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CHEMICAL ANALYSIS OF SOME LESSER  
KNOWN NIGERIAN SAVANNA SEEDS  
BY

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(ii)

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any other University or Institution. Information derived from the published or unpublished work of others has been acknowledged in the text.

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RECOMMENDATION

We hereby recommend that the thesis prepared by  
Mr. G.A. OBODO entitled: "Chemical Analysis of  
Some Lesser Known Nigerian Savanna Seeds" be accepted  
in partial fulfilment of the requirements for the  
award of the degree of master of science in  
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DEDICATION

Dedicated with love to my wife,  
Mrs. Nnenna Obodo, and to my  
children, Uchechukwu, Chidozie  
and Chimere.

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ABSTRACT

The best source of protein is animal, but it is expensive and not always within reach of the common man. In all developing countries, including Nigeria, cereals are still the main source of plant protein, although the quantity and quality of these are often low. Consequently, research has continued in search of better alternative plant proteins.

Although there are locally available protein foods in Nigeria, their widespread use is limited by the virtual lack of adequate information on their chemical and biological values. The present investigation was designed to determine the nutritional values of the following locally available protein seeds: Parkia clappertoniana (PC), Adansonia digitata (AD), Tamarindus indica (TI), and Pentaclethra mycrophylla (PM).

The results showed that the testas (T) had higher values of moisture ( $10.80 \pm 0.03\%$  for PC,  $7.66 \pm 0.15\%$  for AD,  $9.52 \pm 13\%$  for TI,  $21.63 \pm 0.45\%$  for PM), carbohydrate (81.77% for PC, 88.69 for AD, 83.75 for TI, and 68.04 for PM), nitrogen free extracts (53.10% for PC, 48.82% for AD, 60.51 for TI, and 55.06 for PM) and crude fibre (28.67% for PC, 39.80 for AD, 23.24 for TI and 12.97 for PM) than their corresponding nuts (N) (Moisture Content:  $7.5 \pm 0.0\%$  for PC,  $5.18 \pm 0.02$  for AD,  $7.04 \pm 0.11$  for AIP,  $5.59 \pm 0.02$  for TI and  $13.35 \pm 0.20$  for PM); (Carbohydrate: 24.76% for PC, 26.25 for AD, 75.89 for AIP, 41.15 for TI and 8.36 for PM), (Nitrogen free extracts: 23.52% for PC, 23.19 for AD, 66.94 for AIP, 38.23 for TI, and 3.57 for

PM,); and (crude fibre: 1.24% for PC, 3.06 for AD, 8.95 for ADP, 2.92 for TI, and 4.79 for PM).

The nuts had higher levels of ash ( $4.11 \pm 0.31$  % for PC,  $5.53 \pm 0.18$  for AD,  $5.11 \pm 0.68$  for ADP,  $2.45 \pm 0.14$  for TI and  $2.06 \pm 0.50$  for PM); lipid ( $23.46 \pm 1.12$  % for PC,  $24.66 \pm 2.60$  for AD,  $11.52 \pm 1.83$  for ADP,  $12.90 \pm 2.10$  for TI and  $52.09 \pm 4.8$  for PM); crude protein ( $40.17 \pm 0.32$  for PC,  $38.38 \pm 0.00$  for AD,  $0.44 \pm 0.02$  for ADP,  $37.91 \pm 0.05$  for TI and  $24.14 \pm 0.02$  for PM); calorific value ( $470.86$  calories for PC,  $480.46$  for AD,  $409.10$  for ADP,  $432.34$  for TI and  $598.81$  for PM) and minerals (sodium:  $0.03$  % for PC,  $0.04$  for AD,  $0.02$  for ADP,  $0.05$  for TI, and  $0.09$  for PM), (potassium:  $2.35$  % for PC,  $2.96$  for AD,  $4.10$  for ADP,  $1.41$  for TI, and  $1.21$  for PM), (calcium:  $0.13$  % for PC,  $0.07$  for AD,  $0.09$  for ADP,  $0.04$  for TI and  $0.05$  for PM), (magnesium:  $1.15$  % for PC,  $1.22$  for AD,  $0.30$  for ADP,  $0.36$  for TI, and  $0.47$  for PM), (phosphorus:  $0.08$  % for PC,  $0.17$  for AD,  $0.02$  for ADP,  $0.06$  for TI and  $0.05$  for PM), (iron:  $79.00 \mu\text{g/g}$  for PC,  $80.40 \mu\text{g/g}$  for AD,  $96.50$  for ADP,  $68.20$  for TI and  $53.60$  for PM), (copper:  $1.65 \mu\text{g/g}$  for PC,  $2.10$  for AD,  $1.63$  for ADP,  $1.52$  for TI and  $1.60$  for PM), (zinc:  $19.70 \mu\text{g/g}$  for PC,  $27.70$  for AD,  $22.30$  for ADP,  $14.70$  for TI and  $17.70$  for PM), (manganese:  $1.87 \mu\text{g/g}$  for PC,  $1.30$  for AD,  $0.44$  for ADP,  $0.82$  for TI and  $1.40$  for PM) than their corresponding testas (ash :  $2.32 \pm 0.27$  % for PC,  $1.80 \pm 0.18$  for AD,  $0.94 \pm 0.45$  for TI and  $1.91 \pm 0.39$  for PM); (lipid:  $1.91 \pm 0.00$  % for PC,  $1.62 \pm 0.01$  for AD,  $1.89 \pm 0.01$

