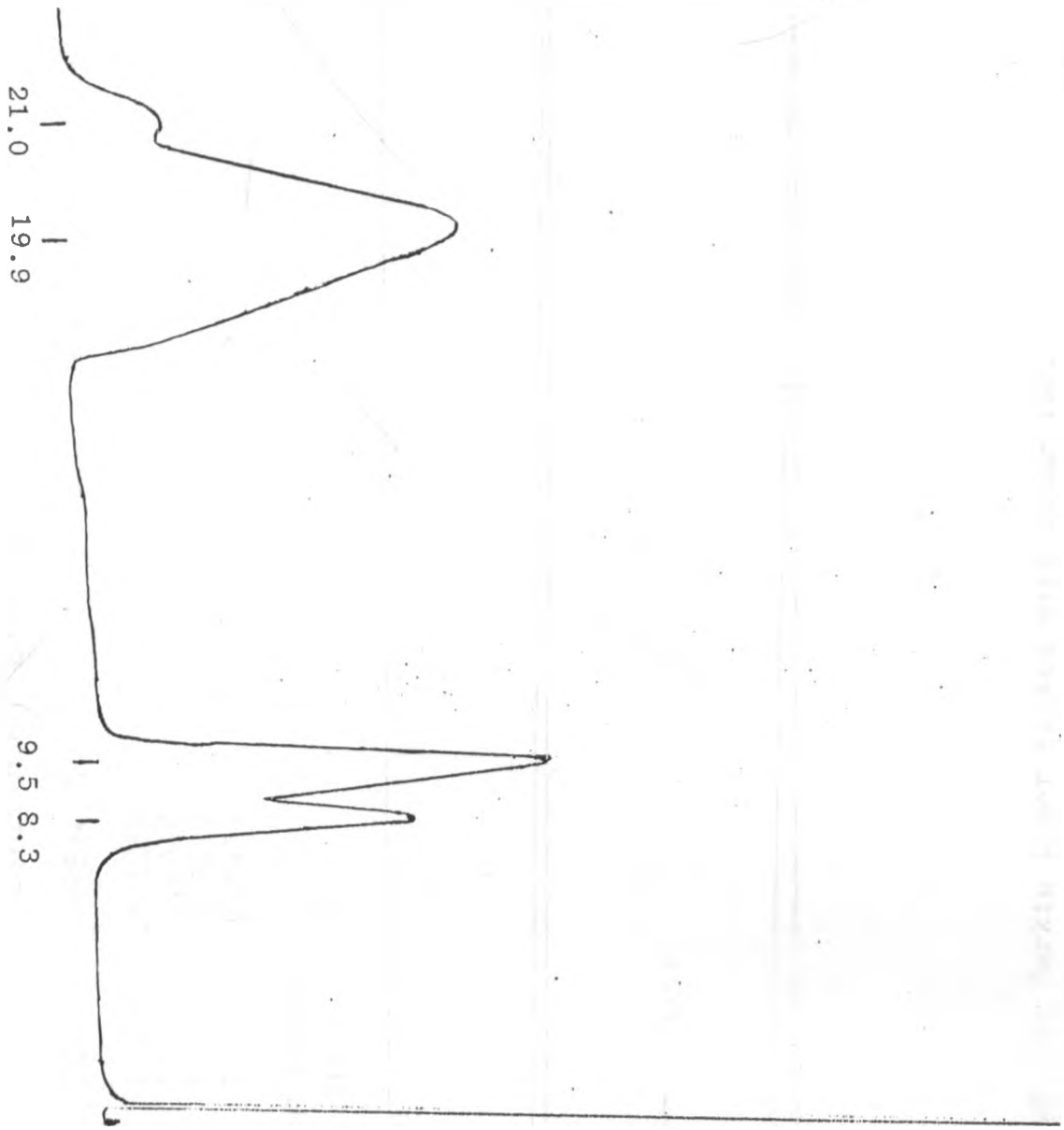


Figure 11. GC result for Gow-Mac packed with Apiezon-L.

