CHEMICAL COMPOSITION OF AVOCADO FRUIT

BY

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented in any previous application for a higher degree. All quotations are indicated and the sources of information are specifically acknowledged by means of references.

A. S. EKOP

DATE: 11/7/83

DEDICATION

TO MY PARENTS

ACKNOWLEDGEMENT

I am very grateful to Dr. M.S. Kazmi who patiently supervised this work throughout the duration with interest, invaluable suggestions and encouragement. Through him, I have acquired a wider research techniques in Analytical Chemistry.

I also wish to acknowledge Dr. H.T. Bozimo, the Head of Chemistry department for making facilities available for this work.

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I am, indebted to my parents, wife and dependants who bore my long absence patiently and kindly, and to the College of Education, Uyo, for granting me the study leave.

A. S. EKOP

ABSTRACT

Some physical properties and chemical composition of avocado fruit (Persea amerlcana L) collected from different parts of Nigeria were Investigated. The fruits were Identified and grouped Into three ecological races(Mexican, West Indian and Guatemalan) for easy study. Physical parameters as mass, length,width, relative density and percentages of different major portions of each fruit were determined. The major portions were seperately analysed for proximate and mineral composition. Ascorbic acid (Vitamin C) and pectin values were also determined for the fruit pulp and skin (exocarp) respectively.

The mineral elements (metals) were determined using Atomic Absorption Spectrometer, (AAS) PYE Unlcam SP 1900 model except sodium and potassium which were determined with flame Emission spectrometer FES) where they are more sensitive.

The results showed all the fruits studied had the same relative density of 0.91 Irrespective of the specie, dimensions and geographical locations. Some physical properties of the lipid (ether/ ethanol extract)were also determined.

It has been revealed in this work that the avocado fruit Is a complete fruit of Its own among other tropical fruits; its edible portion (pulp) contains all the major minerals needed in the human body in a more or less substantial amount,viz:

Minerals	:	K	Na	Ca	Mg	Fe	Zn	P	CI
Mg/IOOg (AV)	:	156.0	3.0	3.05	4.0	0.85	1.9	3.0	3.4

The avocado fruit is found to have a high fat content of 8 to 28%; low sugar content of 1.0 to 2.5%, average crude protain

of *2.4%* and very low ascorbic acid (Vitamin C) of 12.0 to l6.lmg/100g.

The chemical composition of avocade fruit makes it a more recomendable source of energy especially for diabetic patients among common fruits.

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ABBREVIATIONS

DCPH	2,6-dichlorophenylindophenol
DNPH	2,4-dinitrophenylhydrazine
LAA	L-Ascorbic Acid
DAA	Dehydro-L-Ascorbic Acid
EDTA	Ethyldiamine tetraacetic acid
AAS	Atomic Absorption Spectrometer
FES	Flame Emission Spectrometer
T.L.C	Thin Layer Chromatography
A.R	Analytical grade Reagent.

CHAPTER ONE

1.0 INTRODUCTION.

1.1 Importance of Fruits.

Fruits and fruit products play a very important role in human diet, supplying some of the essential substances in which other food materials are deficient (1). They are important in neutralizing excess basic substances produced in the course of disgestion of some alkaline food materials. The pulp of fruits are of value as roughages which promote digestion and helps prevent constipation. They are important source of mineral elements needed by the body, being especially rich in calcium and iron. Fruits are valued source of vitamins, the surest source of the most needed vitamin C which is not synthesized by man and other primates is the fruit and citrus fruits, in particular. Components of most fruits have some pharmacological activities and fruit juice is often employed as suitable solvent base for drugs (2).

The significant role of fruits and fruit products in the maintenance of life had been recognised since the genesis of life on earth. The biblical first beings were believed to have lived in a garden of fruits. Equally, the historical primitive man and other wild lives had been known to survive mostly on wild fruits as their staple food for years before the advent of civilization which brought in other alternative sources of food. The settled life of these ancestors was only a reality when they succeeded in cultivating fruit plants in their settlements. Presently the bush-men of the Kalahri 'desert' around South East Africa still live mostly on wild fruits.

Today fruits and fruit products are commercial source of revenue. They are marketed in various forms all over the world. The fruit juices are mainly sold, as bottled or canned soft drinks and wines. A sorted types of mineral fruit drinks are flushing Nigerian markets. The industries engaged in this production are multiplying as the profit derived thereof is huge and attractive. With the increasing knowledge of the nutritive value of fruits, the million of Naira allocated for agriculture in the national burget is substantially utilized in fruit production (3). Local and organised fruit orchards are on the increase in all parts of the country. But for the ephemeral Petroleum oil that is sustaining the economy of the country, the attention to agriculture and horticulture in particular, would have been more popularized as the next alternative source of national revenue. Fruits are available everywhere. The cost of production and protection of fruit is minimal compared with the profit derived from this. In this country, majority of the people (less privilaged masses) find it difficult to afford for the first class sources of the essential nutrients in their diet; to this group of people, the economic sources of these substances are fruits and vegetable taken in their cheapest forms (3).

1.2 Research Justification and Objective.

With the alarming increase in the population of Nigeria and the World as a whole in the face of limited natural sources of nutrients, the ultimate pending consequences of malnutrition seem to bid the imagination of every rational citizen. The need to search for and perhaps improve upon alternative sources of nutrients is highly obvious

and mendatory. On this justifiable ground, Schmidt (11) proposed an intensification of research in hurticulture for improvement of present nutritional status of many people in the tropics.

In Nigeria large amount of human and animal nutrients are imported or formulated with imported ingredients to supplimont our megre sources. The foreign exchange involved here could better be channeled in other pressing projects, if our knowledge of the nutritional sources of our environment is a bit more widened.

The objective of this work is to study the physical properties and to determine quantitatively the composition of avocado fruit so as to shed more light on its nutritional value as another cheap, but rich source of valuable nutrients among Nigerian fruits. Efforts shall also be made to compare the results obtained with some data given in the literature for similar fruits in Nigeria and other places.

1.3 Factors affecting composition of Fruits.

The quality and composition of fruit seem to be affected by a number of factors. These include genetic factors, use of fertilizers, age of the fruit at time of harvest, geographical location and time of cultivation, handling and transportation, care and application of pesticides; age of the fruit plant.

Literature records show that these factors are significant in determining the composition of a fruit; the generic factor had be shown in an experiment by Bergman (4) of the Department of Horticulture in the Pennsylvania State University, that this govern the ability of different species of the same plant to take up and concentrate minerals from the soil The use of nitrogen fertilizers have been extensively investigated by many authors, and the following results about the quality and composition of fruits have been recorded to include:

(a) increase in fruit yield and size (5,6,7),

(b) fruit content of soluble solid maybe lowered by heavier nitrogen fertilizer application (8),

(c) has no effect on the flavour (9),

The age of the plant also has effect on composition of fruits. Some elements (minerals) decrease while other increase with age of fruit and plant. Similarly, alkalinity and acidity of the soil affect plant composition (10). The presence of some ions such as potassium also affect the up take of other ions. Schmidth (11)showed that calcium and potassium ions are taken up by Amaranthus cruevis maximally at 240ppm potassium ion concentration in the nutrient solution, while for magnesium and chloride ions maximal at potassium ion concentration of less than 60ppm in the nutrient solution. The result of course varied from plant to plant.

1.4 The Avocado fruit.

1.4.1 Historical background.

The avocado (Persea americana L) is originally a fruit of Mexico in central America. It has also been found to grow wild all over South America and West Indies and has lately spread to many other tropical

countries like Hawaii, Philippine, Islands, Coba, the British West Indies, Ceylon, India and West Africa.

The most important places growing avocado on commercial scale are Florida and California. It is not exactly known when it was first introduced into Nigeria; it might have been introduced into the country in the 18th Century during the influx of European into West African Coasts. In India it is assumed to have been introduced in the South and West Coasts of India about 52 to 78 years ago from Ceylon (12). In South India, it is at present grown only experimentally in a few orchards at Bangalore, Shevroys, Nandi Hills, Lower Plains and the Kallar and Burliar Fruit Station in the lower foot hills of the Nilgiris. In Western India, avocado was first time imported from Ceylon in the year 1941 and planted at the Ganeshkind fruit Experiment Station, Poona.

In Nigeria, avocado is not yet a very popular fruit compared with other fruits like banana, where Nigeria produces fifty percent of the world production; and others like pine-apple, mango and citrus fruits (oranges). The avocado is not cultivated for commercial purpose but are found to grow widly in the Rain forest and Savanna areas of the country. Avocado tree of all types are seen to thrive well in all parts of the country. Only few and small avocado orchards are managed by individuals and some schools in the Southern parts of the country for local consumption.

The avocado for all purposes is a salad fruit and is eaten when fresh. It is highly nutritious compared with other fruits and contain oil which is as easy to digest as milk fat (12).

The generally accepted name "avocado" is preferred; the synonyms 'alligator pear' and "avocado pear" so frequently used in this country and other countries are incorrect and should be avoided (13). The strangeness of avocado as a very nutritious fruit in Nigeria is substantiated by the fact that it has no local name in the major languages spoken in this country. The derived English name 'pear' is adopted in Hausa, Yoruba and Ibo.

1.4.2 Ecological Description.

The horticultunist distinguishes three general ecological groups or races of the avacodo: Mexican, Guatemalan and West Indian (14). The Mexican race is characterised by an anise odour of the fruits, leaves and young growth. The skin is smooth and thin. The mature fruit has a yellowish green colour. It is oblong in shape. The Mexican race of the avocado is believed to have originated in the mountains of Maxico and Central America. They are also found to grow wild in West Africa and other tropical countries.

The avocado of the Guatemalan race is easily distinguished by the thick skin of its fruit. It is green in colour when ripe. The fruits of the Guatemalan race are generally oval in shape. They are believed to be native to Central America and California as those of the Mexican race.

The West Indian race of avocado has a smooth peel that is leathery and sometimes glossy. The race is native to the low lands of central America. It has a light green colour when botanically mature. Generally the fruits of the Mexican is larger than a typical West Indian race obtained from the same environment; the latter in turn is

slightly bigger in size than the Guatemalan fruit. However, the differences in size of the fruit of different races have been attributed to the variation in soil fertility (15); thus this characteristic cannot be used rigidly to classify fruits from different environments or locations.

The avocado tree of any race is a typical evergreen tropical and subtropical plant with a dense symmetrical crown with even spreading at about two-thirds the total height of the tree from the ground. The height of the tree in Nigeria ranges from 18 to 21 meters (60 - 70ft). Avocado of any race can grow in any type of soil and does better in loamy soil but is adversely sensitive to excessive moist soil (14, 15). The fruiting season for avocado in Nigeria is from January to August. Unlike other fruits, avocados can be stored on the tree after maturation. Under suitable conditions, fruits of all varieties can be stored on the tree some few months beyond the fruiting season since no softening takes place in attached avocado so long as the stem remains healthy.

1.4.3 Fruit Anatomy.

The anatomy, morphology and cellular development of several varieties of the avocado have been studied by Cummings and Schroeder (16); Schroeder (17) and by Valmayor (18). Botanists refer to the fruit as a berry consisting of a single carpel and single seed. The pericarp, which is the fruit tissue proper excluding the seed, comprises the rind known as exocarp, the flesh edible portion or mesocarp, and a thin layer next to the seed coat, the endocarp. (See plates I and II).

The outer layer of the exocarp is a thin wax-like cuticle covering the surface of the fruit. In addition to this film-

the exocarp consists of a layer of epidermis, 1 - 3 layers of brick-shaped hypodermal cells, several layers of perenchyma cells and a layer of Sclerenchyma, or stone cells limiting the inner surface of the peel. The epidermal cells wall was found by Schroeder (19) to be impregnated with cutin, while Valmayor (18) reported cellulose in the epidermal wall of the florida varieties of the Mexican avocado.

The large seed of the avocado consists of two fleshy cotyledons, plumule, hypocotyl, radicle and two thin seed coats adhering to each other. The endosperm disappears in the course of development (16). The cotyledons consists of parenchyma tissue interspersed with oil cells or idioblasts and contain starch as the main storage material.

1.5 Literature Review.

1.5.1 Chemical Composition

Several reports have been made on the major components of avocado fruit. The high nutritive value and unusual composition of the avocado fruit were proclaimed early by some researchers of the fruit. In a review by Wardlaw (20) the statement was made that the avocado has 'calorific value three times that of banana and one and one-half times that of beefsteak, with abundant amount of vitamin A, B, and E and mineral matter higher than other fuits". Most other fruits are low in fats, protein and minerals and are high in sugars. Early studies by Church (21) indicated the unusual composition of the avocado fruits. Jaffe and Gross (22) made a critical survey of the composition of sixty-eight californio avocado from several locations. Hall et al (23) took the average of 5 - 14 determinations during one season only; and Wolfe et al (24)worked on the Florida varieties and all come to the conclusion that the avocado fruit has a

high fat content ranging from 8 to 31.6%; 1 - 4% protein and variable % of 'total carbohydrate'. Another author, Bean (25) cited "total sugar" as 1.75% in early season and 0.68% in mid-season for the Mexican race. Jaffe and Gross (22) reported carbohydrate values as high as 10% for unspecified avocado.

The fatty acid composition of avocado lipid has been also studied extensively and was reviewed by Hilditch and Williams (26). Their comparisons of fatty acid composition of lipids from various habitats is probably, not valid as different varieties and different analytical methods were used. These factor would lead to differences greater than those attributed to habitat. Mazliak (27) analysed the fatty acids derived from the hydrolysis of the lipids from various parts of the avocado fruit and has shown that the edible portion of fruit was rich in oleic acid, palmitic, linoleic and palmitoleic acids, while stearic acid is present only in trace amounts. L'ttle difference was noted in the fatty acid complement between mesocarp and endocarp, and even the exocarp. The fatty acid of the seed are markedly different from those of the pericarp, being higher in linoleic acid and linolenic acids as hstypical of seeds. This is noted to be low in fat about 1% on fresh weight basis. Kikuta (28) made an exhaustive study of the lipids of avocado fruits grown in South California, and found that the contents o the free fatty acids was very low while 86% of the lipid fraction consisted of triglycerides.

Mazliak (29) examined the composition of the cuticular wax to show that many acids, alcohols and paraffins were found in trace amount.

The avodado fruit has been shown by Maqunne (30) to be a fertile source of unusual carbohydrates. Davenport and Ellis (31) recorded that a7-carbon alcohol (perseitol); glucose, fructose and sucrose are present at all stages of ripening of the avocado. Charlison and Richtmyer (32) found an octulose, D-Glycaro- D-Manno-octulose and the corresponding alcohol, D-erythro- D-galac to-ocitol.

The free amino acid content of avocado was found by Joslyn and Stepka (33) to be higher than in any other fruit tested (apriocots, apples, prunes and pears). The principal amino acids were found to include, asparagine, aspartic acid, glutamine and glutamic acid, Serine, threonine, alarine, valine and cystine were detected. γ-Amino butyrin acid was shown also to be present.