for TI and  $3.88 \pm 0.01$  for PM), (crude protein;  $3.20 \pm 0.04$  % for PC,  $0.24 \pm 0.07$  for AD,  $3.90 \pm 0.01$  for TI and  $4.55 \pm 0.01$  for PM), (calorific value: 357.07 calories for PC, 370.26 for AD, 367.61 for TI and 325.24 for PM), and minerals (sodium: 0.06 % for PC, 0.03 for AD, 0.02 for TI and 0.04 for PM), (potassium: 0.87 % for PC, 1.17 for AD, 0.07 for TI and 0.81 for PM), (calcium: 0.10 % for PC, 0.04 for AD, 0.07 for TI and 0.07 for PM), (magnesium: 0.36 % for PC, 0.32 for AD, 0.32 for TI and 0.10 for PM), (phosphorus: all had the same value of 0.01 %), (iron: 67.00  $\mu\text{g/g}$  for PC, 57.70 for AD, 159.70 for TI and 66.70 for PM), (copper: 1.20  $\mu\text{g/g}$  for PC, 4.10 for AD, 1.02 for TI and 1.52 for PM), (zinc: 54.70  $\mu\text{g/g}$  for PC, 9.47 for AD, 17.00 for TI and 38.30 for PM), (manganese: 0.26  $\mu\text{g/g}$  for PC, 0.49 for AD, 12.50 for TI and 1.80 for PM).

The higher values of fibre and carbohydrate in the testas may be due to the fact that testa is mainly made up of cellulose and lignin. These materials also tend to decrease the digestibility of foods and feedingstuffs in which they occur, thereby reducing their nutritional values. It was observed that Pentaclethra mycrophylla had higher values of proximate composition than Adansonia digitata, Parkia clappertoniana, or Tamarindus indica. Adansonia digitata had higher levels of minerals than parkia clappertoniana, Pentaclethra mycrophylla or Tamarindus indica.

The values of proximate composition obtained in the investigation were slightly higher than some literature values. These differences might be attributed to the effects of local soil conditions, methods of analytical procedure, and plant species.

Further work on these seeds was suggested on the fermented states, the condition in which they are normally taken by humans. Wet digestion should be preferentially used in determining minerals in order to avoid the losses due to volatilization.

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## CHEMICAL ANALYSIS OF SOME LESSER KNOWN NIGERIAN SAVANNAH SEEDS

### INTRODUCTION

#### 1.1. BACKGROUND.

Nigeria has of now, an estimation of well over eighty million humans and an estimated one hundred and nine million livestock (cattle, goats, sheep, donkeys, pigs, horses, camels and poultry) as of 1964 (Oyenuga, 1968). For nearly two decades, and with increased governmental encouragement in food production, in recent years, the livestock population must have undoubtedly more than doubled since then.

How to feed these teeming millions of men and animals is of immense concern to every body. And yet, the problem seems more compounded by the fact that most of the staple food for humans also serve as livestock feeds, so that there exists a keen competition between human and livestock needs for these scarce food items. However, the basic problem appears to be that of in-adequate and unbalanced-feeding rather than the too much scarcity of the human and livestock feeding stuff. For according to Shaw and Colville (1950), "the basic problem concerning any livestock improvement in Nigeria is not one in the first instance for the geneticist, but for the animal feeder. It is the nutrition of the existing breeds of cattle, sheep and goats that has first to be put on a higher plane before the geneticist and animal breeder can either select or improve".

The need to search for alternative sources of plant nutrition and how to improve them have been intensified.

Associated with in-adequate and unbalanced feeding is the virtual lack of the nutritive compositional data of the many varied protein sources indigenous to Nigeria. Among them are:

- (i) Parkia Clappertoniana (Dorowa-Hausa),
- (ii) Adansonia Digitata (Kuka - Hausa),
- (iii) Tamarindus Indica (Tsamiya - Hausa),

all of which are seeds of the Savannah vegetation, and common to the northern States of Nigeria, and

- (iv) Pentaclethra Mycrophylla (Ugba - Ibo),

an oil seed commonest in the Southern parts of Nigeria. What is needed therefore, is an investigation of their nutritional and chemical composition, so that they could be incorporated into foods for humans and livestock.

#### 1.2. JUSTIFICATION

Most of the proteinous human and animal feeds available in the country are either imported or are formulated within, but partly from imported ingredients. This situation, not only causes the scarcity of these commercial feeds in most parts of the year, but also most importantly fritters away the much needed foreign exchanged which could have been plunged into more pressing developmental projects. This makes it more imperative to either improve, formulate or even

discover other nutritional alternatives. Some of these seeds common to Nigeria, could be used as protein concentrates, because they are rich in protein. They might, in combination with the correct carbohydrate, provide good sources of human and live-stock feeding-stuffs.

1.5. OBJECTIVES

This investigation was designed (i) to estimate the nutritional and chemical composition of some of these lesser known Nigerian seeds, (ii) to discover the difference in chemical and nutritional composition between the nuts and testas (iii) to compare the different methods of mineral determination, such as, dry ashing and wet ashing, and (iv) to be able, probably <sup>to</sup> recommend a more practicable method of analysis, under our prevailing local conditions.

Seeds of Parkia Clappertoniana, Adansonia Digitata, Tamarindus Indica and Pentaclethra Mycrophylla, all locally available, will be analysed for their proximate (bulk) composition and mineral contents. The results obtained will be compared with the few data given in the literature on similar seeds in Nigeria and else where.

1.4. LITERATURE REVIEW

1.4.1. PROXIMATE COMPOSITION

1.4.1.1. MOISTURE CONTENT

Moisture determination is one of the most important and most widely used measurements in the processing and testing of foods (Makower, 1950). Since the amount of dry matter in a food is inversely related to the amount of moisture it contains, moisture content therefore becomes of direct economic importance to the processor as well as the consumer. Of even greater significance, however, is the effect of moisture on the stability and quality of foods. For example, grains that contain too much water are subject to rapid deterioration from mould growth, heating, insect-damage and sprouting (Martens and Hlynka, 1965).