METHOD CURRENT CALCULATION: %

| RT | AREA | BC | AREA % |
|---|---|---|---|
| 2.63 | 344.5185 | T | 11.8250 |
| 2.96 | 104.5441 | T | 3.6010 |
| 4.21 | 2215.3756 | T | 76.0260 |
| 4.96 | 249.1023 | T | 8.5477 |

4 PEAKS > AREA/HT REJECT

RUN 5 11:40 89/09/07

METHOD 4 MUTURI.AUD1

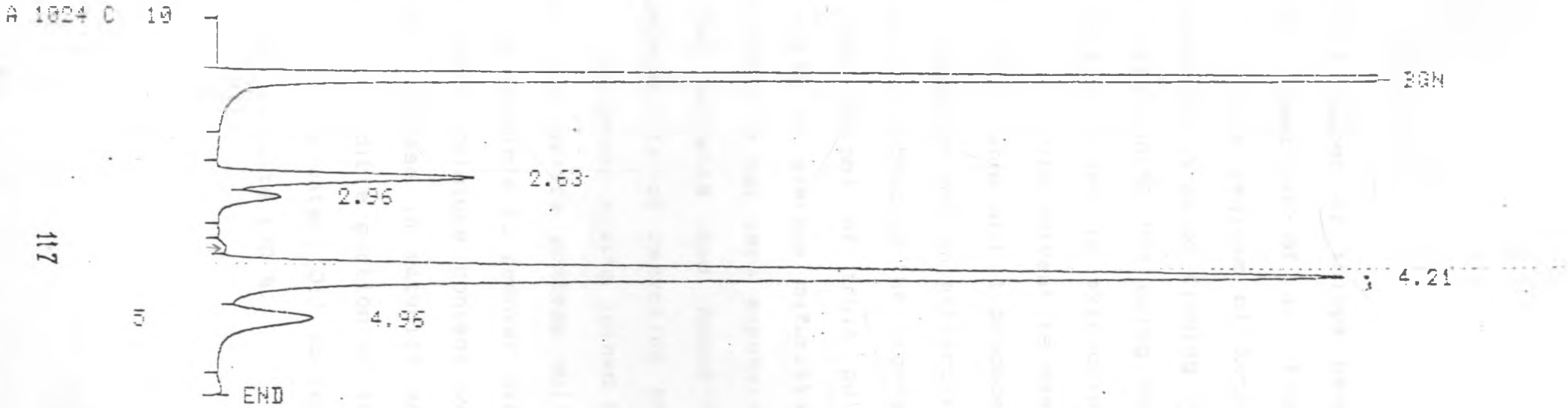


Figure 12. GC result for Perkin Elmer packed with silar 10c.

## CONCLUSION

In this work a number of things have surfaced regarding the properties of oil from avocado fruits grown in three regions of Kenya (Kitale, Kiambu and Murang'a). A major finding is about petroleum ether (PE) (40-60 $^{o}$C) being the best out of eight solvents tried in extracting avocado oil. Distillation of this solvent is easy due to its low boiling point range and it produces oil that has a natural fragrance and an attractive golden colour. This solvent extracted the highest amount of oil per given weight of fruit pulp (about 13.6 % from fruits of average maturity). Petroleum ether (40-60 $^{o}$C) is not very expensive when compared with other solvents used. Added to this fact is the possibility of recycling the solvent by setting up a condenser system joined to a receiver, this makes the entire process quite economical because it is possible to recover over 80 % of the solvent used. Moisture content was found to decrease with increase in maturity and actually about 67 % of the edible portion of the avocado fruit was found to be water. Oil content was highest for most mature fruits (30 %).