Avocado fruit, being a high fat fruit, might be expected to be rich in vitamins, especially the lipid solube ones. In comparison with other fruits, avocado is rather poor in vitamin A, C, K and folic acids, but richer in vitamin B. The pryidoxine level of avocado is highest among fruits, except for banana, which has 0.59mg/100g (34). The vitamin K level of 8µg/100g is low compared to 12µg/100g for tomato fruit and 176µg/100g for spinach leaves. The folic acid level of 11µg/100g is low compared to pears and beans at 50µg/100g. Hall et al (23) showed that there was no significant change in the levels of thiamine and riboflavin through the picking season (January to April).

According to Jaffe and Gross (22) the mineral content of avocate fruit is higher than that recorded for many other fresh fruits. The high phosphate and iron are of interest nutritionally. Weatherby (35) found that rats which had become anaemic on an iron free diet formed

heamoglobin when their diet was supplimented by 1 - 5g of avocado tissue. The copper content was found to be 1.79ppm by Giral and Castillo (36) which is about the same level as in banana pulp and in beans grown in Mexico.

Avocado seeds have been shown to be a rich source of a complex mixture of polyphenolic compounds ranging from simple (+) catechin and (-) epicatechin to nighly polymeric substances (37).

Antibacterial activity of extracts of avocado fruit and seed has been investigated in several studies. Spoehr et al (38) showed some antibacterial activity against stephylococuss accreus, and Gallo and Valeri (39) obtained an extract with, some antibacterial activity against Micrococcus pyrogenes but none against Escherichia Coli or Bacillus Subtillis; on the other hand Valeri and Gimeno (40) reported some activity of certain avocado extracts against Escherichia Coli.

Grant (41) substituted one-half of the dietary fat of male adult hospital patients with avocado fruit. Serum cholesterol declined in the patients on avocado diet compared to patients receiving animal fat. There is still a controversy about this result; it was not clear whether the decrease was due to the unsaturation of avocado fat, the sitosterol present in the unsaponifiable fraction or to some other factor.

Compositional changes associated with development and storage of avocado have also been studied. Changes in fat, sugar and protein have been reported by Church (21); Appleman and Noda (42), Davenport and Ellis (31) Mazhak (29), Dolendo (43) and Kikuta (28). Appleman showed that the weight of the fruit and seed increased rapidly from August to December when it became "horticulturally mature" i.e. attain the

capacity to ripen normally, fruits picked before December 15th shrivelled badly, and did not soften normally and had less than 8% fat. Fat level increased equally from November to March when it tended to level off. Increase in the size of the fruit after December was very slow and moisture content follow the same parttern.

Total and reducing sugars were high and constant until about December, when, they decreased at a linear rate. There was a marked change for perseitol which was highest in May, but this disappeared gradually during storage.

The iodine number, which is an index of degree of unsaturation showed dramatic decrease during the period and slower changes after December (in the mature fruit). Saponification number increased rapidly and became constant after December.

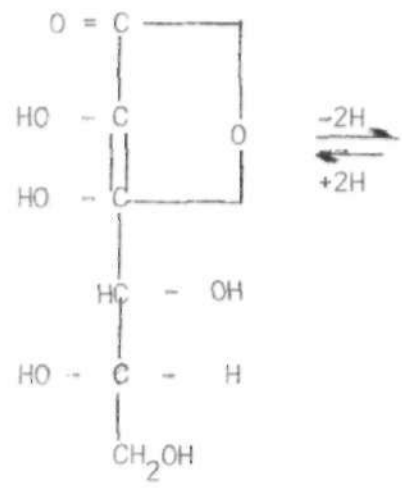
Kikuta (28) showed that the linolenic acid remained unchanged throughout the development period, palmitic acid, palmitoleic and linoleic acids increased slightly while oleic acid showed large increase.

Dolando et al (43) recorded no significant changes in fatty acid distribution during ripeningi torage (11 days at 15^{o}C). Mazliak (29) recorded an increase in palmitic and palmitoleic acid and a decrease in the percentage of oleic acid in an atmosphere high in carbon dioxide.

Davenport and Ellis (31) reported small increase in protein during storage. Dolendo et al (43) also recorded changes in pectin andi stated that softening was accompanied by a rapid decrease in protopectin and an increase in water soluble pectin. There was also a decrease in the degree of esterification of the pectin which contributes to the softening process.

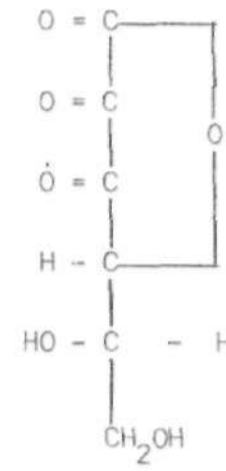
I.5.2 Vitamin C (L-Ascorbic Acid)

I.5.2.1 Properties and physiocological functions.



$(C_6H_8O_6)$

L-Ascorbic Acid



$(C_6H_6O_6)$

Dehydro L-Ascorbic acid

Vitamine C or ascorbic acid (l threo-2,4,5,6 pentohexene carboxylic acid lactone', behaves like a monobasic acid; this property stems from two enol groups (enediol); the two asymetric carbon atoms (C_4 and C_5) in ascorbic acid make four stereoisomers possible. It is soluble in water, alcohol, glycerol, and proplene glycol but insoluble in oils, fats, ether or chloroform. Ascobic acid is unstable to oxidizing agents, alkalis and certain metals e.g. iron and copper salts, it is stable in acid solutions.

Dehydroascorbic acid is the first oxidation product of ascorbic acid as shown above; however, the antiscorbutic potency of the two forms are the same. Most of the commerically available pure vitamin C is synthetic (44). Vitamin C occurs in nature in only one form, L-ascobic acid (45).

Higher plants and most mammals can synthesize vitamin C. Ascorbic acid is synthesized in rats as follows:

D-glucose → D-glucuronic acid →

L-gulonic acid → L-gulonolactonic

→ L-ascorbic acid (46).

Man, primates, guinea Pigs and the fruit eating bats, cannot synthesize this acid because they lack the liver enzyme,"gulonolactone oxidase" required for converting L-gulono-lactone to L-ascorbic acid (46).

Ascobic acid is know cofactor for:

(a) hydroxylation of proline to hydroxyproline in collagen synthesis (47);

(b) the hydroxylation of tryptophan to 5-hydroxytryptophen (48);

(c) conversion of 3,4-dihydroxyphenyl-ethyl amine to norepinephine (49) and

(d) hyaroxylation of p-hydroxyphenyl pyruvate to homogentistic acid in tyrosine metabolism (48). It stimulates the incorporation of iron into hemoglobin (50). Vitamin C deficiency causes scurvy and damages most if not all the tissues and organs of the body (48, 50 and 51)

1.5.2.2 Method of determination.

There are several methods in literature for determination of ascorbic acid in biological samples and in blood serum. The 2,6-dichloroindophenoltitrimetric method (52 and 53) is the most elegant and is regarded as the official method in U.S.A. (54). It is the method adopted in this work.

Other methods include.

(i) photometric methods - there are several modifications of spectrophotometric methods in literature (55, 56, 57, and 58). The use of 2,4-dinitrophenylhydrazine reagent method is the most popular and is the one adopted for this work.

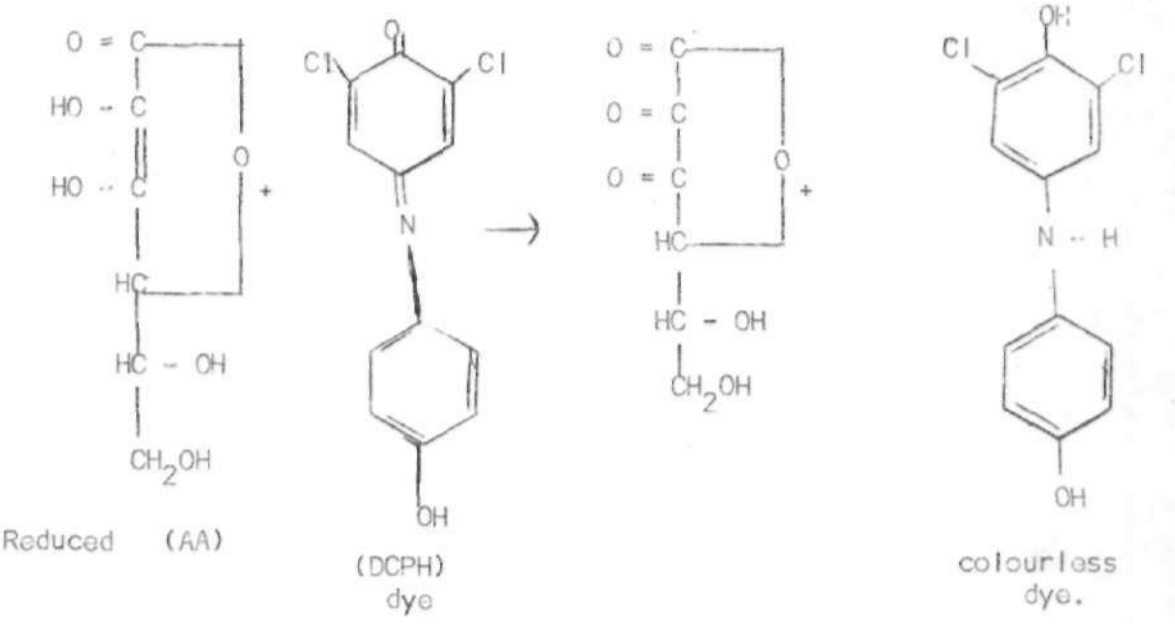
(ii) microfluorometric method (54);

(iii) chromatographic method (59; 60);

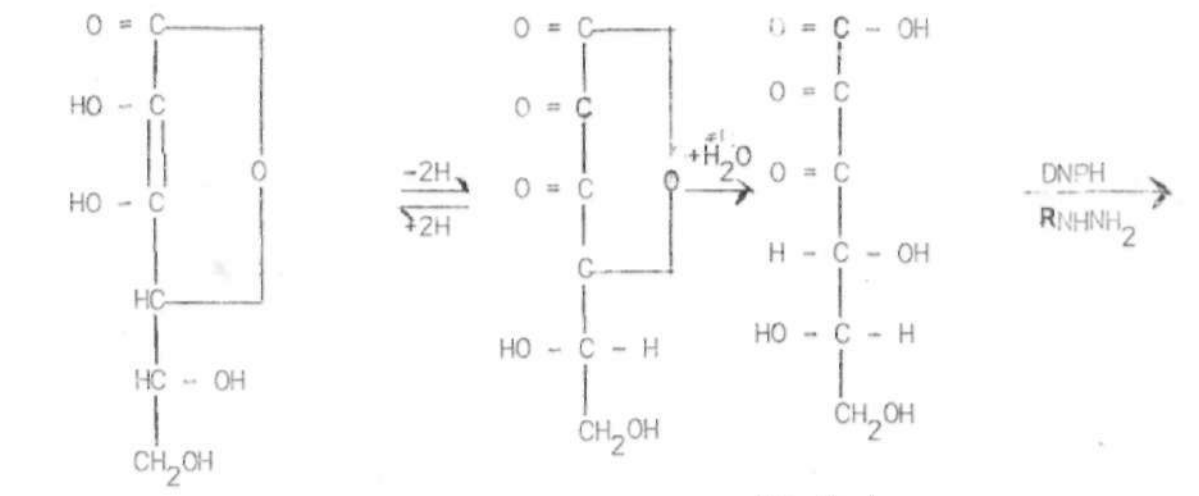
(iv) polarographic method (61, 62, and 63);

(v) Kinetic methods (64).

The principle involved in the dichlorophenol (DCPH) method is that ascorbic acid reduces the indicator dye (DCPH) to a colourless solution. At the end point the excess of dye is rose pink in acid solution. The vitamin is extracted and titration is performed in the presence of a stabilizing acid medium like HPO_3 - HOAC solution to avoid the autooxidation of the vitamin at high pH value. The following equations have been proposed for the reaction (52)



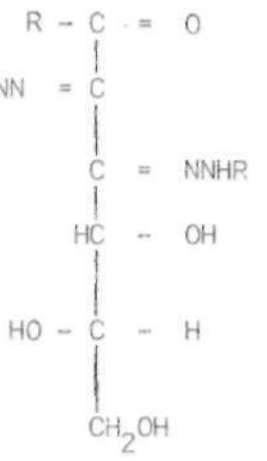
And the proposed reaction for the 2,4,dintrchydrazine reagent (56, 58) is as follows:



L-Ascorbic acid (AA) reduced form

Dehydro-L-ascorbic acid (DAA)

Diketo-L-gulonic acid



Osazone, coloured.

CHAPTER TWO

2.0 EXPERIMENTAL METHODS AND PROCEDURES

Most of the procedures adopted in this work are the standard recommended methods of analysis as described in "Official methods of Analysis" of the Association of the Analytical (Agricultural) Chemists (A.O.A.C.) 13th Edition, 1980, unless otherwise stated.

2.1 Sample collection and locations.

2.1.1 Sample sources and collection.

All the samples of avocado fruits used were collected randomly from different parts of the country between the months of July and September. The fruits are usually sold during this period (season) in local markets at an average cost of 10k to 25k per fruit according to sizes. Some of the samples were obtained through the help of friends and others collected free of charge from the avocado trees in some orchards in Cross River State and Ahmadu Bello University main campus (Staff Club and Area F) by owners' permission. The rest of the samples were bought from local markets and hawkers in the following towns: Uyo in Cross River State; Aba in Imo State, Elema in Rivers State; Ibadan in Oyo State; Benin in Bendel State; Gindiri in Plateau State; Zaria in Kaduna State; and Kano in Kano State.

22 fresh fruits were selected out of the lot in the laboratory and grouped into three main ecological races using their physical characteristics and some unique botanical features.

2.2 Sample Preparation.

After the determination of the physical parameters (dimensions, weight and relative density), each fruit was cut open into two halves to study the cross section and transverse section (shown in plates I and II). The single dicothyldoneus seed was removed, the edible portion (pulp or mesocarp) and the skin (exocarp) carefully separated. Each of the separated portions was made homogenous by a method that preserved the edible nutrients for subsequent analysis.

2.2.1 Materials.

Table knife (stainless steel); Spatular blender and Watch glasses.

2.2.2 Procedure.

The pulp was completely scraped from the skin using the spatular into a weighed watch glass. The weight of each portion (seed, pulp and skin) was noted for each fruit. Each of these portion was then chopped in a porcelain dish with a stainless table knife into fine slices. A suitable portion of each was used for the respective moisture determination. The remaining portion was dried at 80^{o}C for 24 hours in the oven and under the sun for another three hours to ensure adequate dryness. This was then ground into fine powder and stored in dry labelled sample bottles for further analysis.

The remaining fresh fruits were preserved in water-tight cellophene bags in the refrigerator maintained between 5 to 10°C. This could keep well for 14 days or more.

2.3 Determination of Physical Properties.

2.3.1 Fruit dimensions, Weight, Percentage fraction, Weight, Volume and Density.

2.3.2 Materials: Top loading balance (Metoller P1210 model) overflow can, vernier callipers, Meter rule. Measuring cylinder (500 cm^3)

2.3.3 Procedure: The weight (g) of each fruit was noted with the top loading balance. The corresponding volume of each fruit was measured with the over-flow can by allowing the fruit to immerge totally in water in the can with the help of a thin metal rod. The displaced water was collected in a measuring cylinder placed directly below the spout of the overflow can; The total volume of water displaced as noted in the measuring cylinder, measured the volume (cm^3) of the fruit.