Small differences in moisture content may be responsible for the unexpected cases of spoilage in commercially stored grains (Christenson and Linko, 1963). The rate of browning in dehydrated vegetable and fruits or oxygen absorption by egg powders increases with an increase in moisture content. Hence moisture determination is important in many industrial problems. We must know the moisture content, and sometimes its distribution, for optimum processing of foods, like in the milling of cereals, mixing of dough to optimum consistency, and for producing bread with the best grain texture and freshness retention. Most importantly, moisture content must be known in determining the nutrient value of foods, so that results in analytical determinations

can be expressed on a uniform basis and in meeting compositional standards or laws.

And finally, it is often desirable to weigh out samples for analytical determinations on a given moisture basis, and this is specially important if the measured analytical parameter does not vary in a linear or simple manner with an increase in dry matter content.

The rapid and accurate determination of water in foods especially those varying so widely in texture, overall composition and moisture content, continues to present many problems. Many workers have therefore stressed the complexity of analytical procedures for the determination of water in foods. Makower (1950) pointed out that though the literature is replete with methods of moisture determination, we have no methods that are both accurate and practical. According to Matchett and von Lock Loesecke (1953), accurate, rapid and simple method of moisture determination applicable to all types of food materials are continuously sought, but it seems doubtful that such a goal will ever be attained.

In practice, the guiding principle has been to prefer the method that gives the highest value for the moisture content, provided that decomposition of organic components and volatilization of others are negligible, or that such losses can be compensated by incomplete removal, and under fixed experimental conditions, of strongly bound water (Hlynka and Robinson, 1954). Generally, the

the reproducibility and practicability of a method (simplicity, convenience of apparatus and rapidity) have been the important factors in the selection of an analytical method for water determination. Less emphasis is placed on the accuracy of water determination more than the extent that it is significant in establishing conditions that govern food stability.

The usefulness and validity of simple and rapid moisture determinations depends on their calibration against standard and accurate reference methods.

The difficulties involved in developing the reference methods can best be understood by considering the manner in which water is held by various food components.

#### 1.4.1.1.1. Some Basic Considerations

The nature of the forces between water and the component substances of foods was reviewed by Ward (1963); and Hamm (1963).

Water may occur in foods in at least three forms.

- (a) A certain amount may be present as free water in the intergranular spaces and within the pores of the material. Such water retains its usual physical properties and serves as a dispersing agent for the colloidal substances and as a solvent for the crystalline compounds.
- (b) Part of the water may be absorbed on the surface of the macromolecular colloids (starches, pectins, cellulose and proteins). This water is closely associated with the

absorbing macromolecules by forces of absorption attributed to Van der Waals forces or to hydrogen bond formation and

(c) Some of the water is in a bound form-in combination with various substances, that is, as water of hydration.

Though convenient, the above classification is arbitrary. Attempts to determine quantitatively, the amounts of various forms of water in foods have been unsuccessful as a result of the continuous spectrum of the isotherm of all types of water binding in foods. Consequently, the terms Free, Absorbed and Bound are relative and as the true moisture content is not known, the conditions selected for moisture determination are arbitrary.

#### 1.4.1.1.2. Methods for the determination of moisture

Methods for the determination of moisture can be divided into: (1) Drying methods, (2) Distillation procedures, (3) Chemical Assays and (4) Physical procedures. Of all the methods, the drying method will be adopted in this work in accordance with the principle of simplicity, convenience and availability of apparatus. Drying methods are additionally, relatively rapid and permit the simultaneous analysis of large numbers of samples and thus they continue to be the preferred procedure by many food analysts. In the drying methods, the Air-oven drying method is adopted and will only be described in the appropriate chapter. The procedure for determining the moisture content specified in food standards generally involves thermal drying methods.

The material is heated under carefully specified conditions and the loss of weight is taken as a measure of the moisture content of the sample. Consequently, the determination of moisture from the loss of weight due to heating necessarily involves an empirical choice of the type of oven and temperature and length of time of drying - hence the values obtained for moisture depend on the arbitrarily selected conditions, so that some of the methods therefore merely provide approximate rather than accurate moisture values. In an ideal procedure for the determination of water, weight losses should result from quantitative and rapid volatilization of water only. The rate at which water moisture can be removed from the surface of a solid phase is a function of the water vapour pressure and of the drying temperature. Water can therefore be determined at any temperature provided the partial vapour pressure in the air above the solid phase is lower than the vapour pressure of the water in the sample. Thus we can have thermal drying below the freezing point of water as in lyophilization. For accurate moisture measurements, the tendency is to dry the foods at the lowest possible temperatures. Practical considerations dictate that temperatures should be selected at which the decomposition of organic compounds is minimised, and yet the time required for quantitative drying at the selected temperature not unduely prolonged.

1.4.1.1.3. Factors affecting the Precision of Moisture Measurements by Air Drying.

According to Koster (1934), and Oxley and Pixton (1961), the accuracy of moisture determination is affected by (1) the drying temperature, (2) temperature and relative humidity and air movement of the drying chamber, (3) Vacuum in the chamber, (4) depth and particle size of sample, (5) drying oven construction and (6) the number and position of the samples in the oven. Equally, the nature and geometry of the sample holder also influences moisture determination. Guilbot (1955) emphasized that the surface of the material being dried and the rate of diffusion of water vapour in the drying substance also affects the results of water measurements. Results therefore show that the rate of evaporation was higher in aluminium than in glass or porcelain dishes, higher in vacuum than in steam ovens, and higher in shallow than in deep dishes. The rate of heat supply to the bottom of the dish was the most important factor.

1.4.1.1.4. Sources of Error in Dry Oven Moisture Determination.

Hlynka and Robinson (1954) listed the general sources of error in the determination of moisture. They include sampling errors (of the bulk lot or at laboratory subsampling stage), moisture change during subsequent storage of the samples, loss of moisture to or gain of moisture from the air, may take place, if the material for moisture determination must be ground.

1.4.1.2. ASH AND ORGANIC MATTER CONTENTS

The Ash of a biological material is an analytical term for the inorganic residue that remains after the incineration of the organic matter. Its content and composition depend on the nature of the food ignited and on the method of ashing. The ash is not usually the same as the inorganic matter present in the original food since there may be losses due to the volatilization or chemical interaction between the constituents. The value is useful in assessing the quality of or grading certain edible materials; it is a measure of the mineral content of the sample.