Parameters that were determined include: the acid value with a mean of 2.354 mg, mean saponification value of 186.761, mean saponification equivalent of 300.491, the unsaponifiable matter content was 1.49 %, iodine value had a mean value of 87.81, density had a mean of 0.85389 g/ml at 25 $^{o}$C, refractive index a mean of 1.46475 at 25 $^{o}$C, the enthalpy of vapourization as determined from Clausius Clapeyron equation was 23710.463 $JK^{-1}mol^{-1}$, boiling point was estimated using two different methods, one was without decomposition and gave a value of 223.57 $^{o}$C, the other was with decomposition, which gave a range of 232-244 $^{o}$C, most of the oil however distilled at over 238 $^{o}$C in the latter case. The energy value was determined by a complete combustion method and was found to have a mean value of 9896.73 Cal/g. The viscosity of avocado oil was determined by the Ubbelohde viscometer and the values; 19.8957 centipoises, 22.2021 and 23.2901 stokes were obtained for coefficient of viscosity, relative viscosity and kinematic viscosity respectively. The mean fatty acid composition of the oil obtained from two different instruments was: Palmitic 12.257%, stearic 3.865%, oleic 74.649 %,

linoleic 9.254 %, stearic 3.5672 % and palmitic 20.9445 %, oleic 75.3237 %, linoleic 0.1644 %. The two analyses were done on different G.C. instruments, Perkin Elmer series 8500 and a Gow-Mac series 750 respectively. F.I.D. detectors were used in both cases.

Preliminary work, using avocado oil extracted in this work, revealed possible application, particularly for, skin-related defects, minor cuts, mild backache, athlete's foot and hair recovery.

## RECOMMENDATIONS

This research project dealt on avocado oil from fruits grown in three regions of Kenya. Similar work should be done on fruits grown in other parts of our country that were not covered like Embu, Kirinyaga and Naivasha just to mention a few.

Out of the eight different solvents tried for cold solvent extraction process, petroleum ether (40-60 $^{o}$C) emerged with the ideal features. However, more work should be done on other solvents and even solvent mixtures.

The suggestion that oil content increases with increase in maturity has been supported by results of this project. But more indication would surface if many more maturity stages, than the four tried here, were investigated.

Decrease in moisture content with increase in maturity has tentatively been revealed by this work but it is imperative to examine more levels of maturity so that a comparision is drawn between moisture and oil content. This would add reason to the feeling that oil seems to replace water as the fruit matures.

Preliminary work has shown that avocado oil holds medicinal potency. More work needs to be done especially on the oil's ability to revive growth of hair on bald heads, treat skin-related defects, heal minor cuts and mild backaches. This work should also probe and propose a dosage and a style of application for each defect.

If avocado oil is going to be used for the afore mentioned needs, then it will be necessary to preserve it. This is a field for further work in search of preservative methods of avocado oil.

The field of cosmetics is another hot area in need of a prolonged life of avocado oil. Methods for deodorizing and perfuming avocado oil should be devised because if the oil can be a source of income, then, the idea is welcome.

For industrial purposes, oil extracted by solvents seem to be limited due to the solvent's volatility, a property that might lead to disastrous results. Thus, a more robust extraction process should be evolved specifically for our own avocado fruits. Centrifugal principle has been successfully employed in California and would be a good starting point.

It has been suggested by several workers that the magic of avocado oil lies within the unique content of its unsaponifiable matter. So it is very important, and interesting too, that work is done aimed at quantifying and characterizing the compounds present in the unsaponifiable matter. Additional information should be sort on how the contents of the unsaponifiable matter vary with maturity.

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# APPENDIX

App. 1 Infrared spectrum of avocado oil before (a) and after (b) distillation using SP3-300 Pye Unicam Spectrophotometer.

App. 2 Proton Nuclear Magnetic Resonance spectrum of avocado oil before distillation using Perkin Elmer R12 60 MHz instrument.