The dimensions (length and width) of each fruit was measured with a vernier callipers and confirmed with a meter rule measurement of the vertically cut section. Weight of each portion was noted and express as % of total fruit wt purchased.

2.3.4 Calculation.

The Relative density (R.D.) of each fruit was calculated using the relationship.

$$R.D. = \frac{\text{mass of Sample of fruit}}{\text{Volume of Water displaced by Sample}}$$

2.4 Proximate Analysis.

2.4.1 Water Content (Moisture) and Total Solid.

The term moisture is used in analytical chemistry for total volatile matter present in sample under analysis (15). These are mainly free water vapour and bound water.

The weight of any food has little significance unless taken with water content. Whether naturally present, added through fraudulent intent, or present in excess amount because of insufficient drying, water, is usually a dead loss to one who buys by weight or measure (65). It is also on added expense for transportation if present in excess amount above the optimum requirement. Moisture determination therefore is one of the most important and widely used parameter in the processing and testing of food.

2.4.1.1 Methods for Moisture Determination

Several methods for moisture determination have been reported in literature (65,66). These include:

(i) Indirect distillation (or dry) method (67).

This involves the measurement loss in weight of sample due to evaporation on drying.

(ii) Direct distillation methods (68).

The distillation is carried with denser immicible solvents like toluene xylene and heptane with higher boiling point than water. The weight of distillate obtained at 100 ± 1^{o}C represents the moisture.

(iii) rapid method employing electrical moisture meter (69) (Thermobalance).

(iv) Chemical methods (71, 70, 72, 73, 74).

This makes use of the chemical reaction of water with iodine or sulphur dioxide in pyridine-ethanol solution in a volumertic titration.

(v) the use of Nuclear Magnetic resonance (N.M.R.) spectroscopy had been reported by Miller and Kaslaw (75).

(vi) Gas liquid chromatographic methods (75).

This has also been reported to give reproducible results.

(vii) Infra Red (I.R.) method (76) has also been reported for the determination of moisture.

The indirect distillation (dry method) was adopted for this work because of the convenience and availability of apparatus.

Apparatus: Mettler balance, P2010 model (sensitive to 0.1g). Oven (thermostated); platinuim dishes, dessicators with effective dessicants.

Procedure: The platinum dish was dried in an oven at 80°C for 20 minutes, cooled in a dessicator and weighed (W_1)g. 5g of each sample portion was placed in the dish and reweighed $(W_2)_g$. The dish with the sample was then dried in the over at 80°C for 24 hours until a constant weight was obtained after successive cooling in dessicator and weighing. It was finally transfered from the oven to the dessicator to cool; and then quickly weighed with minimum exposure to the atmosphere to give mass (W_3)g

The moisture and total solid contents were calculated as percentage of weight of the sample used using, the formulae:

$$\% \text{ moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

and the

$$\% \text{ Total solid} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

3.4.2 Ash and Organic Matter (71)

The ash of a biological material is an analytical term for the inorganic residue, that remains after the organic matter has been incinerated.

2.4.2.1 Materials.

Silica dish, dessicator, Muffle furnace, Pair of tongs.

2.4.2.2 Procedure.

The flat bottomed silica dish was dried by holding in the Bunsen flame for about one minute then transfered into a dessicator to cool before weighing(W_1)g. About 5g of the each pulped sample was weighed inside the dish (W_2)g.

The Silica dish with sample was then heated gently in a Bunsen burner in a fume cupboard until smoking ceased, then this was transfered to the Muffle furance preheated at 500°C. The heating continued until all the carbon has been burnt away. (usually 24 - 48hrs) The furnace was switched off and the silica dish taken out with a pair of tongs and immediately covered and placed in an efficient dessicator to cool before weighing (W_3)g.

The weight of the ash and organic matter that burnt away were calculated and expressessed as percentage of the sample used as follows:

$$\text{Ash } \% = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

and

$$\text{Organic matter } \% = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

The arithmetic mean of at least three determinations was often taken as the result.

2.4.3 Lipid Content.

The lipid content of a biological material can be estimated by exhaustive extraction of the dry sample with a suitable lipid solvent. Lipids generally represent the water insoluble component in food stuff which are soluble in organic solvents (fat solvents e.g. ether, ethanol, petroleum ether etc).

The method adopted in this work was the direct but exhaustive Soxhlet method (69) of extraction using diethyl ether/ Ethanol mixture (2:1).

2.4.3.1 Apparatus.

Soxhlet extractor unit. Heating mantle.

2.4.3.2 Chemicals:

Diethyl ether and ethanol 95% (A.R.)

2.4.3.3 Procedure.

About 5g of the powedered dry sample was put into a thimble of known weight (W_1)g. The thimble with the sample (W_2)g was placed inside the Soxhlet extractor. $300cm^3$ of diethyl ether/ethanol mixture (2:1) was used for the extraction. The gentle heating was done on an electrically controlled heating mantle. The process was allowed to continue for 24 hours before

the thimble with the content was removed, dried in oven at $50^{o}C$ for another 6 hours, cooled in a dessicator and reweighed (W_3)g. The extracted oil and solvent in the flask was distilled to about 20 cm^3 and then evaporated on a water bath until all the solvent evaporated and the oily lipid left in the flask. The percentage lipid was obtained by the formula:

$$\% \text{ Lipid} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

The extractible fat could also be calculated from the weight of lipid obtained after evaporation divided by the original weight of the sample and multiplied by one hundred. Usually the latter method giveslower results as some of the lipid might have escaped during evaporation.

2.4.4 Nitrogen and Crude Protein.

The amount of protein in a biological material is obtained by determining the amount of nitrogen present which is then multiplied by known and accepted emperical factors (based on the proportion of nitrogen in the proteins). Most protein contain about 16 percent nitrogen (67, 69). Total nitrogen estimated by the Kjeldahl method is multiplied by 6.25 to express the average crude protein; for milk and egg this factor is 6.38 and 6.68 respectively. The factor of 5.7 is used for proteins in flour and in wheat.

In this work the usual volumetric method of Kjeldahl method (67) with semi-micro modification was used. The method is less time consuming, convenient and simple in technique. It is more accurate than other methods. Nitrates and nitrites are not determined by this procedure. The determination is done in 3 stages - first the sample is completly digested with concentrated sulphuric acid to convert all the nitrogen into ammonium sulphate, $(NH_4)_2SO_4$; secondly ammonia is liberated by making the mixture containing the ammonium sulphate alkaline, the amonia is distilled and thirdly, the ammonia is determined by titration.

Some other modifications of the Kjeldah method have been reported in literature and these include -- Use of Con. Sulphuric acid - Hyrogen peroxide mixture (73) and elimination of the distillation process by addition of alkali to the sulphric acid digest followed by hypobromite solution and potassium iodide. The liberated iodine is titrated with standard thiosulphate (74).

Other methods of protein determinat ion include the formal titration (69, 75); colorimetric method (76) and Turbidimetric method (77) using sulphosalicylic acid.

2.4.4.1 Materials.

(a) Apparatus:

100 cm^3 Kjeldahl flask

Markham-: micro nitrogen still.

Kjeldahl distilling bulb.

Straight-tube condenser

Heating mantle.

(b) Reagents.

All the reagents used were of analytical reagent grade and free from nitrogen.

2.4.4.2 Procedure.

(a) 250mg of the dry sample was accurately weighed into a clean dry 100cm^3 Kjeldahl flask. About 1g of mixed catalyst (consisting of 160g anhydrous K_2SO_4, 10g $CuSO_4.5H_2O$ and 3g selenium powder, properly mixed). 6cm^3 of conc. H_2SO_4 was added and a few chips of Pumice Stone.

(b) The mixture was carfully digested over on electric heater in the hood initially with low flame until frothing subsided and at a higher temperature until contents were clean greenish in appearance. The digestion was continued for further 60 minutes.

(c) The heat was put off and the flask allowed to cool then 15cm^3 of distilled water was added. The contents were quantititively transfered into a 50cm^3 volumetric flask using more distilled water to rinse out, but leaving the pumic stones behind. The volume was made to the 50cm^3 mark and shaken to mix well.

(d) Exactly 10cm^3 of the digest was transfered with a pipette into the Markham semi-micro nitrogen still. 10cm^3 of 40% sodium hydroxide solution was added to the digest and closed immediately to prevent any escape of ammonia. The ammonia liberated was steam distilled into the 5cm^3 $\left(\frac{N}{70}\right)$ boric acid solution containing 4 drops of mixed indicator taken in the conical flask.

(e) The distillate was removed and titrated with the standard $\frac{N}{70}$ hydrochloric acid, the end point was reached when the indicator changed from green through faint to a definite pink. The amount of acid consumed was noted.

(f) A blank was run through the whole procedure and the burette reading substracted from that in (e) above to get a corrected volume of standard HCl.

CALCULATION

Let the mass sample used = Mg

Volume of HCl in blank titration = V_1 cm^3

Volume of HCl in 5cm^3 test sample = V_2 cm^3

Then,

% total Nitrogen in the sample is given by

$$\text{Nitrogen}(\%) = \frac{(V_2 - V_1) \times 14 \times 5 \times 100}{1000 \times 70 \times M}$$

$$\text{i.e. } N(\%) = \frac{\text{Corrected litre } (cm^3)}{10 \times \text{sample wt.(g)}}$$

So crude protein (%), = 6.25 x Nitrogen (%).

2.4.5 Crude Fiber Determination.

"Crude fiber", is the organic residue which remains after the material has been treated under standard conditions with light petroleum, boiling dilute H_2SO_4, boiling dilute sodium hydroxide solutions, dil HCl, ethanol and ether.

Crude fiber consists, largly of cellulose together with little lignin. As the recovery of cellulose using the specified procedure seldom exceeds four fifths of that actually present, the crude fiber does not represent a measure of specific group of substances. Also as the figures obtained tend to vary with the conditions employed (78), it is important to adopt a standardised procedure in order to obtain consistent results.

2.4.5.1 Reagents.

Sulphuric Acid (0.255N)

Sodium hydroxide (0.313N)

Pethyl alcohol (95%)

Diethyl ether (

Hydrochloric acid (1% V/V)

2.4.5.2 (b) Apparatus.

500cm^3 beakers.

Round bottomed flasked condenser unit

Buchner flask 1dm^3

Buchner funnels

Crucibles silica with porous base

Rubber bungs to fit (5) above.

2.4.5.3 Procedure.

2g of the dried, fat-free sample was weighed into a 600cm^3 of round bottomed flask. (For convenience the residue from ether extract for lipids was used). 200cm^3 of boiling dil. H_2SO_4 was added and the condenser fitted and brought to boiling within one minute. The boiling continued gently for exactly 30 minutes. Distilled water was used to maintain the volume at 600 cm^3 while boiling and to wash down particles adhering to the sides of the flask.

The mixture was filtered through a Whatman No. 541 ashless paper in a Buchner funnel using suction pump and residue washed well with distilled water. The residue was then transfered back into the flask and 200$_{cm}^3$ boiling NaOH solution added. The reflux condenser was replaced and the mixture brough to boiling within one minute.

After boiling for exactly 30 minutes, it was filtered through porous crucible and washed with boiling distilled water, 1% HCl and then again with boiling water. It was then washed twice with ethanol ether. The final residue was dried over night at 100°C in the oven, and then allowed to cool in a dessicator and weighed.

The dried residue was ashed in silica crucible at 500°C in the Muffle furnace for 3 hours; after cooling in the dissicator the ash was weighed. The weight of fiber was calculated by difference.

2.4.5.4 Calculation.

Let:

Weight of Sample used = a_g

Weight of crucible + dried residue = b_g

Weight of crucible After ashing = c_g

Then;

$$\text{Crude fiber } \% = \frac{b - c}{a} \times \frac{100}{1}$$

2.4.5 Determination of Sugar content.

Sugars are generally estimated by their ability to reduce copper II ions in alkaline solution or by their ability to rotate plane polarised light (optical activity).

Two approches can be adopted for the reduction of copper (II) ions. Firstly, the cuprous oxide produced by the reduction of fellings solution under standard conditions is weighed while in the

second method, the volume of sugar solution required to reduce completely 25cm^3 of mixed fellings solution using methylene blue as redox indicator in measured (79).

There are two main classes of sugar i.e. reducing sugar (e.g. dextrose maltose, lactose) and non-reducing sugar (e.g. sucrose). The latter class, after inversion by acid hydrolysis can also be determined by the method of reducing sugar.

The method adopted in this work is due to Luff - Schoorl (inderect volumetric method). It is similar in principle to the method of Lane and Eynon (79).

2.4.6.1 Luff - Schoorls Method.

(a) Apparatus: Magnetic stirrer

(b) Reagents: All reagent are of Analytical reagent grade or best quality commercially available.

(i) 40% Ethanol (V/V)

(ii) Carrez Solution I: 21.9g zinc acetate and 3g glacial acetic acid in 100cm^3 water.

(iii) Carrez Solution II: 10.6g Potassium ferrocynide in 100cm^3 water.

(iv) Luff-Schoorl Reagents: a, b and c -

(a) 25g copper sulphate in 100cm^3 water

(b) 50g citric acid in 50cm^3 water

(c) 143.8g anhydrous sodium carbonate dissolved in about 300cm^3 warm water then cooled.

The citric acid solution (b) was poured into the sodium carbonate solution (c) with stirring. Copper sulphate solution (a) was added and then made up to $1dm^3$ with distilled water. (pH of solution is approximately 9.4).

(iv) Sodium thiosulphate solution (0.1M).

(v) Starch solution: 1.0g soluble starch in $5cm^3$ water was poured into 200ml boiling water boiled for three minutes and allowed to cool.

(vi) Potassium iodide 30% W/V

(vii) 3M sulphuric acid.

2.4.6.2 Procedure:

(a) Extraction of Sample.

Exactly 2.5g of the pulp sample was weighed into a $250cm^3$ volumetric flask. $200cm^3$ 40% (V/V) ethanol was added and mixed for one hour with magnetic stirrer, $5cm^3$ of Carrez solution I and $5cm^3$ of Carrez solution II was added and stirred for another one minute. The mixture was made up to volume with 40% ethanol, homogenised and filtered. $200cm^3$ of the filtrate was removed, evaporated to almost half volume in order to get ride of most of the ethanol. The residue was transfered quantitatively to a $200cm^3$ volumetric flask with warm water, cooled and made up to the mark. This solution was then used to determine the amount of reducing sugar; then the amount of "total" sugar, after inversion.

(b) Determination of Reducing sugar.

25cm^3 Luff-Schoorl reagent was pipetted into a 300cm^3 Erlermeyer flask and 25cm^3 of the sugar sample solution added together with some anti-bumping grannuds. This was heated to boiling over Bunsen-burner within three minutes with constant stirring. A reflux condenser was fitted to the Erlenmeyer flask and the liquid was then boiled for exactly ten minutes. It was then cooled immediately in cold water; and after about five minutes, it was titrated as follows:

10cm^3 potassium iodide solution was added carefully, followed by 25cm^3 3M sulphric acid. This was titrated with 0.1M sodium thiosulphate until a dull yellow colour developed. Five drops of the starch solution was added and the titration continued to the end point when blue iodine-starch complex colour was discharged.