Ash in foods is determined by weighing the dry mineral residue of organic materials heated at elevated temperature-around 550<sup>0C</sup> the details of which will be described in the next chapter.

The form of the mineral constituent in ash differs considerably from the form of those components in the original food. Thus calcium oxalates are converted into carbonates and upon further ashing, to oxides. Some minerals, linked to biologically active systems, are converted to inorganic components, yet the total mineral component is a useful analytical parameter; and dry ashing is used prior to elemental analysis.

In dry ashing, the selection of ashing dishes (crucibles) depends on the nature of the food to be analysed and on the analyses to be performed on the ash.

The materials used include quartz, vycor, Porcelain, steel, nickel, platinum and a gold-platinum alloy. Silica dishes are very commonly used too. Quartz dishes smooth on the inside are resistant to halogens, neutral solutions and acids (except hydrogen fluoride and phosphoric acid) at most concentrations and temperature. Resistance to alkali is relatively poor. The crucibles are stable at high temperatures (up to 1100<sup>0</sup>C, for routine work) and can be cleaned with hot dilute hydrochloric acid. Porcelain crucibles resemble quartz in physical and chemical properties and have even a higher temperatures resistance, are easily washed with dilute acids, have a good weight constancy and low price, but they are liable to alkali attack and crack with sudden high temperature changes. Steel is sometimes used in ashing large samples and nickel crucibles are least used as they easily deteriorate due to nickel carbonyl formation in reducing atmospheres.

Platinum is the best-but is still not used because of its very high price-for normal routine work. They have a high melting point (1773<sup>0</sup>C), good heat conductivity and high chemical inertness. But they can corrode, and as <sup>such</sup> ~~me~~, should only be touched with platinum-tipped tongs and placed after ashing on clean porcelain asbestos, or marble surfaces. Silica dishes combine most of the good qualities of the platinum but has the added advantage of cheapness and less delicacy.

For total ash determinations, where recovery and determination

of the individual metals is not necessary, ashing in porcelain crucibles and at temperature ranging from 400°C to 700°C but most commonly around 550°C, is satisfactory. But if the ash component are to be determined, the biological material that is to be ashed and the elements that are to be determined must be considered individually as different metals prefer different crucibles. Generally, a furnace with a rheostat for temperature control is used for most routine work.

If prolonged ashing fails to give a carbon-free ash, the residue should be moistured, dried and reheated, until a white-gray ash remains. In some cases, it may be necessary to dissolve the ash in a small amount of water, filter the carbon containing residue through a small low-ash filter paper, dry the two parts and ash separately.

If water fails to break up the material the residue may be treated with a few drops of hydrogen peroxide, nitric acid and/or sulphuric acid; but in the latter cases, the composition of the ash is changed, and special precaution should be taken to report the correct type of ash.

The method has many advocates and attractive because many samples can readily be handled and reagent blanks are generally low, so that it is advantageous for trace element work.

Nevertheless, dry ashing for the destruction of organic matter prior to the determination of trace elements is not used extensively,

because it is generally believed that losses occur from volatilization. According to Lynch (1954), dry ashing, is the most satisfactory method if no losses occur at temperatures up to 500°C.

The literature contains, contradictions regarding alleged losses mainly because the factors controlling the volatilization and retention mechanisms which lead to low recoveries are not always assessed. The most important factors include the temperature and time of ignition, the composition of the sample and the nature and composition of the crucible. All non-metals are potentially subject to volatilization. The method cannot be used for Arsenic and mercury; its usefulness for lead is uncertain, Iron can sometimes be troublesome owing to the difficulty of getting the metal into solution after ashing is completed.

Thiers (1957), summarized reported losses during dry ashing (Table 1). Most of the losses can be minimized at least if proper ashing conditions are used-such as ashing at minimum temperature required to ensure a reasonably complete combustion,

Table 1.: Summary of losses reported during Dry Ashing\*

<u>Metal</u>	<u>Conditions</u>
Copper:	Volatilizes as porphyrin compounds when petroleum samples are burned. Volatilizes from vinegar, possibly as copper acetate at low temperatures. Reduces to metal which is not dissolved by hydrochloric acid.

Table 1: Contd.                      Conditions

Iron: Volatilizes as ferric chloride at 450°C. Volatilizes as porphyrin compounds when petroleum samples are burned. When materials with a high phosphorus-to-iron ratio are ashed, an unidentified compound is formed which resists solution or hydrolysis, causing low results.

Phosphorus: Volatilizes, presumably as one of the oxy acids, especially when sulphate is present, except in the presence of excess magnesium.

Zinc: Volatilizes, presumably as the chloride, above 45°C.

\* From Pomeranz et al: Food Analysis: Theory and Practice. P.558

introducing samples into the muffle furnace cold and the temperature allowed to rise gradually; using oxidative additives such as nitric acid (to facilitate oxidation) or magnesium nitrate (to raise base status), should be cut to a minimum. Losses by retention have long been suspect and are usually attributed to the formation of complex silicates. This may arise through combination with the silica wall of the crucible, if silica crucibles are used and for the silaceous residue of the sample itself. Trace elements, particularly copper and zinc together with manganese and iron are the most likely to be affected.

It is therefore evident that in spite of its advantages and apparent simplicity, dry ashing cannot be universally recommended for solution preparation, and the choice will be governed by the elements required and the type of materials to be used.

#### 1.4.1.3. LIPID CONTENT

The term lipid is used to denote fats and fatlike substances, and is synonymous with the terms lipoids or lipins used in earlier literature. They are usually defined as food components that are insoluble in water and that are soluble in fat solvents. Lipids can also be defined as chemical constituents of living organisms or are derived from such constituents, and most commonly possess fatty acids as part of their moiety - which fact greatly arouses interest in fats and oils. In this work, the terms lipids, fats and oils are used inter-changeably to denote one and the same component.