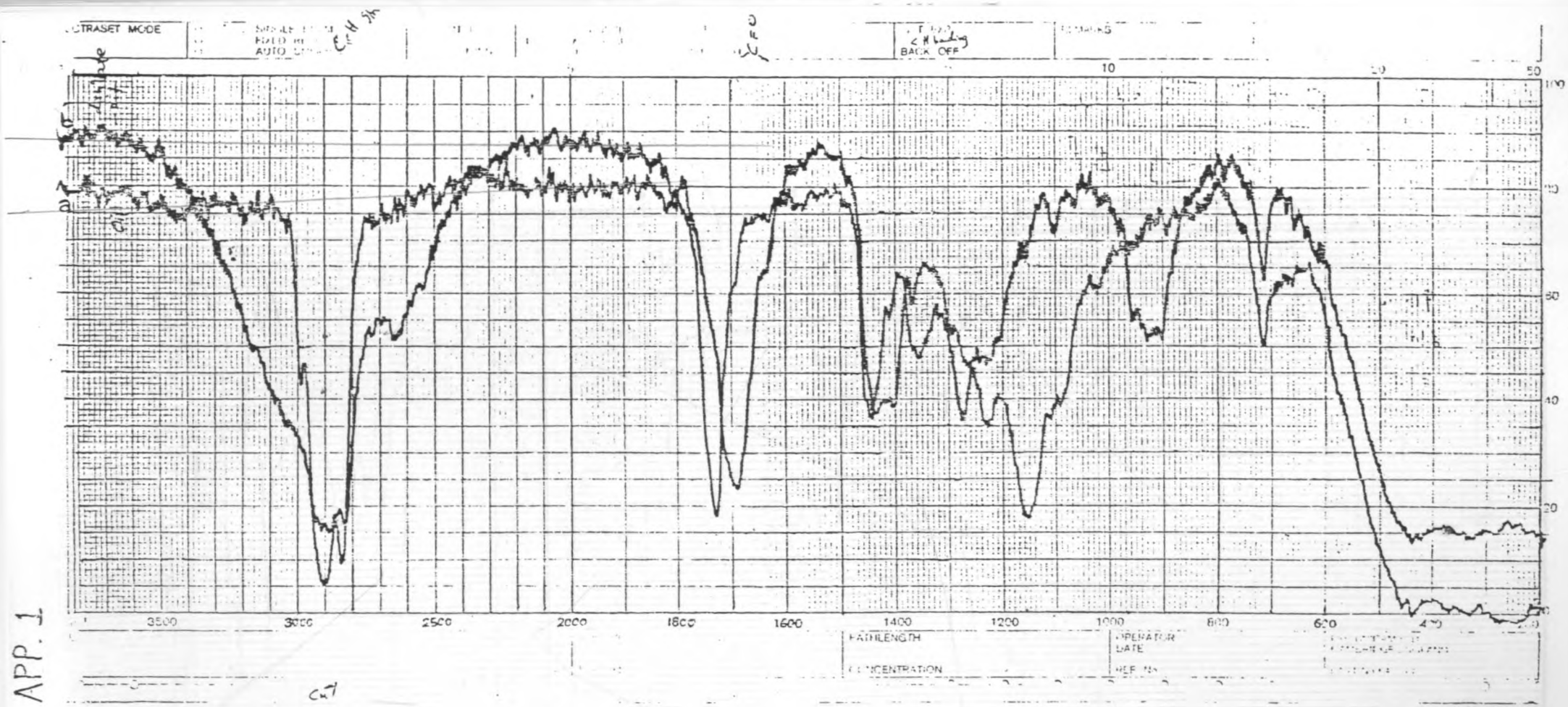
App. 3 Proton Nuclear Magnetic Resonance spectrum of avocado oil after distillation using Perkin Elmer R12 60 MHz instrument.

App. 4 Proton Nuclear Magnetic Resonance spectrum of avocado oil using a Bruker instrument.

App. 5 Carbon-13 Nuclear Magnetic Resonance spectrum of avocado oil using a Bruker instrument.

App. 5′ Carbon-13 Pulse Nuclear Magnetic Resonance spectrum of avocado oil using a Bruker instrument.