A blank titration was carried out with water plus 10cm^3 potassium Iodine and 25cm^3 3M sulphuric acid without any sample solution.

2.4.6.3 Calculations:

Using the conversion table in appendix I, the equivalent amount of glucose in mg which corresponds to the difference between the values of the two titrations is obtained, and the result expressed as percentage of the sample weight (i.e. mg sugar/ 100g sample).

2.4.6.4 Determination of "Total" sugar after Inversion.

50cm^3 of the clarified sugar solution was taken with pipette into a 100cm^3 volumetric flask. 3 drops of methylorange indicator solution was added. 4M hydrochloric acid solution was also added carefully with stirring until the liquid turned a definite red. 15cm^3 of 0.1M HCl was added and the flask immersed in a fast boiling water bath and allowed to stand there for 30 minutes. It was then cooled rapidly to about 20^{o}C. 15cm^3 of 0.1M NaOH was added and diluted to 100cm^3 with distilled water. 25cm^3 of this was titrated as described for reducing sugar by the Luff-Schoorl method above.

2.5 Determination of Ascorbic Acid (vitamin C) by 2,6-Dichloroindophenol (DCPH) method.

This method is only applicable in the determination of reduced ascorbic acid in the absence of iron (III), tin, Copper(II), sulphur dioxide and thiosulphate as possible interferences.

2.5.1 Reagents:

(a) Extracting solution: Metaphosphoric acid - acetic acid solution (HPO_3 - HOAC). 15g of glacial metaphosphoric acid pellets and freshly pulverized stick was dissolved with shaking in 40cm^3 acetic acid and 200cm^3 distilled water. This was diluted to about 500cm^3 and filtered rapidly through fluted paper into g-s bottle.

(HPO_3 slowly changes to H_3PO_4, but if stored in refrigerator, solution can keep satisfactorily for 7 - 10 days).

(b) Ascorbic Acid Solution Standard ($1mg/cm^3$ or 1000 ppm)

50mg of USP Ascorbic acid Reference standard was accurately weighed into a $50cm^3$ volumetric flask and diluted immediately to volume with HPO_3 - HOAC solution (a) above.

(c) Indophenol Standard Solution: ($0.125g/50cm^3$)

50g of DCPH sodium salt(Eastman KODACK Co.No.3463) was dissolved in $50cm^3$ water to which has been added 42mg sodiumbicarbonate and shaken vigorously and when the dye dissolved it was diluted to $200cm^3$ with distilled water, filtered thru flutted paper into amber g - s bottle. It was preserved stoppered and out of reach of direct sunlight in refrigerator.

2.5.2 Procedure:

$5cm^3$ of the extracting solution containing excess ascorbic acid was added to $15cm^3$ of dye reagent. (If reduced solution was not practically colourless, it was discarded and a new stock prepared). Replicate of $2.0cm^3$ aliquots ascorbic acid standard solution were transfered to each of $50cm^3$ Erlenmeyer flasks containing $50cm^3$ HPO_3 - HOAC solution (a). This was titrated rapidly with the indophenol solution from $50cm^3$ buret until light but distinct

rose-pink persisted within seconds. Each titration required 15.1cm^3 indophenol solution). Three similar blank titrations were carried out with blank solutions containing 7.0cm^3 HPO_3 - HOAC solution (a) plus volume of water about equivalent to the volume of indophenol solution used in the direct titrations. After substracting the average blanks from the standard end points. The number of mg ascorbic acid equivalent to 1cm^3 reagent (F) was calculated. The indophenol solution was standardised daily with freshly prepared ascorbic acid standard solution.

(b) Preparation of sample Assay Solution.

Sample was pulverized by gently grinding then added HPO_3 - HOAC solution and triturated until sample was in suspension. It was then diluted with HPO_3 - HOAC solution to 50 cm^3. 10cm^3 of the extracting solution/dry sample was used.

2.5.3 Determination.

3 sample aliquots were titrated and blanks were titrated simutaneously for end point correction. Using the average of 3 titrations; the mg Ascorbic acid per gramme sample was calculated using the formula:

$$\text{mg Ascorbic acid/g sample} = (A - B) \times \frac{F}{E} \times \frac{V}{Y} \times \frac{100}{T}$$

where A = average cm^3 for sample titration

B = average cm^3 for blank titrations

F = mg Ascorbic acid eqivalent to 1.0cm^3 of indophenol standard solution.

E = number of grammes of sample used.

V = inital volume of assay solution ($50cm^3$)

Y = volume of sample aliquot titrated ($10cm^3$)

2.5.4 Determination of 'Total' Ascorbic acid by the 2,4-Dinitrophenylhydrazine Method (79).

2.5.4.1 Procedure describes the determination of total vitamin C-like compounds (henceforth refered to as 'total ascorbic acid) and is represented by ascorbic acid, dehydroascorbic, and diketogulonic acid if present. The method is accurate for antiscorbutic assay of many fresh foods when ascorbic acid and dehydroascorbic acid occur in the foods and diketogulonic acid has not been formed in appreciable amount (78). Thus, the method is valuable in determining the original ascorbic acid content of the fruit at the time of harvesting.

(i) Extraction.

(a) 20g of the dry pulped sample and 20g of 10% HPO_3 was blended for 3 minutes to a homogenous slurry.

(b) 10g of the slurry was weighed into a $25cm^3$ flask and diluted to the mark with 5% HPO_3 solution and mixed thoroughly; $10cm^3$ of this was further diluted to $100cm^3$.

(c) The suspended particles were removed by filtering thru a Whatman No. 12 fluted paper.

(II) Oxidation to Dehydroascorbic acid.

(a) To the solution from C above was added 3 drops of bromine from a medicine dropper in a fume cupboard. This was shaken gently until the solution was slightly yellow, excess bromine was decanted off and Nitrogen (saturated with water) was passed through the solution until the dissolved bromine was expelled.

Roe (80) has also recomended the use of acid washed Norit as the oxidising agent rather than the poisonous bromine. Norit is particularly advantageous in highly pigmented extract; it can decoloruise the extracts while simulteneously oxidising them. Unavailability of Norit during the period of the work made the use of bromine unaviodable. Other oxidising agents like 2,6-dichlorophenolindophen has been recomended by Bolin and Book (65) but this reagent is limited to colourless extracts.

(b) To a $10cm^3$ aliquot of the oxidized extract $10cm^3$ of 2% thiourea in 5% HPO_3 was added and mixed thoroughly to yelld a dilute sample of $30cm^3$.

(c) To a $5cm^3$ aliquot of oxidized extract was added $10cm^3$ of 2% thiounea in 5% HPO_3 and $5cm^3$ of 5% HPO_3 and thoroughly mixed.

(iii) Formation of Osazone.

(a) $4cm^3$ aliquot of each of the sample dilutions (1-b) and (2 - c) above was pipetted into each of three test tube (celoremeter tubes).

(b) One tube of each dilution was set aside to serve as blank.

(c) To each of the remaining tubes was added $1cm^3$ of 2% 2,4-dinitrophenyl hydrazine reagent.

(d) All the tubes were placed in a water bath at $37^{\circ}C$ for 3 hours.

(e) At the end of this period, the tubes were removed from the bath and placed in ice bath.

(iv) Treatment with 85% sulphuric acid.
(Formation of soluble pigment)

(a) While the tube were still standing in the ice bath, $5cm^3$ of 85% H_2SO_4 was added to each. The addition was made one drop at a time and this lasted for at least one minute. The essence of this slow addition is to obviate a darking of solution containing sugar due to rapid rise in temperature of the heat of hydration of the acid.

(b) With the tubes still in ice bath, $1cm^3$ of 2% 2,4-dinitrophylhydrazine reagent was added to the blank. Without removing from the ice bath, all the three tube were shaken to mix the contents.

(c) The tubes were then removed from the ice bath, and allowed to stand for 30 minutes at room temperature.

As the colour continues to develop to maximum within this period many interfering Osazones would slowly decompose in the H_2SO_4 - Thus enough waiting time of not less than 30 minutes is essential.

(v) Measurement of Colour.

(a) Stable photoelectric colorimeter (spectronic 120) was used at 520 wave length with suitable filters.

(b) With the blank, the instrument was set at 100% transmittance reading. This corrected for colour other than that produced from the Osazone formed during the 3 hours in the water bath at $37^{\circ}C$.

(c) The percentage transmittance (% T) was read for the samples.

(vi) Calibration of the Colorimeter.

(a) To $50cm^3$ of the standard ascorbic acid (100ppm) was added 3 drops of liquid bromine; shaken and the excess bromine seperated.

(b) The oxidised solution was aerated with nitrogen to remove excess bromine. Then $10cm^3$ of the aerated solution was put into a $500cm^3$ volumetric flask. 5g thourea added and diluted to volume with 5% HPO_3.

(c) Final diluted dehydroascorbic acid solutions containing 1, 2, 4, 5, 8, 10 and 12 µg per cm^3 were prepared by drawing 5, 10, 20, 25, 40, 50 and 60 cm^3 of the diluted solution into seven labeled $100cm^3$ volumetric flasks and diluting each to the mark with 1% thiourea in 5% HPO_3.

(d) Each of the seven standard ascorbic acid solutions was treated in the same manner as samples in steps I (IIIa - vc) in the previous sections, using a seperate blank for each tube.

(e) The calibration curve was prepared by ploting % transmittance (% T)as ordinate and the concentration of ascorbic acid "C" (µg per cm^3) as abscissa. (Ref. standard curve for determination of "total" ascorbic acid).

.5.4.2 Calculations:

(a) Total ascorbic acid content of each aliquot was calculated according to the formula:

$$\frac{R}{W} \times \frac{100}{1000} = \text{mg "total" Ascorbic acid per 100g Sample.}$$

where

R = µg of total Ascorbic acid per cm^3 of solution obtained by interpolation from the calibration curve.

W = g of sample per cm^3 of the diluted solution.

$\frac{100}{1000}$ or 10^{-1} = conversion factor from µg per g to mg per 100g.

2.6 Pectin Determination (89):

2.6.1 (a) Apparatus: Gooch, crucible.

(b) Reagent. 2^M calcium chloride.

1^M acetic acid

0.1^M sodium hydroxid.

2.6.2 Procedure: A suitable quantity of dry fruit skin was macerated and extracted with cold water; the mixture was then boiled and filtered. An aliquot of the filtrate was diluted to $300cm^3$, and $100cm^3$ of 0.1M NaOH was added, then allowed to stand overnight. After which $50cm^3$ of 1M acetic acid was added followed after 5 minutes by $50cm^3$ of 2N $CaCl_2$ solution. This was allowed to stand for one hour then boiled for a few minutes and filtered. The residue was washed with boiling water until quite free from chloride. It was then boiled with water and filtered on a Gooch Crucible. The residue was washed, dried and weighed as calcium pectate (88).

$(C_{17}H_{22}O_{16}Ca)$.

2.6.3 Pectic Acid (81, 87).

2.6.3.1 (a) Extraction of Sample.

30g of skin powder was macerated in 80cm^3 distilled water in a beaker then boiled for one hour followed by addition of 3 lumps of refined sugar. This was filtered into a 200cm^3 flask and made up to the mark with distilled water.

(b) The above solution was evaporated to about 25cm^3 and immediately 200cm^3 of 95% ethanol was added with stirring. The precipitate was allowed to settle, then filtered through a 15cm qualitative paper and residue washed with ethanol.

The precipitate was transfered to the original beaker with hot water and then evaporated to about 40cm^3 and cooled to room temperature; few drops of HCl (1 + 2.5) was added and warmed, cooled again and diluted with 30cm^3 of 1% NaOH. Allowed to stand for 15 minutes then followed by 40cm^3 of distilled water and 10cm^3 of the HCl (1 + 2.5) and then boiled for 5 minutes. Filtered and the pectic acid washed with hot water.

The pectic acid was washed into a platinium dish and dried on steam bath and finally in oven at 100°C to a constant weight. This was then ignited and reweigted. Loss in weight equals to the pectic acid.

$$\% \text{ Pectic acid} = \frac{\text{loss in wt. on ignition pectin}}{\text{original sample wt.}}$$

2.6.3.2 Chromatographic Seperation of Pectin.

(a) Procedure. (Paper chromatography and TLC) (92) concentrated water solution of the pectin was spoted on a chromatogaphic plate by means of a micro pipette. This was allowed to dry then a decending chromatography was run using water - Acetic acid - Pet. ether Ammonia (10:5 2:1) as the mobile phase. Running time was one hour. The chromatogram was visualised in Iodine tank for 1 second; the Rf values of the distinct spots recorded.and compared with literature values for identification.

2.7 Determination of some properties of Extracted Oil Boiling point and Melting point.

2.7.1 Melting Point. Since the fats are semi-solid at room temperature, there is a choice od determining the melting point after cooling to strict solidification or the solidifing point, after melting to a clear liquid. However the two methods are not identical. In this work the capillary tube method was used to determine the melting point.

2.7.1.1 Capillary Tube Method.

Some melted fat was aspirated into a capillary tube (1mm diameter) and one gently sealed with a low flame without burning the oil. The tube and the fatwas cooled in a refrigerator overnight. A thermometer

was fastened to the tube so that the column of the fat and the bulb of the thermometer were at the same level. These were then heated slowly (0.5°C per minute in a water bath and the temperature at which the fat liquifies was noted as the melting point.

2.7.2 Boiling point. Emrich's method (82) Apparatus.

(i) A capillary tube 10cm long and 1mm bore with one and drawn into a very fine capillary and about 2cm long.

(ii) Liquid paraffin a melting point apparatus.

(iii) thermometer.

2.7.2.1 Procedure.

The capillary tube was dipped into melted oil, so that the oil rose slowly by capillary attraction, the tube was removed when the oil has filled the narrow conical portion. The capillary end was sealed by merely touching with a minute flame. A small air bubble was formed in the point of the capillary. This was easily seen with a lens. The capillary tube was then attached to a thermometer as in the melting point determination above. The v arrangement was heated slowly in the paraffin oil in the open bath which is kept well stirred. When the bubble enlarged and began to show signs of upward motion, the flame was considerably lowered or removed as the need demanded. The temperature at which the bubble

reached the surface of the bath liquid was taken as the boiling point of the oil. The bath was allowed to cool to about 10°C below the first observed boiling point then slowly heated again, and a second determination of the boiling point was made. The averge determination was taken.

2.7.3 Acid Value.

The acid value of a lipid is the number of milligrammes of potassium hydroxide (KOH)) required to neutralise One gramme of fatty material.

2.7.4 Procedure.

2g of ether extracted lipid sample was dissolved in 50cm^3 (1:1%) ethanol/diethyl ether mixture in a 250 cm^3 Erlenmeyer flask. This was titrated with 0.1N ethanolic potassium hydroxide solution using phenolphthalein indicator.

2.7.5 Calculation.

$$\% \text{ Acidity} = (V_a - V_b)\frac{MN}{10W}$$

where,

V_a = number of cm^3 of ethanolic KOH required for end point.

V_b = End point of blank

M = Molecular weight of fatty acid adopted (e.g. 282 for oleic acid).