##### 1.4.1.3.1. Uses of LIPIDS

Lipids have at least three important uses in foods:-  
Culinary, Physiological and Nutritional.

The ability of lipids to carry odours and flavours, and their contribution to palatability of meats, tenderness of baked products, and richness and texture of ice creams are examples of the first kind. As lipids serve as a convenient <sup>means</sup> of rapid heat transfer, they have found increasing use in commercial frying operations. Dietary lipids represent the most compact chemical energy available to man. They contain twice the caloric value of an equivalent weight of sugar.

In the second place, lipids are essentially vital to the structure of and biological function of cells. Dietary lipids provide the essential linoleic acid which has both a functional and structural role in animal tissue.

Thirdly, lipids are carriers of the nutritionally essential fat-soluble vitamins.

#### 1.4.1.3.2. SOLVENT EXTRACTION OF LIPIDS

As indicated, lipids are characterised by their sparing solubility in water and their considerable solubility in organic solvents-physical properties which reflect their hydrophobic, hydrocarbon nature. In practice, the wide range of relative polarities of lipids, as a result of their various structures, makes the selection of a single universal solvent impossible. Successful extraction requires that bonds between lipids and other compounds be broken so that the lipids are freed and solubilized, and generally such solubility is attained when polarities of the lipid and the solvent are similar - and this is the guiding principle in the choice of solvents for extraction.

#### 1.4.1.3.3. PREPARATION OF SAMPLE FOR EXTRACTION

There is no single standard method for lipid extraction. The method used depends on the type of analysed material and the nature of the subsequent analytical problem. Thus extraction of lipids from milk is relatively simple compared to the extraction of lipids from plants or animal tissue - as these require fragmentation such as mechanical grinding, sonic disintegration, homogenization

or compression-decompression. During these steps, it is important that the chemical, physical and enzymatic degradation of lipids are kept to a minimum and this is usually accomplished by the control of temperature, chemical environment and time of exposure of the material to each solvent.

For best results, and especially if the extracted lipids are to be characterized, the following requirements must be met:

(1) All procedures must be carried out in an atmosphere of nitrogen. The tissue should be removed from the source and subdivided as soon as possible. Heating should be minimized. The extract should be purified to remove non-lipid components and the purified lipids should be stored under conditions that minimize alteration.

The moisture content of the material to be extracted is an important factor. Only part of the lipid can be extracted with ether if the material is moist as the solvent cannot penetrate the tissues and the extractant becomes saturated with water and inefficient for lipid extraction. On the other hand, drying at elevated temperatures is undesirable as some lipids become bound to proteins and carbohydrates and are thus rendered inextractable. The extraction of dry materials depends on particle size and consequently efficient grinding is important. The classical method of determining fats in oil seeds involves extracting the ground seeds with a selected solvent after repeated grinding in a mortar with sand,

while soft materials are grated in a grating mill without expressing oil during the grinding. To promote rapid extraction, the solvent and sample could be premixed together in a high speed blender, whose cutting device further reduces the particle size thereby accelerating extraction.

(2) The solvent(s) used must be peroxide-free and must be used in the proper solute-solvent ratio.

1.4.1.3.4. SOLVENTS USED FOR EXTRACTION

Ethyl and petroleum ether are the commonest solvents, but there is a growing tendency to prefer petroleum ether because it is more selective towards true lipids. Ethyl, however is a better solvent for fats than petroleum ether as it dissolves all forms of fats including oxidised lipids. It has however several disadvantages; it is more expensive; there is danger of explosion and fire hazards are somewhat greater; and it picks water during extraction and even dissolves non-lipid materials like sugar. Again, dried ether tends to form peroxides. So that combination or alternate extraction with ethyl and petroleum ether are used often in extraction of lipid from dairy products. Mixtures of alcohol and ether or alcohol and acetone are similarly employed as lipid extraction solvents.

1.4.1.3.5. APPARATUS USED FOR EXTRACTION

Direct extraction is often carried out in a Soxhlet type extractor. The sample in a thimble (which is made from filter paper or alundum) covered with defatted (by soaking in ether) cotton wool

to prevent small particles from finding their way into the flask, is placed in the middle part of the apparatus. The flask is filled with solvent to a convenient level; the three parts of the apparatus are thereafter assembled; the condenser attached to a tap; and the heating of the solvent in the flask started. The condensing vapours fill the middle part containing the sample and carry the dissolved lipid into the flask by a siphoning action each time the height of the siphon is attained. The process is continuously repeated until extraction is exhausted and the apparatus disconnected, the solvent from the weighed or tared flask is evaporated and the weight increases (due to the lipid) calculated. Though other modifications of this process abound in the literature, the above method will be used in this work.

#### 1.4.1.4. PROTEIN AND AMINO ACID CONTENTS

The problem of providing adequate protein for an expanding world population is second only to the overall food problem. Apart from their nutritional significance, proteins play a large part in the organoleptic properties in foods (Rhodes 1973; Schultz and Anglemier 1964). Proteins exert a controlling influence on the texture of foods from animal sources. Proteins often occur in foods in physical or chemical combination with carbohydrates or lipids. The glycoproteins and the lipo-proteins affect the rheological properties of food solutions or have technical applications as edible emulsifiers. The ageing of meat is associated with the chemical changes in the

protein (Whitaker, 1959). Pure native proteins have little flavour. During heating (boiling, baking, roasting) the Amino acid side chains are degraded or interact with other food components like lysine and reducing sugars and give typical flavours (Danehy and Pigman, 1951). Excessive heating may on the other hand reduce nutritive value (Rice and Beuk, 1953).

The food analyst most commonly wishes to know the total protein content of a food, eventhough that content is made up of a complex mixture of proteins. At the moment, all methods, of determining the total protein content of foods are empirical in nature. Isolation and direct weighing of the protein would provide an absolute method. Such a method is sometimes used in Biochemical investigations, but is completely impractical for food analysis.