App. 6 Ultra violet spectrum of avocado oil in carbon tetrachloride before (a) and after (b) distillation using SP8-150 Pye Unicam Spectrophotometer.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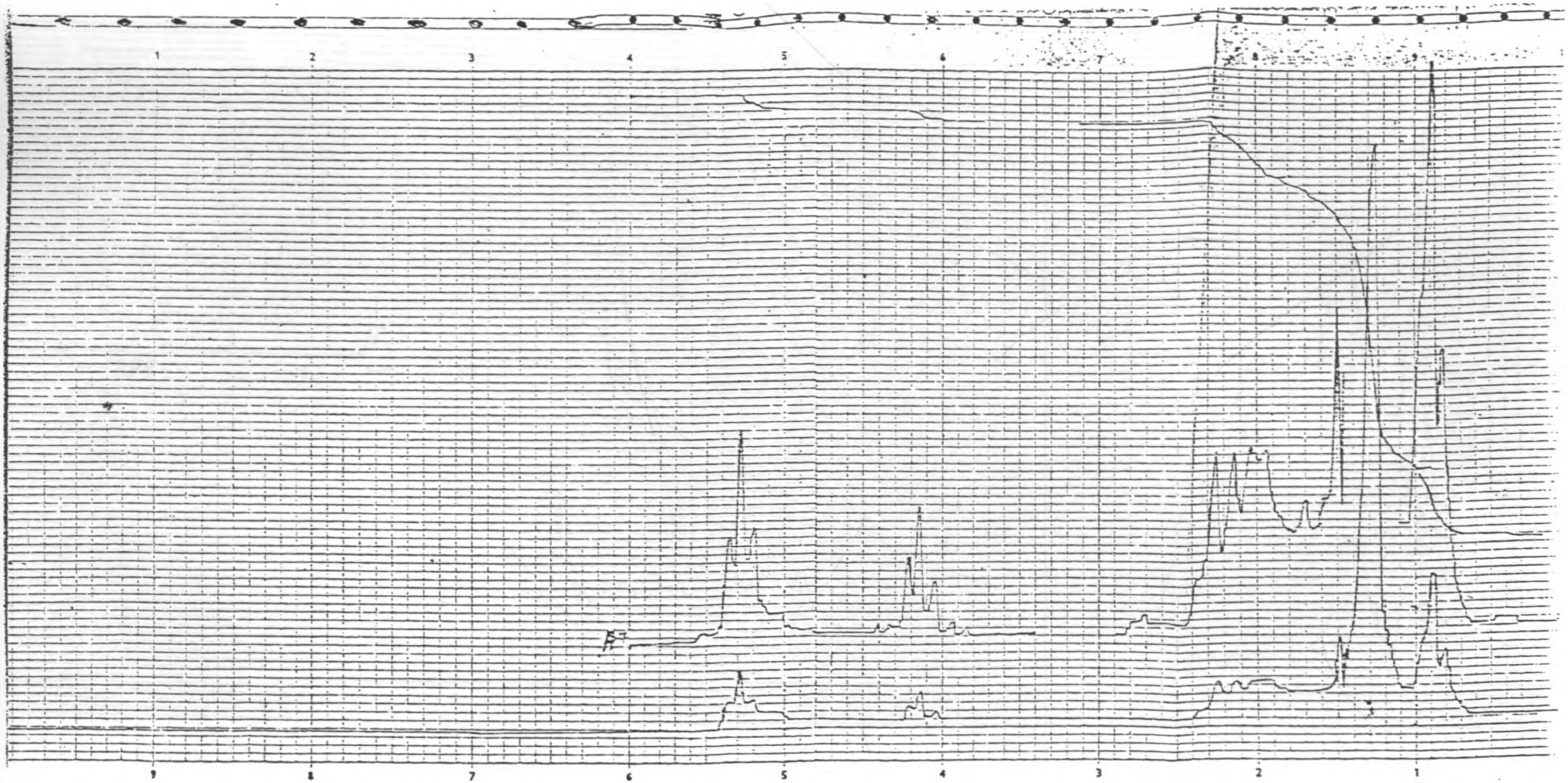