N = Molarity of the ethanolic KOH

W = weight of sample used in grammes.

$$\underline{\text{Acid Value}} = \frac{56.1\ (V_a - V_b)\ N}{W}$$

2.7.6 Saponification Value, (61, 83, 84).

Saponification value denotes the mass in milligrams of potassium hydroxide required to saponify one gram of the lipid. (i.e. the amount of sodium hydroxide to neutralize the free fatty acid and fatty acid combined in the lipid).

2.7.6.1 Procedure.

To 1g of lipid in a 250 cm^3 Erlenmeyer flask, 25cm^3 of 1M ethanolic potassium hydroxide solution was added with some antibumping granules. The flask was then fitted with a reflux condenser and boiled gently, swirling from time to time to ensure complete mixing; the heating continued for about two hours. Few drops of phenolphthalein were added; and the excess potassium hydroxide in the hot soap (glyceride ester) was titrated with 0.5M hydrochloric acid solution.

A blank titration on the ethanolic potassium hydroxide was also carried out simultaneously under similar conditions.

2.7.6.2 Calculations.

$$\text{Saponification value (S.V.)} = \frac{56.1N\ (b - a)}{W}$$

Where,

a = number of cm^3 of HCl used in sample test

b. number of cm^3 of HCl used in blank.

N = Normality of HCl solution

W = weight of oil sample in grams.

2.7.7 Iodine Value (Hanus) (67)

The iodine value of a lipid is a measure of its degree of unsaturation. In practice it is determined by the amount of halogen absorbed by 100 parts of weight of the lipid.

2.7.7.1 (a) Materials.

(i) Hanus Iodine Solution (13.2 Pure Iodine in 1L HOAC + 3cm^3 Br)

(ii) Liquid Bromine

(iii) Acetic acid

(iv) 0.1N sodium thiosulphate

(v) 15% Potassium Iodide

(vi) Carbon tetrachloride

(b) Apparatus:

(vii) 500cm^3 Reagent bottles (g - S flask)

(viii) Pipette 25cm^3.

2.7.7.2 Procedure.

0.5g of the fat was weighed in 500cm^3 g-s flask and dissolved in 10cm^3 carbon tetrachloride. With pipette, 25cm^3 Hanus Iodine solution was added and allowed to stand for 30 minutes in the dark cupboard and shaken occasionally.

10cm^3 of 15% KI solution was added and shaken thoroughly. Then followed by 100cm^3 of freshly boiled and cooled water,

washing down and free Iodine on the stopper.

The Iodine was then titrated with standard 0.1N $Na_2S_2O_3$, gradually with constant shaking until yellow solution turns almost colourless; A few drops of starch indicator were then added and the titration continued until the blue (Iodine-starch complex) colour was discharged. Towards the end of titration, the flask was stoppered and shaken vigorously, so that the Iodine remaining in the carbon tetrachloride layer may be taken up by KI solution. Replicate titrations were made and two blank titrations also conducted alongside.

2.7.7.3 Calculation.

$$\text{Iodine value (No.):} = \frac{12.69\ N(b - a)}{W}$$

where

W = mass of lipid used

a = av. cm^3 of 0.1N $Na_2S_2O_3$ used for lipid sample.

b = av. cm^3 of 0.1N $Na_2S_2O_3$ used for blank test.

N = Exact Normality (Molarity) of $Na_2S_2O_3$ after standarisation.

2.8 Determination of Mineral (Ash analysis) (67, 69).

All the mineral metals in ash solution were determined with Atomic Absorption spectrometer (AAS); (Pye Unicam S.P. 1900 model) (85), except for the alkaline metals (Sodium and potassium) which were determined with Corning-Eel Flame Emission Spectrometer (FES) where they are more sensitive.

Phosphorous was determined by the Molybdovanadate Colonimetric method; and chloride was estimated by the Morh's direct titration method (86).

In the Atomic Absorption spectrometer, the solution is sprayed into the flame of the spectrometer already set as described in the Data Book (85). Radiation of characteristic wave length from a selected hollow cathode discharge lamp is passed through the flame, and the atoms of the element absorb some of the radiation and become excited. The decrease in the intensity of the radiation of the Cathode lamp is monitored using a monochromator and detector system. The extent of absorption of the cathode radiation by the element is dependent on the number of atoms of the element in the ground state and thus is a linear function of the concentration of the atoms of the element in the sprayed solution.

In the case of flame Emission spectrometer (87), after spreading the investigated solution in the flame of the photometer, the atoms of the metal are excited by absorbing radiant energy of the flame. On returning to the ground state,

they emit the absorbed radiation. The intensity of the emitted radiation is directly proportional to the concentration of the element in the solution.

2.8.1 Sample Solution Preparation.

The ash obtained as in the determination of ash-content was treated with $10cm^3$ of 6M HCl to wet it and then gradually evaporated on a hot plate to dryness. Then $12cm^3$ of 3M HCl was added and heated until the solution just boiled. This was then allowed to cool then filtered through a small filter paper into a $100cm^3$ standard flask. Any solid left on the evaporating dish was treated with some 3M HCl boiled, cooled and filtered again into the flask. The dish and the filter paper were properly washed through into the flask leaving any impurity on the paper. About $5cm^3$ of 0.1M Lanthanium Chloride ($LaCl_3$) solution was added to the flask, to prevent interference in the case of calcium determination.

2.8.2 Preparation of Standard (calibration) curves.

Stock solution of 1000 ppm ($1000\mu g/cm^3$) of each metal to be determined was prepared as described in the PYE Unicam AAS Data Book (85) given in appendix 2. All the reagents used were of A.R. grade and deionized water or Hydrochloric acid was used as the solvent in all cases. Appropriate dilutions were then made to correspond with the limit range of each mineral element present in ecological materials as given by Allen et al (91) and shown in appendix 3.

To ensure the exact strength of each stock solution, it was further standardised with EDTA using Eriochrome Black T Indicator.

2.8.3 Determination of Phosphorous

2.8.3 (a) Reagents:

(i) Molybdovanadate reagent - 40g of Ammonium Molybdate.$4H_2O$ dissolved in $400cm^3$ hot water; 2g ammonium metavanadate in $250cm^3$ hot water, added $450cm^3$ of 70% perchloric acid. Then gradually mixed and made up to $2dm^3$.

(ii) phosphorous standard stock solution - (1000 ppm or $1000\mu g/cm^3$) - 8.788g potassium dihydrogen orthophosphate dissolved in water and made up to $1dm^3$.

(b) Apparatus:

(i) Spectrometer to read at 400 mμ.

(ii) Graduated $100cm^3$ flasks.

2.8.3.2 Procedure.

Aliquots of ash solution of each sample was pipetted into a $100cm^3$ flask and $20cm^3$ of the molybdovanadate reagent was added and the solution made up to the mark and allowed to stand for at least 10 minutes.

The standard stock solution of the phosphorous was then diluted accordingly with dionized water to obtain standard working solution containing 0.5, 0.8, 1.2, 1.4 and 1.5mg were placed in

seperate 100cm^3 flask and treated as above with Molybdovanadate reagent.

Reading were taken with the spectrometer set at 100% transmitance for the 0.5mg standard. The readings of each sample were taken along side with the standards. Average of 3 readings were taken. A standard curve was plotted and the concentration of phosphorous in the samples interpolated. (ref. calibration for phosphorous. fig. 8).

CHAPTER THREE

3.0 RESULTS AND DISCUSSION.

3.1 Results.

Table I

Physical Properties of 3 types of Avocado Fruits

Race (type)		MEXICAN	WEST INDIAN	GUATAMALIAN
Weight (g):	Max.	323.00	289.10	271.50
	Min.	321.00	288.00	270.50
Length(cm):	Max	14.30	15.30	13.51
	Min.	14.27	15.00	13.00
Width:	Max.	7.21	7.60	7.65
	Min.	7.10	7.40	7.00
Relative density				
mean ± s.d.		0.98 ±0.1	0.98 ± 0.1	0.98 ± 0.0
% Pulp (Eadible portion)				
per fruit M_3 ± s.d.		57.5 ± 2.5	62.0 ± 3.0	60.0 ± 2.1
% Seed per fruit				
$Mean_4$ ± s.d		38 ± 1.0	30.5 ± 1.1	30.1 ± 1.2

Table 2

Proximate Composition of Avocado Pulp

(dry wt. basis)

RACE:	MAXICAN	WEST INDIAN	GUATEMALAN
	mean of 5 ± s.d	mean of 3 ± s.d	mean of 3 ± s.d
*Moisture%	72.3 ± 1.1	77.3 ± 1.0	77.8 ± 1.4
Total water Soluble solid %	71.8 ± 3.4	71.6 ± 3.2	71.4 ± 2.8
Total sugar %	2.63 ± 0.16	3.07 ± 0.05	2.00 ± 0.04
Reducing sugar %	1.7 ± 0.07	1.4 ± 0.05	1.25 ± 0.04
Lipid %	29.2 ± 0.68	25.8 ± 0.68	27.6 ± 0.49
Crude Fiber %	11.16 ± 0.03	11.40 ± 0.43	10.95 ± 0.15
Crude protein (%N x 6.5)	2.57 ± 0.14	2.6 ± 0.15	2.1 ± 0.18
Ash %	1.15 ± 0.10	1.15 ± 0.05	1.14 ± 0.08
"total" Vitamin C (mg/100g)	12.2 ± 1.3	12.3 ± 1.7	16.0 ± 1.00

*Fresh weight basis

Table 3

Proximate Composition of Avocado Seed

RACE:	MAXICAN	WEST INDIAN	GUATEMALAN
	means of 5 ± 0.31	mean of 3 ± s.d	mean of 3 ± 0.20
Moisture %	70.5 ± 0.31	69.3 0.25	70.2 0.20
Total water soluble solid %	80.2 ± 1.5	79.1 ± 2.0	78.0 ± 1.8
Total surgar %	1.8 ± 0.14	1.6 ± 0.12	1.75 ± 0.11
Reducing sugar :	0.8 ± 0.01	0.91± 0.02	0.90 ± 0.01
Lipid %	22.0 ± 0.10	20.1 ± 0.15	18.0 ± 0.21
Crude Fiber %	8.0 ± 0.2	8.6 ± 0.25	7.9 ± 0.21
Crude Protein (%N x 6.5)	2.2 ± 0.12	2.00 ± 0.16	2.1 ± 0.15
Ash %	2.00 ± 0.15	1.96 ± 0.1	2.00 ± 0.10

Table 4

Promixate Composition of Avocado-Skin or Exocarp

	MEXICAN	WEST INDIAN	GUATEMALAN
	Mean of 5 ±s.d.	Mean of s ± s.d	Mean of 3 ± s.d
Moisture %	80.1 ± 2.1	78.9 ± 2.6	82.0 ± 2.8
Total water soluble solid %	69.5 ± 1.1	71.1 ± 2.5	70.1 ± 2.3
Total sugar %	0.10 ± 0.01	0.20 ± 0.01	0.12 ± 0.02
Reducing Sugar %	-	-	-
Lipid %	5.1 ± 0.1	6.3 ± 0.09	3.0 ± 0.12
Crude fiber %	14.0 ± 0.0	15.1 ± 0.3	13.8 ± 0.2
Crude protein %N x 6.5	0.00	0.00	0.00
Ash %	0.14 ± 0.02	0.17 ± 0.04	0.2 ±0.03
Pectin %	52.50 ± 0.21	52.45 ± 0.44	52.51 ± 0.20

Table 5

Mineral Composition of Avocado (Pulp)

Minerials:	K	Na	Ca	Mg	Fe	Zn	Cu	P	Cl
				m /100g					
MEXICAN=	156	3.0	3.1	4.0	0.88	0.8	Tr	3.0	3.7
WEST INDIAN	153	3.1	3,1	3,9	0.88	2.0	Tr	3.1	3.6
GUATEMALAN	160	2.7	2.9	4.2	0.87	0.9	Tr	2.9	3.6

Table 6

Mineral Composition of Avocado Seed (mg/100g)

Minerials:	K	Na	Ca	Mg	Fe	Zn	Cu	P	Cl
MEXICAN	115.0	21.0	9.0	17.0	0.4	-	0.1	10.1	3.1
WEST INDIAN	116	23.0	9.1	16.8	0.4	-	0.1	9.8	3.1
GUATEMALAN	118.0	21.0	8.6	19.1	0.5	Tr	0.1	10.2	3.1

*To convert from mg/100g to % divide by 1000.

To convert m,/100g to µg/g or ppm multiply by 10.

Table 7

Vitamin C Content of Avocado Pulp

	DNPH Method (mg/100g)		DCPH method (mg/100g)	
MEXICAN	12.21 13.22 12.20	Mean of 3 12.21	9.50 9.30 9.40	Mean of 3 determinations 9.40
WEST INDIAN	12.3 12.35 12.3	Mean of 3 12.33	6.80 6.70 6.75	Mean of 3 6.75
GUATAMALAN	16.0 16.1 16.0	Mean of 3 16.03	8.82 8.81 8.75	Mean of 3 determinations 8.79

Table 8

Some Physical Properties of Avocado

Oil (Ether/Ethanol extract)

Melting point	=	5.0 - 6.5°C
Boiling point	=	70 - 80°C
Iodine value (Hanus)	=	880.0 g Iodine/100g sample
Saponification value	=	180.2 MgKoH/100g sample
Relative density	=	0.98
Acid value =	=	1.0 - 7.5 (2% oleic acid)

Table 9

T.L.C. Seperation of Avocado Skin Pectin

Solvent front = 13.0cm^3

Component	Eront (cm)	R_f Value	Identification
I	7.6	0.585	Cholestenol (C_{27})
II	8.5	0.653	aliphatic saturated alcohol C_{25} - C_{35}
III	11.2	0.862	aliphatic Esters (C_{20} - C_{34})
IV	12.0	0.920	Glycarides (C_{27} - C_{32})
V	13	1.00	Saturated hydrocarbon

3.2 Discussion.

3.2.1 Physical Properties of Avocado Fruit.

Table I gives the physical properties of the tree types of avocado fruits studied. The fruit size (weight) ranges from 270 to 321g per fruit. In all cases the Mexican race is more massive than a typical West Indian which in turn is more massive than the Guatemalan fruit. The length and width show the Guatemalan to be the shortest and the West Indian the largest. Dispite the variation in the dimensions and sizes, all the avacoda races have a constant relative density of 0.98.

The West Indian type has the highest percentage edible portion followed by the Guatemalan type. The percentage ratio of the seed to whole fruit weight is the same for both Guatemalan and the West Indian but is slightly higher for the Mexican type.

The variations in physical properties seem to be due to fruit species rather than ecological locations since there is only a small deviation for each specie compared to the marked deviation with other species.

3.2.2 Proximate (bulk) composition of Avocado Pulp.

The proximate composition of the edible portion (pulp) is given in table 2. The moisture content ranges from 72 to 78%.

The West Indian and the Guatemalan fruits have nearly the same moisture content (77 - 78%) which is higher than that

of the Maxican (72%). Apart from other genetic factors, this difference can be accounted for by the fact that the latter specie has a thinner and smooth fruit skin which can allow faster evaporation of volatile components even at room temperature.

The total water soluble portion of the pulp is nearly the same for the three races; this indicates a similar chemical composition. The total sugar content ranges from 2.0 to 3.0% with the West Indian having the highest and the Guatemalan the lowest value. The level of the reducing sugar show a similar trend.