#### 1.4.1.4.1. AMINO ACIDS

The primary nutritional importance of proteins is as a source of amino acids. Twenty four amino acids are generally thought to be constituents of proteins. Some amino acids are essential to good physical and mental health. Of the amino acids in food, eight are known to be essential to man, that is, they must be supplied in the diet to maintain health and growth. These proteins are:

Lysine, Tryptophan, Phenylalanine, Methionine, Threonine, Leucine, Isoleucine and Valine. Proteins from some plant sources like cereal grains are deficient in certain amino acids like Lysine. Deficient proteins must be combined with those from other sources to provide

an adequate balance of the essential amino acids. Such a balance can be accomplished by a combination of wheat flour with dry skim milk or soya flour which have high percentages of the eight essential amino acids.

1.4.1.4.2. TOTAL PROTEIN CONTENT

The most common procedure for a protein assay depends on determining a specific element or group in the protein and calculating the protein content by using an experimentally established factor. Methods based on the analysis for constituents of proteins include those, for determining carbon or nitrogen, certain amino acids or the peptide linkage. In some proteins, certain constituents (iron in haemoglobin, iodine in thyroglobulin) can serve as a basis for protein assay. In all the above methods it is assumed that the constituent determined is present entirely in the protein fraction. Thus any non-protein carbon-containing matter must be removed, if the protein content is to be determined from the carbon content. Similarly if the Kjeldahl's method is used, protein-nitrogen only should be measured. The common <sup>practice</sup> of estimating the protein content of foods from the total nitrogen assay, is therefore, not always correct. The presence of non-protein-nitrogen compounds is as a rule generally small compared to the protein content of most sound foods.

1.4.1.4.3. ELEMENTARY ANALYSIS

Carbon analysis has several advantages for determining the protein content of foods. The digestion can be accomplished easier than for a nitrogen determination, and high percentage of carbon minimizes experimental errors and provides a relatively constant conversion factor. However, the difficulty of a complete and quantitative separation of protein from non protein carbon-containing components is practically unsurmountable.

And so, a nitrogen determination for a protein assay is the most commonly used procedure. It is generally assumed that a mixture of pure proteins will contain 16% of nitrogen. Thus the protein content of a sample is obtained by multiplying the determined nitrogen by a factor of 6.25, which was obtained by dividing one hundred by sixteen.

For the approximate analysis of foods containing an unknown distribution of proteins of unknown composition, this is a practical and widely accepted procedure. Much confusion has resulted in reporting protein content: Protein levels above 100% in pure or highly concentrated protein fractions, and differences among laboratories originating from the use of various conversion factors. Much of the confusion could be eliminated by reporting the nitrogen rather than the calculated protein content. But the food industry and trade will probably be reluctant to make such a change and will continue to report the calculated protein content. Yet, the

inherent limitation of the procedure must be realised; the results are affected by non-protein nitrogen, the nitrogen content of a particular protein mixture is seldom known precisely, and the methods of nitrogen determination are wrought with some difficulties. For practical purposes, unless shown to the contrary, the effects of non-protein nitrogen can be assumed of little consequence. The general factor of 6.25 is used for most foods. For those foods in which the protein is known to differ from this figure, the specific figures for converting nitrogen to protein are as given in table II.

Table II: Nitrogen-Protein Conversion Factors\*

<u>Food Type</u>	<u>Factor for Converting Nitrogen to Protein</u>
Egg	6.68
Milk	6.38
Rice	5.95
Barley, oats and Rye, wheat (whole kernel)	5.83
Soy beans	5.71
Wheat flour, refine and other cereals	5.70
Gelatin	5.55
Peanuts, Brazilnuts	5.46
Nuts and other seeds	5.30
Almonds	5.18

\*Compiled from:

(1) Pomeranz et al; Food Analysis: Theory and Practice,  
Revised Edition P.672, and

(2) Wood-Tsuen Wu Leung (Editor), Food Composition Table for  
Use in Africa P.4.

The availability of better methods for protein isolation and characterization including amino acid composition provides the basis for a continuous reexamination of the conversion factors (Tkachuk, 1966). Although extremes in nitrogen content range from 4.2 % in beta lipo proteins to 30 % in protamines, most foods contain about 16 % nitrogen.

1.4.1.4.4. Kjeldahl Method

The Danish investigator, Kjeldahl worked out in 1883, a method for the determination of organic nitrogen in his studies on protein changes in grain used in the brewing industry. Since the first publication of kjeldahl, the method has undergone many changes.

Basically, the sample is heated in Sulphuric acid and digested till the carbon and hydrogen are oxidised, and the protein-nitrogen is reduced and transformed into ammonium sulphate. Then concentrated Sodium hydroxide is added and the digest heated to drive off the liberated ammonia into a known volume of a standard acid solution. The unreacted acid is determined and the results are transformed, by calculation; into a percentage of protein in the original sample.

Kjeldahl had originally used potassium permanganate for the oxidation, but this was discontinued as the results were unsatisfactory. In 1885, Wilforth found that a digestion with sulphuric acid was accelerated by adding some catalysts. Gunning in 1889 suggested adding Potassium sulphate to raise the boiling point of the digestion

mixture in order to shorten the reaction time. The test has therefore generally come to be known as the Kjeldahl-Wilforth-Gunning method.

Various factors are known to influence the completeness and speed of the conversion of protein-nitrogen into ammonia by the sulphuric acid digestion. Thus, in some proteins, it is more difficult to convert the organic nitrogen to ammonia. Histidine and Tryptophan-rich protein, generally require long or harsh digestion conditions.

Excessive ratios of potassium or sodium sulphate (added to raise the boiling point) to acid may result in heat decomposition and the loss of ammonia.

Generally, digestion temperatures of  $370^{\circ}$  to  $410^{\circ}\text{C}$  are best.