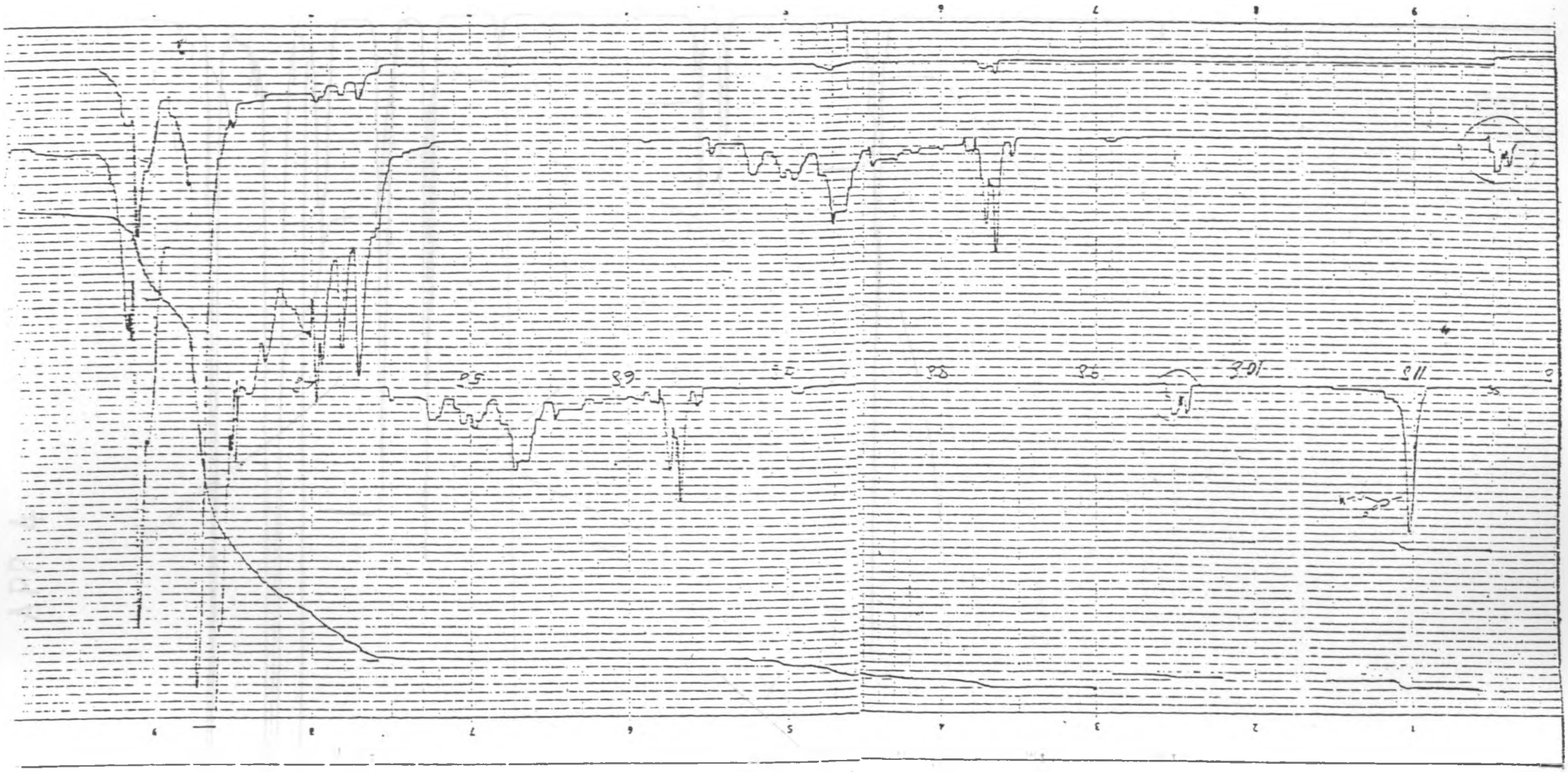
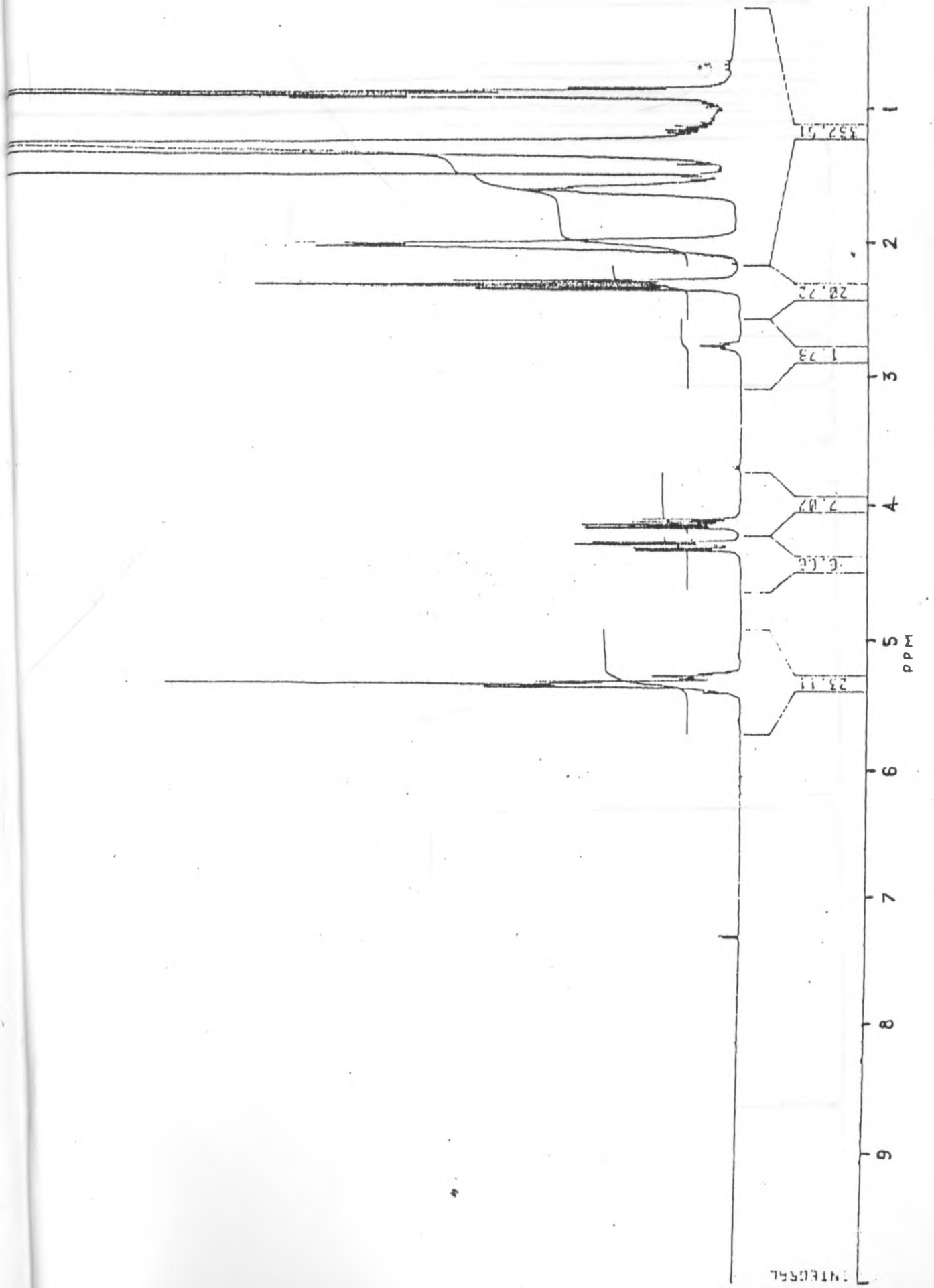
APP. 1



APP. 3



APP. 4



APP. 5

APP. 5'