The lipid content of the avocado fruit is higher than all values recorded for other tropical fruits. The values obtained here are similar to that given in liberature (vide appendix 7) This high fat content gives avacode fruit a unique value among fruits. Its energy content of 88% is correspondingly high compared to 77% recorded for banana. Banana has been considered one of the highest energy source among fruits as shown in appendix 6. In the United States, avocado ice cream and salad are very popular because of its special digestible oil content.

The crude fiber content is almost the same (11%) for all the species. The value is similar to that of paw-paw and pinapple which means that most of the edible portion is digestible in the human system.

The crude protein value ranges from 2.1 to 2.6% The West Indian has the highest value while the Guanthamalan has the least. However, these values are higher than those of most tropical fruits as shown in appendix 6. Fruits generally are not good sources of protein compared with the seeds and vegetables.

The ash content is consistent with some literature values for some avocado species in U.S.A. (see appendix 7). The average ash percentage of 1.15 is suggestive of the corresponding fair inorganic nutrients in the fruit. The slight deviation among the different species reflects the ability of the species to retain different inorganic nutrients, which altbough in traces, are very useful.

3.2.2 "Total" Vitamin C content.

The value for this ranges from 12.2 to 16.0 mg/100g with the Guatemalan having the highest. The result is generally low compared with other fruits like baoba (300 mg/100g) and citrus fruits (200 - 400 mg/100g). Hence, the avocado fruit is not a good source of this vitamin.

3.2.3 Proximate Composition of Avocado Seed)

Table 3 gives the proximate somposition of the three races of avocado seed. The moisture content shows only a slight deviation for all the races. This is so, probably because all the

seed were protected, inside the fruit until time of experiment, which means the condition for the moisture loss for all of them was almost the same. This result here is quitecomparable with that given by McCane in appendix 5. It is interesting to note that the avocado seed has the highest moisture content of 69 - 70.5% among Nigerian common seeds, next to this is coconut with 46.3%.

The water soluble portion ranges from 78% for the Guatemalan to 80.2% for the Mexican race. This shows that the seed is more water soluble than other major portions of the fruits.

The sugar content is low (1.8% max.). Equally low is the lipid content. This low lipid value for the seed seems to be compensated for by the high value for the pulp. The value for th- crude fiber is higher than those for most tropical seeds except for cotton seed. The result indicates that the avocado seed would be less digestible in human and animal systems. The avocado seed is a poor source of protein. The protein level is about 2 to 2.6%. Values as high as 30% are recorded for Walnut and cotton seed as shown in appendix 5.

The ash value of 2.0% is comparable with other seeds. This is an indication of a fair mineral content in the seed.

3.2.4 Proximate Composition of Avocado Skin (Excocarp)

The composition of the skin as given in table 4, reveals that this portion of the fruit is nutritionally poor. Apart from water content, water soluble solid and crude fiber which are

considered economically wasteful when purchasing the fruit are high. Other values like protein, fat sug[illegible]d ash are very small.

3.2.5 Mineral Composition of Avocado Pulp.

Table 5 gives the mineral (inorganic elements) composition of the three Nigerian races of the fruit's edible portion. Chlorine, phosphorous, Zinc and Iron seem to show no signiffication variation in the content level for all the species. Potassium content varies from 153 mg/100g for the West Indian type to 160 mg/100g for the Guatemalan type. Sodium level ranges from 2.7mg/100g for Guatemalan to 3.1 mg/100g for the West Indian. The variation in calciumand Magnesium contents is less than 0.2% for the three races.

The results for the mineral composition of the fruit pulp when compared with those of common Nigerian fruits as given by Onyenuga and Fetuga in appendix 4, reveals the following about the avocado:

(i) It is poor in K compared to other common fruits;

(ii) Has low Ca and Mg contents;

(iii) It is high in and best source of Iron;

(iv) Also contain low Zn which is totally absent in other common fruits.

(v) Very low in P and Cl; and Cu is totally absent.

However, the mineral components are needed only in traces in the human body. Thus this study has shown avocado as a complete fruit in this respect as it contains most of the essential minerals needed in human body.

Table 6 gives a similar composition for the seed. It is equally indicated that the seed could be nurishing if the absence of trace poisonous constituent is ascertained.

3.2.6 Vitamin C Contents by Different Methods.

The values of vitamin C content determined by the 2,4-dintrohydrazine (DNPH) reagent and indophenol (DCPH) methods are presented in table 7. The results obtained by the DNPH method vary from 12.21 to 16.03mg/100g for the three races of the fruit studied. This variation is not unexpected since the vitamin is very susceptible to a lot of interferences as enumerated in chapters one and two. The results obtained by the DCPH method are lower than those obtained by the DNPH method since the former measured only the reduced form of the ascorbic acid (Vitamin C) which is easily oxidizable during the determination processes and storage. The same reasons can be given for the wide deviation between 9.4 and 6.75mg/100g obtained for the fruits by this method.

The highest ascorbic acid level recorded here is low when compared to other fruits level, this confirms the avocado fruit as a poor source of Vitamin C.

3.2.7 Some other Properties of the Fruit Components.

Table 8 gives some physical properties of the avocado oil (ether/ethanol extract). The high iodine value of 880 and saponification value of 180.2 indicate a high degree of unsaturation. Thus the oil can be suitable for the production of butter by hydrogenation of the fat. The temperature range between the melting point and the boiling point is narrow enough to make it favourable for the above production too.

All the results presented in the data (tables 1 - 8) were simple averages of not less than three determinations unless otherwise stated. The results for the mineral determination were more reproducible than those of the proximate analysis. The small variation could be due to many factors which include

(I) non uniformity of freshness in portions studied,

(II) Small differences in the age of the fruit which this work could not take into account because the fruits were purchased randomly from local markets and

(III) Slight changes in the composition during processing and storage periods. However, the results obtained for ascorbic acid (vitamin C) were rather variable as the acid is very susceptible to changes. The formation of osazones of minor interfering substances like glucose in the sample solution could not be totally obviated, hence the results obtained here for 'total' vitamin C were rather unreliable too, even though the deviation from 'true' value might not be high.

Table 9 shows the likely components of the pectin extracted from the fruits skin (excocarp). The pectin is shown here by chromatographic separation to be a complex composition of hydrocarbons, alcohols (aliphatic) glycerides and chloresterol. High pectin value is suitable for fruit jam and Jelly production.

3.2.8 Availabllibility and Eating Habit.

The eating habit of avocado in Nigeria seems to be influenced by its abundancy in the country and secondly by its taste. Since the fruit tree is not commonly found in aboundance as other popular Nigeria fuits like oranges, mangoes, banana and pappaw people tend to show little demand for it. Moreso, the other mentioned fruits have higher sugar content and therefore sweeter than the flat and rather insipid taste of avocado. It is noteworthy that more people pay greater attention to fruit taste rather that nutritional and medicinal values of it. The comsumption of avocado fruit is higher in the southern parts of the country than in the North, the reasons for this are two folds; firstly, the southerners are more familiar with the fruit due to their ealier contact with the Europeans who might have been the first to introduce the fruit into the country. (There are many small avocado orchards in schools and premises occupied by colonial masters in Calabar). Secondly the fruit growth is more favoured by the Rain forest climate of the South than the climate of the Savana areas of the north; hence the population density of the fruit tree is greater in the south

and the fruit is commonly seen here in the markts during the season.

The fruit is more popularly eaten by the educated Nigerias as a dessert after meals than the illiterate majority despite the low price of the fruit. The price of one avocado range from 10k to 25k per fruit depending on the size and irrespective of the specie. The highest price was noted in Kaduna in September where five fruits were sold for N1.50k.

The fruit is eaten raw when ripe (soften). Here it is cut vertically into two halves so that the seed can be removed and the pulp eaten on the skin using a teaspoon. It is also used widely as a salad fruit with the addition of lime juice, salt or salad dressing. Mashed and seasoned avocados are used as a sandwich filling or as a spreadу on salted crackers and in the tropics they are added to soup just before serving (93).

Fully riped fruit has been used successfully to make avocado ice cream and this adumbrates some promise as a minor outlet for disposal of good quality fruit. Documented records in this approach include that of Cruess et al, (94) in California who have reported that the macerated flesh of avocodo can be mixed with either sugar or vinegar, frozen quickly and stored successfully in glass containers at 15^{o}C or lower. The sweetened frozen pulp can be used in ice cream. and the vinegar and pulp mixture can be used in sandwich spreads and salads. Macfic and Stahl (95) also reported satisfactory storage of quick frozen

macerated avocado pulp mixed with salad dressing, salt and spieces in anamel lined cans which were frozen immediately after filling and sealing.

3.3 Conclusion:

The proximate analysis as well as the determination of mineral contents of the avocado fruit have shown the fruit to be a 'complete' one with high nutritional value. The pulp being more nutritious followed by the seed. The skin is of low nutritional value. The avocado has the highest fat content among other fruits and consequentlyia high energy value; hence good for growing children. By the virtue of its high energy value and low sugar content, the avocado also stands a more recommendable source of energy for diabetic patients.

The fruit is seen to grow well in all parts of the country without any significant variation in nutrient composition. The fruit tree needs no special core for proper growing and it takes about five to seven years to bear the first fruits. It is not easily destroyed by pests and changes in wheather.

The low consumption of the fruit presently in Nigeria is due to ignorance of it's nutritional value, scarcity of the fruit, and moreso, it's insipid and non-sugary taste discourages a lot of people who do not know the alternatives ways of consuming the fruit as a palatable sauce.

3.4 Suggestion for further work.

1. A weekly study of the variation in the physical properties and composition of each specie of avocado with the age of the fruit during its major fruting period (February to September).

2. Analysis for nutrients and possible toxic substances in non-edible portions of the avocado fruit.

3. Changes in the composition and physical properties of avocado lipid (ether/Ethanol extract) with the age of the fruit.