Nearly all of the likely elements of the Periodic Table have been tried for their effect on Kjeldahl digestion. Mercury, copper and selenium have been widely employed. Mercury is superior to copper, though an additional step is required, precipitation of mercury with sodium thiosulphate to decompose the mercury-ammonia complex formed during digestion. The most controversial catalyst is selenium. It has a more rapid effect than mercury, and unlike mercury, it requires no further treatment before distillation. Nevertheless, loss of nitrogen can occur if too much selenium is used or the digestion temperature is not carefully controlled; the conditions are more critical than with mercury or copper.

So that, a compromise catalyst mixture, consisting of 160 grams anhydrous potassium sulphate, 10 grams copper sulphate pentahydrate, and 3 grams selenium powder is now widely used. The boric acid modification is accurate and has the advantage that only one standard solution (of titrating acid) is required. Neither the amount nor the concentration of boric acid in the receiving bottle need be precise. The Kjeldahl-Wilforth-Gunning method will be preferred in this work.

#### 1.4.1.5. CARBOHYDRATE CONTENT

Carbohydrates are the most abundant and widely distributed food component. The term carbohydrate embraces a broad spectrum of compounds ranging from simple mono- and di-saccharides to complex polysaccharides. In addition to their nutritional and metabolic function, carbohydrates are important as natural sweeteners, raw materials for various fermentation products, including alcoholic beverages. In food composition tables, the most common approach to determine carbohydrate content is usually given as 'Total Carbohydrate' by 'difference' that is, the sum of the percentage of water, crude protein, fat and ash subtracted from hundred. Another widely used term is 'Nitrogen-free Extract' calculated by deducting the sum of the percentage of water, crude protein, crude fat, crude fibre and minerals (ash) from one hundred.

The increasing awareness that specific carbohydrates play significant metabolic and functional roles, and the availability of analytical

tools to determine individual components aroused interest in investigations on their distribution in many foods. However, in this work, we are only concerned with the total carbohydrate and despite objections (like vulnerability to the inaccuracies associated with the determination of the other constituents, negligence of other minor components such as lignin which may be present, and from the nutritional point of view, not differentiating between carbohydrates that are available to man and those that are not) to the use of the difference method, it is going to be employed here.

#### 1.4.1.6 CRUDE FIBRE CONTENT.

Crude Fibre is the organic residue which remains after the material has been treated under standardised conditions with light petroleum, boiling dilute sulphuric acid, boiling dilute sodium hydroxide solution, dilute hydrochloric acid, alcohol and ether. The crude fibre includes, theoretically, materials that are indigestible in the human and animal organism. It consists largely of cellulose together with a little lignin. Crude fibre determinations are greatly affected by manipulations and procedures. Particle size is very important; the finer the material is ground, the lower the determined crude fibre content. Also apparent crude fibre is lowered by defatting, though the low lipid content of some foods (white flour) affects the results little. The rate of heating to the boiling point and the rate of boiling must be controlled. Filtering after each digestion

must be completed within a given time. Delays in filtering after acid or alkali digestion generally lower results.

In general, the outer protective coatings of many foods contain considerably more fibre than the softer, more edible inner tissues. The fibre figure can be employed therefore for assessing the proportion of shell which is present in foods such as cocoa and pepper. Similarly the fibre figure is of value in calculating the amount of the shells of nuts, the stones of fruits and saw dust which may be present in foods, as all these adulterants have a high fibre content. As the fibre content increases with age in plants, the figure may also be of value in assessing the maturity of legumes. The digestibility of feeding stuffs varies inversely with the fibre content. Though the crude fibre is a mixture of cellulosic materials and does not represent any specific compound or group of compounds, yet the crude fibre is a useful parameter in food and feed analyses. Crude fibre is commonly used as an index of the feeding value of poultry and stock feeds; seeds high in crude fibre content are low in nutritional value. The Weende method for the determination of crude fibre will be adopted in this work.

1.4.1.7. CALORIFIC VALUE.

The calorific value of food is the amount of energy it contains. It is calculated, usually, from the amounts of protein, fat and available carbohydrate that it contains, these amounts being derived from appropriate methods previously described. The amounts of protein fat and carbohydrates are then multiplied by factors representing the number of kilocalories produced by one gram of the material in the body. The sum of these gives the Calorific value of the food. According to Oyenuga (1968), these factors are; Protein 4.0, Fat 9.0 and Carbohydrate 4.0.

Energy consumption by the body is related both to heat energy associated with basic body function and temperature and mechanical energy associated with the movement of organs and limbs. The energy value of foods is measured in kilocalories ( 1 kilocalories = 4.18 kilojoules). Even when at rest, the human body needs energy. The amount required has been determined experimentally to be about one kilocalorie per kilogram of body weight per hour or 1500-2000 kilo calories per day. However, this varies with an individuals metabolism and may be as low as 1200 kilo-calories or as high as 2200 kilocalories. Thus a large part of human energy consumption via food is used just for maintaining essential life processes and body temperature. Over and above satisfying the basic energy requirements of the resting body, we need extra energy

to be able to carry out the movements associated with our daily jobs and leisure activities.

When the body derives energy from food, it is less than the amount of energy produced when the food is burnt (completely oxidised) in a calorimetry. This is because the caloric-producing nutrients, which are mainly protein, fat and carbohydrate, are not completely digested, absorbed or oxidised to yield energy in the body. Thus in a calorimetry, the protein is completely converted into carbon dioxide, ammonia, oxides of nitrogen and sulphur and water and yields 5.6 kilo-calories per gram, but in the body, the amount of energy available is only 4 kilocalories per gram. Similarly, the Calorific values for fat and starch as measured by physical techniques are 9.4 and 4.2 kilocalories per gram respectively, but the physiological values are 9.0 and 4.0 kilocalories per gram respectively. Allowances are therefore made for this when calculating the calorific value of foods.