4. The detail study of fat and separation of various esters present.

PLATE - I

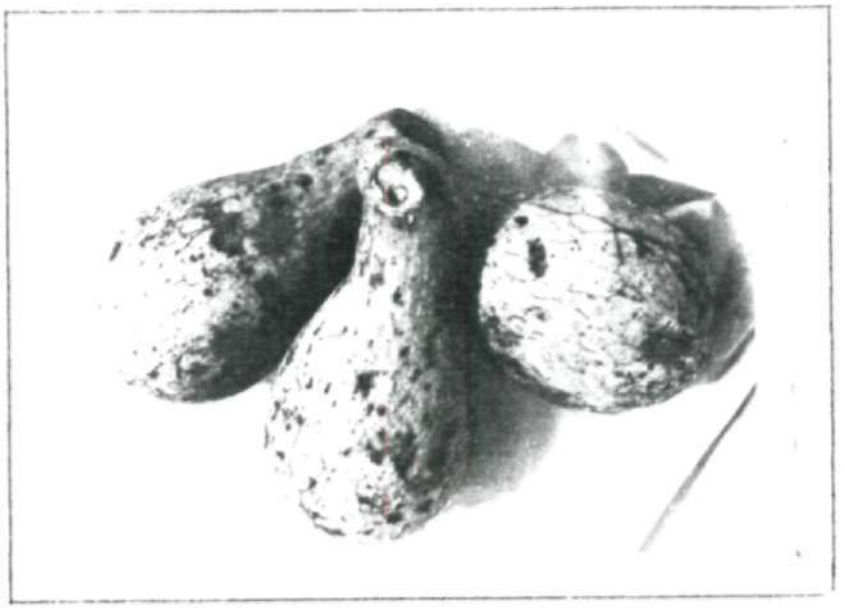


FIG. I : FRESH AVOCADO FRUITS



FIG. II T.S. AND L.S. OF AVOCADO FRUIT

Fig. 2 CALIBRATION CURVE FOR SODIUM

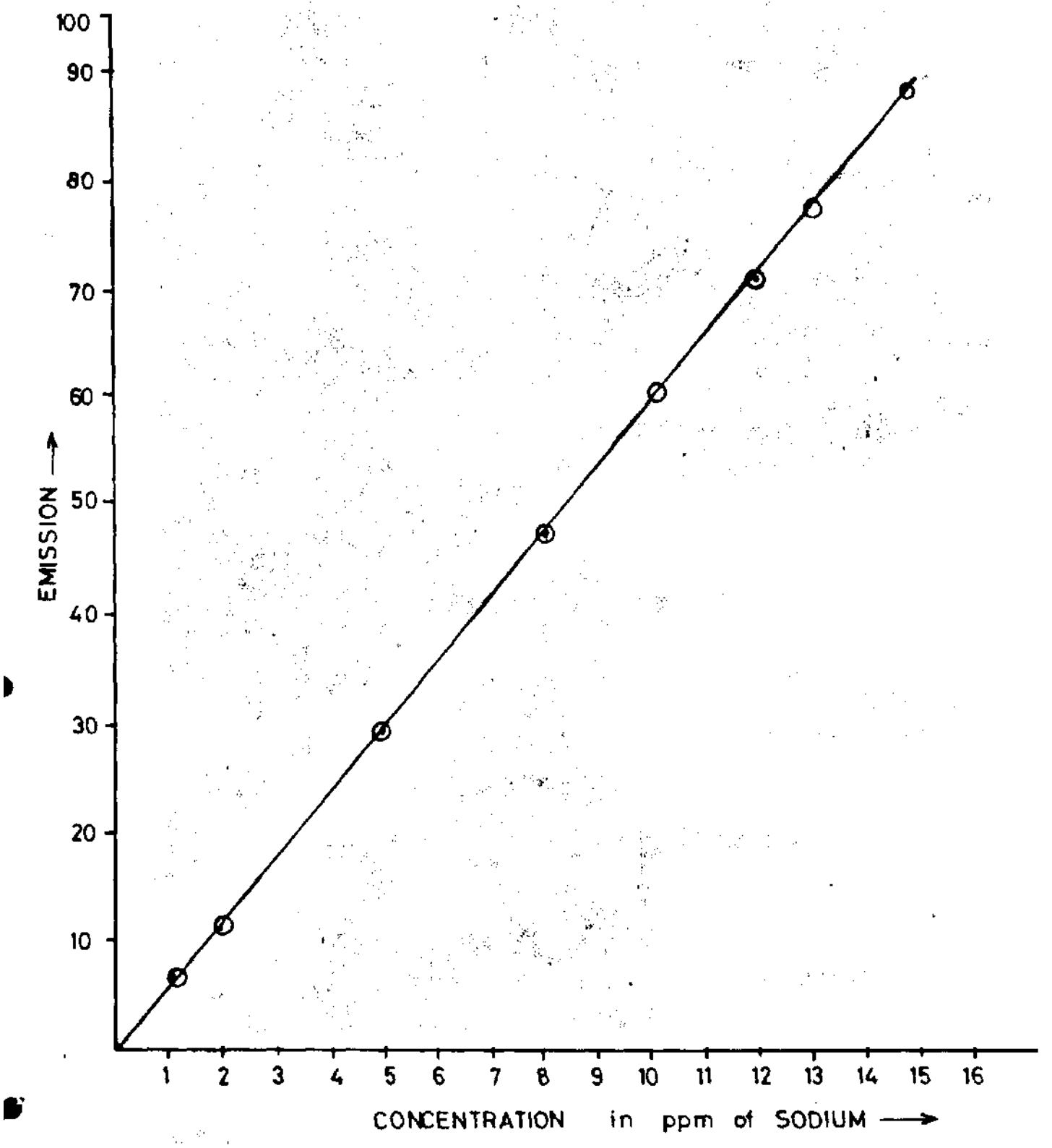


Fig. 4

A CALIBRATION CURVE FOR DETERMINATION OF POTASSIUM

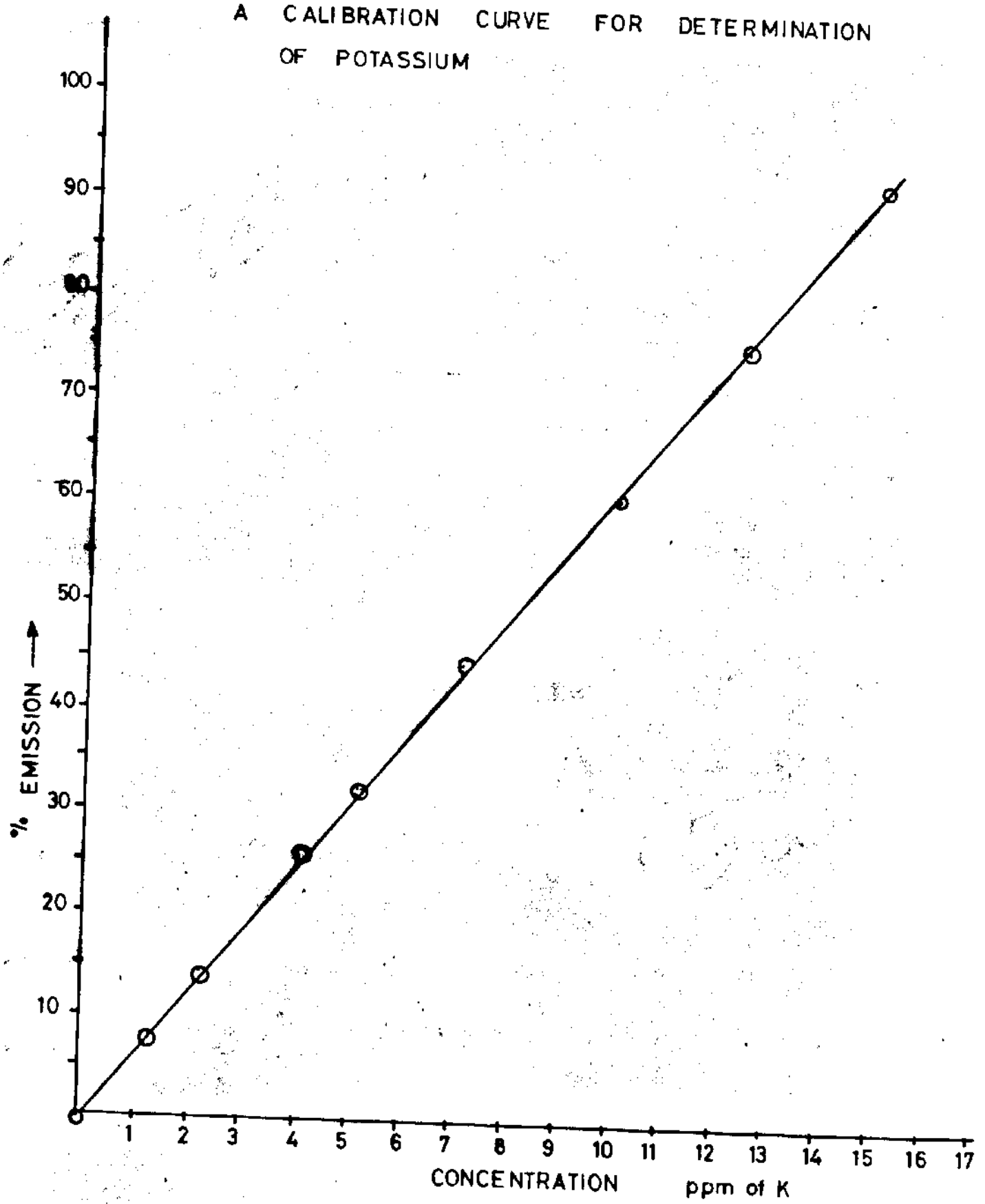


Fig 5 CALIBRATION CURVE FOR CALCIUM DEDTERMINATION

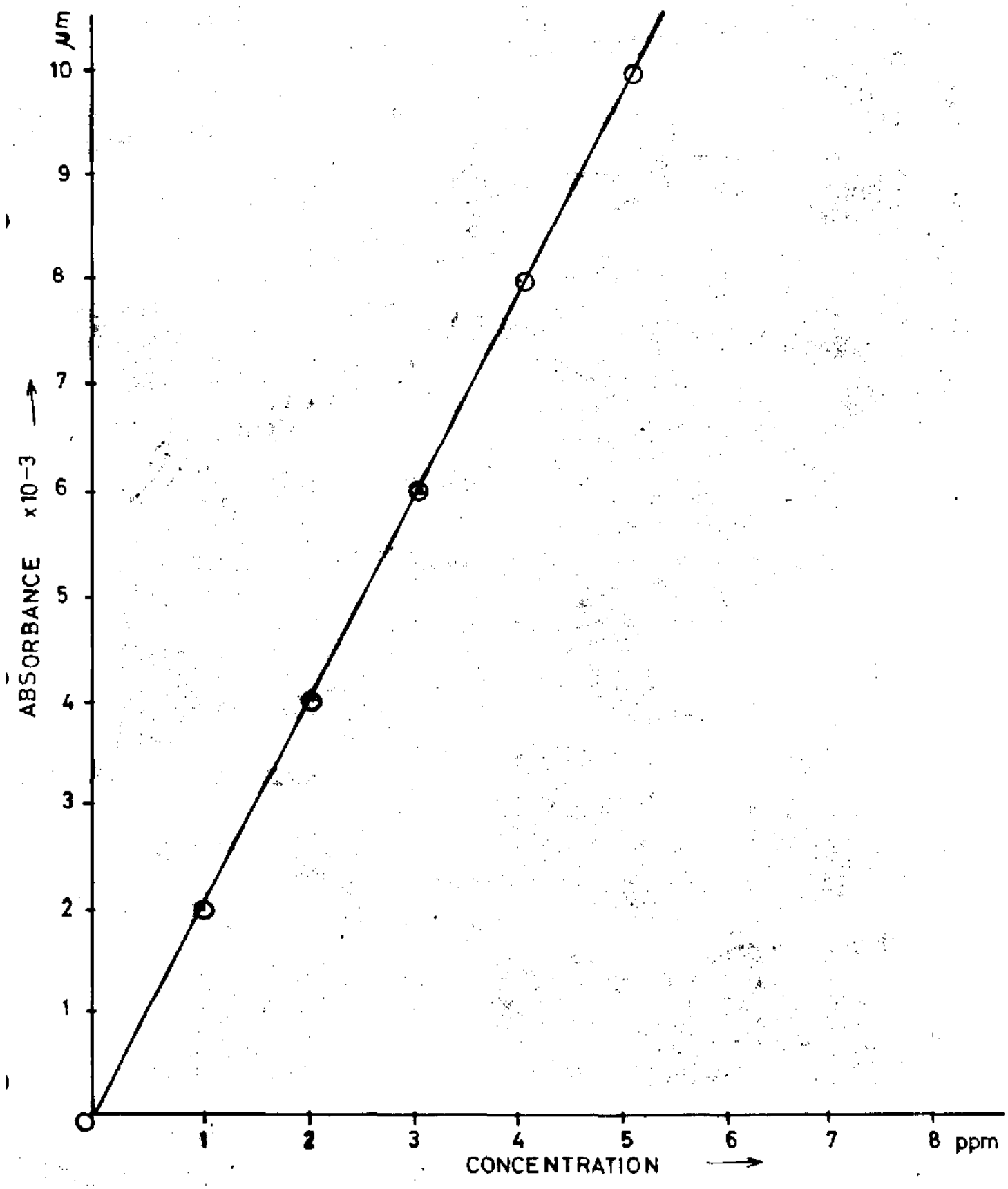


Fig. 6

A CALIBRATION CURVE FOR DETERMINATION OF IRON.

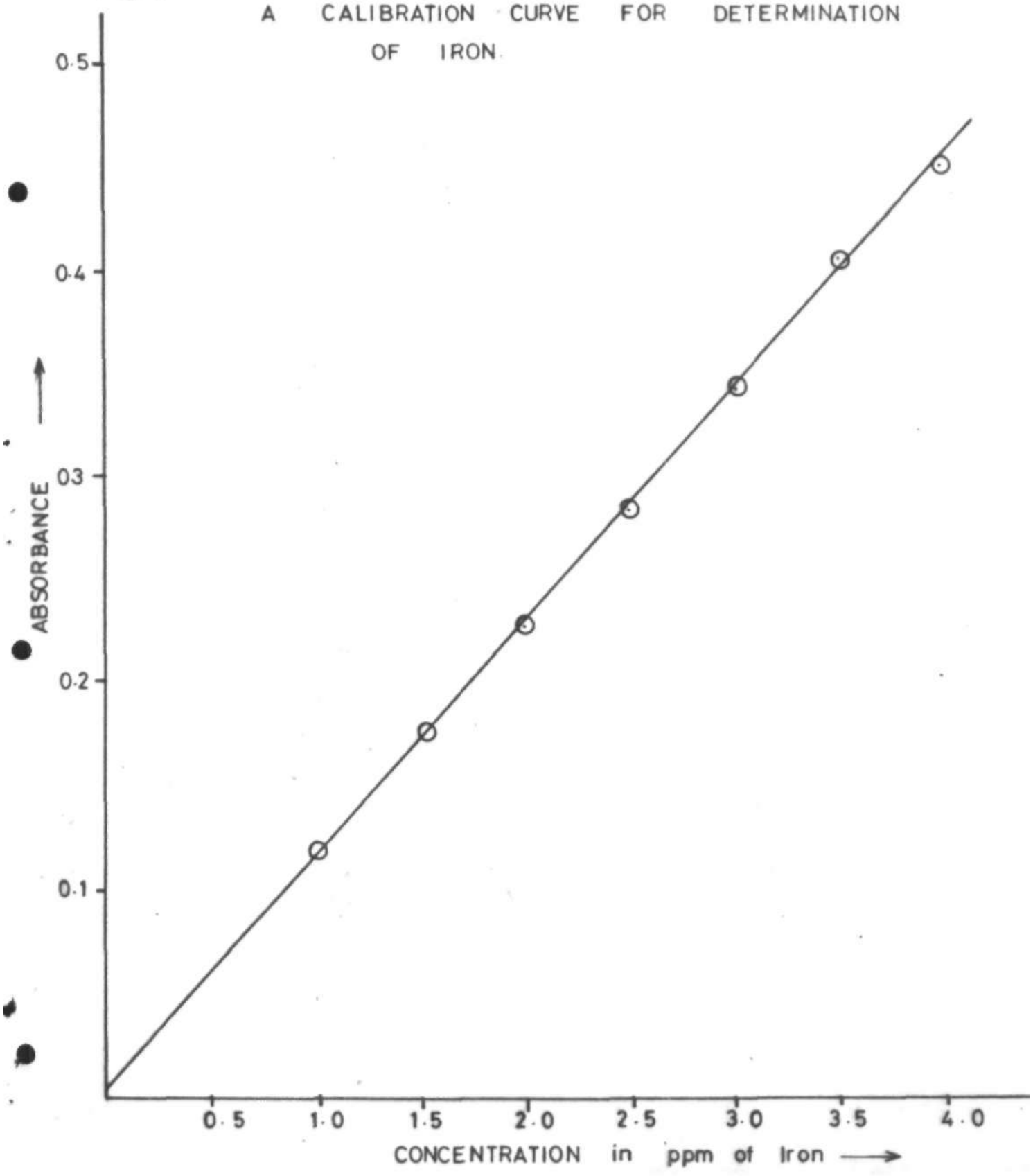


Fig. 7

A CALIBRATION CURVE FOR MAGNESIUM

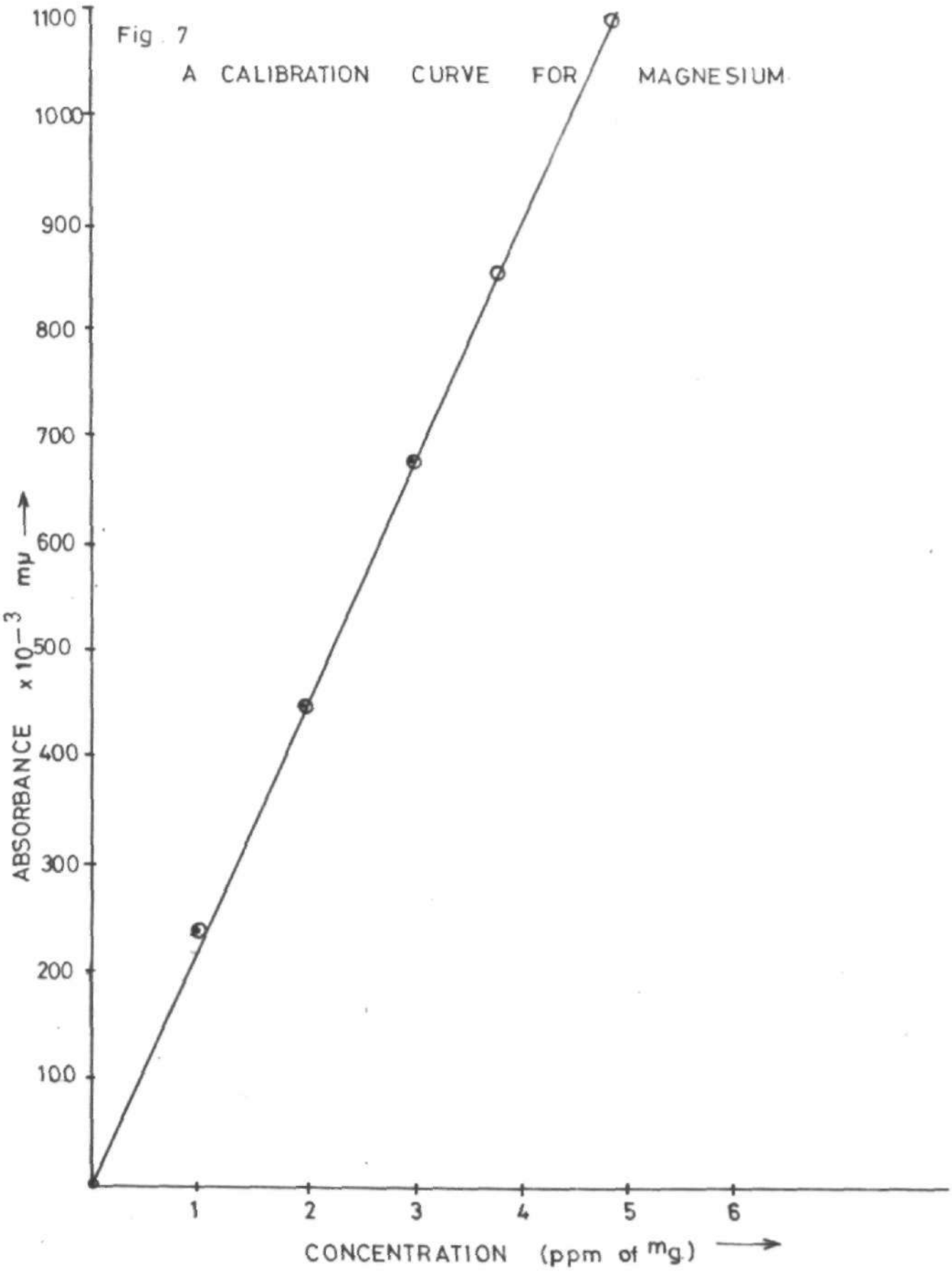


Fig. 8

A CALIBRATION CURVE FOR DETERMINATION OF PHOSPHORUS.

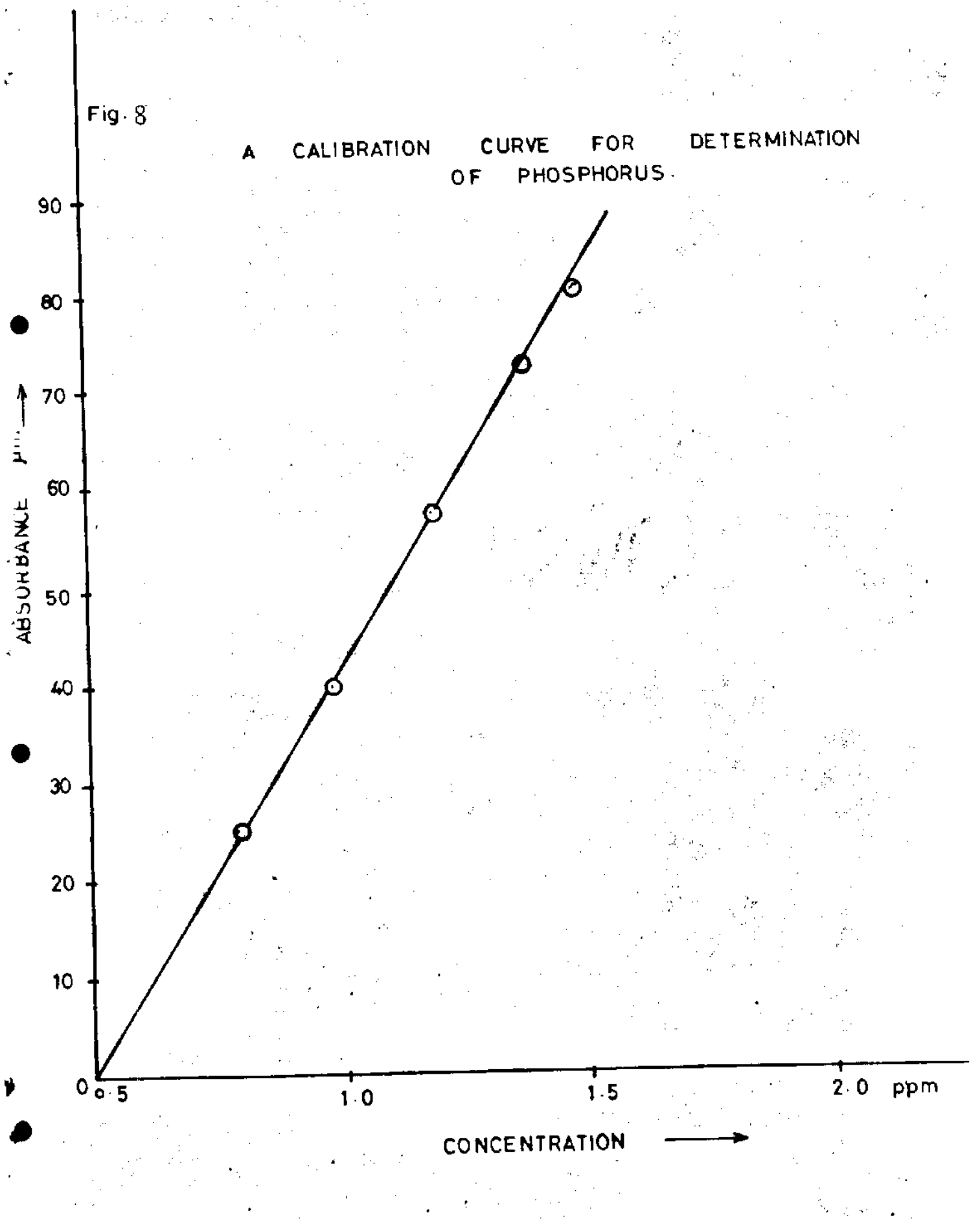
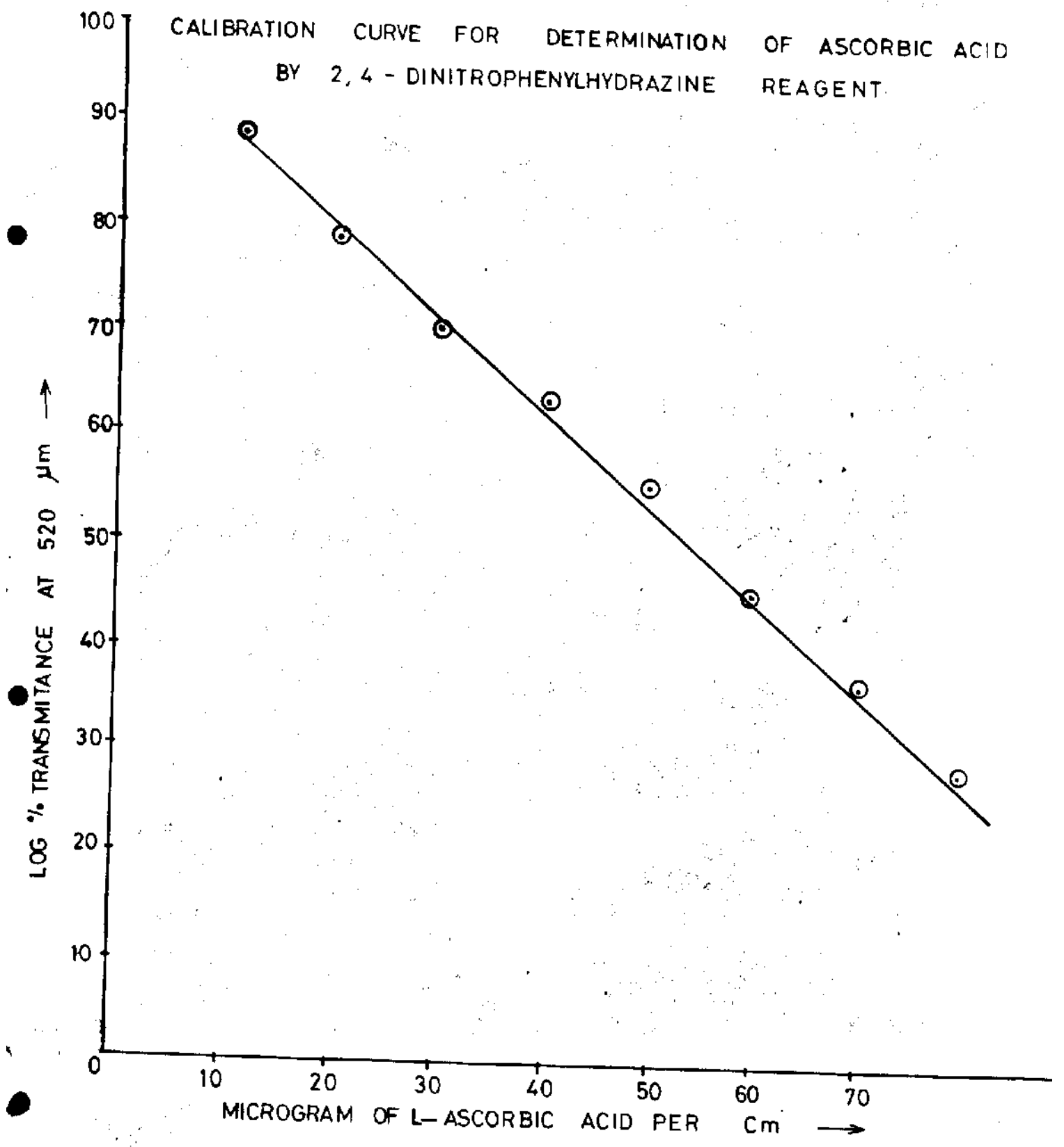


Fig.9

CALIBRATION CURVE FOR DETERMINATION OF ASCORBIC ACID BY 2, 4 - DINITROPHENYLHYDRAZINE REAGENT



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APPENDIX ONE

Conversion Table of cm^3 Sodium Thiosulphate in the Determination of sugar for $25cm^3$ Luff-Schoorl Reagent

0.1M Sodium Thiosulphate ($Na_2S_2O_3$) cm^3	Sugars $C_6H_{12}O_6$ mg	0.1M Sodium Thiosulphate $Na_2S_2O_3$ cm^3	Sugars $C_6H_{12}O_6$ mg
1.0	2.4	13.0	33.0
2.0	4.8	14.0	35.7
3.0	7.2	15.0	38.5
4.0	9.7	16.0	41.3
5.0	12.2	17.0	44.2
6.0	14.7	18.0	47.1
7.0	17.2	19.0	50.0
8.0	19.8	20.0	53.0
9.0	22.4	21.0	56.0
10.0	25.0	22.0	59.1
11.0	27.6	23.0	62.2
12.0	30.3	24.0	65.1

From: Official Journal of European Communities, p.41, 1974.

Appendix 2

Preparation of Standard Stock Solution (1000µg/cm^3) of Elements*

	Mineral Elements	Reagents	wt/dm^3	Solvent
1.	Calcium	$CaCO_3$(drd)	2.4973g	1MHCl 25cm^3
2.	Magnesium	$MgCl_2.6H_2O$	3.9160g	D.Water
3.	Zinc	ZnO	1.2450g	5cm^{3}D.water + 25cm^3.5MHCl + D. water.
4.	Iron	$FeCl_3.6H_2O$	4.8400g	D. water.
5.	Copper	$Cu(NO_3)_2.3H_2O$	3.7980g	D. Water
6.	Manganes	$MnCl_2.4H_2O$	3.6007g	50cm^3.Con.HCl + D. water
7.	Sodium	NaCl(dry)	2.4440g	D. water
8.	Potassium	KCl(dry)	1.5040g	D.Water

*Pye unicam AAS Data Book, 18th Ed. (1975)

APPENDIX 3

Plant-Life Mineral Ranges*

MINERALS	RANGE
Sodium	0.02 - 0.3%
Potassium	0.5 - 3.0%
Calcium	0.3 - 2.5%
Magnesium	0.1 - 0.5%
Iron	40 - 500µg/g
Copper	2.5 - 25µg/g
Zinc	15 - 100µg/g
Maganese	50 - 1000µg/g
Phosphorous	0.05 - 0.3%

*Allen et al (1974) *Chemical Analysis of Ecological Materials*

Appendix 4

The Mineral Composition of Some Common Nigerian Fruits mg/100g Edible Portion

	K	Na	P	Mg	Fe	Cu	Ca	Cl
Banana	346	1.5	29.4	41.9	0.52	0.24	7.9	79.8
Grape	344	2.1	16.1	5.2	0.43	0.09	4.2	1.8
Guava	295	4.2	46.2	13.8	0.94	0.32	230.0	52.4
Lime	184	7.2	21.4	12.3	0.37	0.22	122.0	4.9
Mango	192	6.4	13.8	11.3	0.43	0.31	11.4	24.2
Orange	201	3.2	24.8	11.8	0.42	0.08	42.6	3.1
Pine Apple	263	2.1	10.2	17.3	0.54	0.09	12.2	32.4
Pawpaw	-	-	11.5	-	2.24	0.22	178.4	-

Data from: Oyenuga, V.A. and Fetuga, B.L. (1975)

Appendix 5

A Food Composition of Some Oil Seeds

	Moisture %	Protein %	Fat %	Fiber %	Ash %
Cocoanut (Karnel)	46.30	4.08	37.29	3.39	1.03
Walnut (Kernel)	2.50	30.30	57.80	1.60	2.00
Palm (Kernel)	8.40	8.41	48.75	5.82	1.75
Soy Beans	10.80	33.98	16.85	4.79	4.69
Cotton seed	0.00	30.29	25.79	14.37	3.24
Avocado	69.00	1.8	22.0	7.2	1.40

Data from: McCance, R.A. (editor): Medical Research Council Special report Series No. 235 1946, p.82-3

Appendix 6

Food Composition of Common Fruits (Raw)

Food	% Edible portion	Moisture %	Sugar %	Protein %N x 6.5	Fat %	Energy %
1. Apple English Eating	79.0	84.5	11.4	0.3	Tr	45
2. Banana(fresh)	59.0	70.7	16.2	1.1	Tr	77
3. Grape fruit (fresh)	48.0	90.7	5.3	0.6	Tr	22
4. Lemon(Juice)	36.0	91.3	1.6	0.3	Tr	7.0
5. Orange(fresh)	75.0	86.1	8.5	0.8	Tr	35
6. Mango	48.0	88.0	10.0	0.7	0.1	15
7. Pine Apple	53	84.3	11.6	0.5	Tr	46

From: R.A. McCance (editor): Medical Research Council Special report series No. 235 1946, p78 - 79.

Appendix 7

Composition of Avocado Fruits From Different Location In U.S.A.

100% Fr. Wt.

Location	Fruit wt. g.	Edible portion	Moisture	Fat	Carbo-hydrate	Ash
Altadena, Califonia	256	71.3	1.51	26.6	4.62	1.60
Yorba Linda "	556	73.5	1.36	24.2	4.82	1.27
California	200	75.0	1.80	20.0	7.80	1.20
Califonia	254	70.0	1.56	20.4	4.69	1.35
Florida	496	63.3	1.21	13.6	1.78	0.92
Boston	422	72.2	0.90	6.3	1.56	0.64
Sonoran, Mexico City	298	64.8	1.40	13.0	1.52	0.87

Data from: A.C. Hulm (editor): The Biochemistry of Fruits and their Products Vol. 2. p.7 Academic press London and New York; 1970.

Appendix 8

Recomended Conditions for Metal Analyses Using The AAS or The FES*

Element	Wave-length	Absorption on Emission E	Max. current MA	Limit of Detection	Working range µg metal cm^3	Possible interference	Control of addition of:
Calcium	442.7	A	6	0.01	0.05 - 5	Al^{3+}, S^{2+}, SO_4^{2-}	$LaCl_3$
Copper	324.8	A	6	0.005	0.05 - 5	-	-
Iron	248.3	A	15	0.03	0.05 - 5	-	-
Magnessium	285.2	A	6	0.001	0.02 - 5	Al^{3+}, S^{2+}, SO_4^{2-}	$LaCl_3$
Manganese	279.5	A	12	0.005	0.02 - 3	-	-
Potassium	-	E	-	0.002	1 - 20	Na^+	1000µgNa
Sodium	-	E	-	0.002	1 - 20	K^+	1000µgK^+
Zinc	213.9	A	10	0.004	0.1 - 1	-	-

Flame	Air-Acetylene
Mode	A
Sensitivity	513
Slit	126
Curvature	000
Scale Expansion	000

*Osborne et al 1978. *The Analysis of Nutrients in foods*, Academic press., London.