Since no two foods and no two people are exactly the same, the physiological correction factors are based on averages and do not have the same accuracy as the values for protein, fat and carbohydrate found by chemical means. This has resulted in many sets of conversion factors being derived by different workers in the field, though using different experimental approaches to the problem. Another complicating factor is the "availability" of calories in certain food ingredients, that is, there is often a difference

between the number of calories that a diet would provide were the protein, fat and carbohydrate in it completely digested and the number of calories that it does in fact provide. This is mainly due to the so-called "unavailable carbohydrate" which are naturally contained in plant food. The calculation of calorific value is thus, not straight forward, and there is no clear cut answer to it. It is however important to have a consistent approach to the interpretation of data and for the analyst to state the basis of his calculation.

1.4.2. MINERALS.

All forms of living matter require many minerals for their life processes.

And as if to confirm this, virtually all the elements of the Periodic Table have been found in living cells, even though not all are essential to life. The study of mineral nutrition is complex and like the proteins, fats, carbohydrates etc, the minerals do not function independently but are inter-related and balanced against one another. For example, calcium and phosphorus are in a defined relationship in the formation of bones and teeth. Sodium, Potassium, Magnesium, Phosphate, and chloride ions serve individual and collective purposes in the control of body fluids. Many elements act alone or in conjunction with others as catalysts for essential enzymic processes in the body.

The animal body requires seven minerals in relatively large (gram) amounts. These are: Calcium, sodium, magnesium, potassium, phosphorus, chlorine and sulphur, and at least seven others in trace amounts and these are:- Cobalt, copper, iodine, iron, manganese, molybdenum and zinc. In addition, though not fully established as essential, fluorine appears to have an important prophylactic action in bones and teeth, chromium has been claimed to be a glucose tolerance factor, and selenium has been shown to protect against liver necrosis and other disorders in animals. There are also more recent reports that vanadium, tin, nickel, silicon and several others are important in the proper development of experimental animals, such as rat but the importance of these in human nutrition term is yet to be fully established.

Of all the metals, this work is going to investigate to what extent there abounds in the reference samples, the following metals: Sodium, Potassium, Calcium, Magnesium, Copper, Zinc, Iron, Phosphorus, Cobalt and Manganese.

1.4.2.1. DETERMINATION OF MINERALS:

1.4.2.1.1. Dry Ashing: The process of dry ashing has been described before. Thereafter this ash is dissolved in dilute acid. The solution is sprayed into the flame of an atomic absorption apparatus and the absorption or emission of the metal to be analysed is measured at a specific wavelength. Dry ashing for the destruction of organic matter prior to the determination of minerals is not used extensively,

however. This is because it is believed that losses occur from volatilization.

1.4.2.1.2: WET ASHING: Wet ashing is used primarily for the digestion of samples for determining trace elements and metallic poisons. The use of simple acid is desirable, but usually not practical for the complete destruction of organic material.

Sulphuric acid is not a good oxidising agent, and the time required for decomposition is long. In this case, adding a salt, Potassium sulphate, raises the boiling point of the acid and accelerates decomposition. The technique is particularly useful for samples in which adding nitric acid causes the formation of insoluble oxides.

Nitric acid alone is a good oxidant, but it usually boils away before the sample is completely oxidized. Middleton and Stuckey (1954) described a method of destroying organic matter at temperatures below 350°C by digestion with nitric acid as the sole major reagent. Small amounts of sulphuric acid are added at the initial charring stage to prevent the ignition of fat-rich materials when only nitric acid is added. Gorsuch (1959) found the method generally satisfactory, though recoveries were slightly lower than with conventional wet oxidation methods. Selenium and mercury were lost almost completely. Moreover, the procedure is tedious and time consuming.

Mixed acids are the usual reagents for the decomposition of organic materials. The use of a mixture of sulphuric and nitric acids is recommended by many workers and the most acceptable procedure. Suggested quantities of each acid, order and rate of addition, vary with different biological materials and investigators.

To avoid excessive foaming in the digestion of fat-rich or sugar-rich materials, it may be advisable to add sulphuric acid, allow it to soak in, add nitric acid in small portions, with heating in between. In latter digestion stages, hydrogen peroxide may be added to complete digestion. The use of perchloric acid with nitric acid, or with nitric-sulphuric acid mixtures has been suggested by Smith (1953) for the rapid decomposition of many organic compounds that are difficult to oxidise.

Perchloric acid is an excellent oxidant at elevated temperatures. It is used routinely by many laboratories and is the method to be used in this investigation. Digestion with perchloric acid should be performed under a special hood containing no plastic ingredients and no glycerol-containing caulking substances.

The digestate, like that of the dry ashing, is also sprayed into the flame of an atomic absorption apparatus and the absorption or emission of the metal sought for is measured at its particular wavelength.

1.4.2.1.3. WET ASHING VS DRY DIGESTION

Dry ashing is the most commonly used procedure to determine the total mineral content of foods. It is used to determine water soluble, water insoluble and acid insoluble ash. Dry ashing is applicable also to the determination of most common metals. Dry ashing requires no attention, is simple and is well suited to handle routinely large numbers of samples. Generally, no reagents are added, and no blank subtraction required. Dry ashing takes a long time but can be shortened by using accelerated methods or taken care of by ashing over-night. High temperature, and the relatively expensive equipment limit somewhat, the usefulness of the method. The main objection, however, is the interaction between components themselves or the receptacle material. In the estimation of certain trace elements in foods, the use of either silica or porcelain crucibles leads to the absorption of the element by the vessels. If the temperature of ashing is not excessively high the absorption loss may be faster than the volatilization loss. Excessive heating makes certain metallic compounds (of tin) insoluble. Foods with a high phosphorus to base ratio fuse to a dark melt in which carbon particles are trapped, and will not burn. Foods with a high alkaline balance show progressive decomposition of the carbonates and the volatilization of chlorides. High hygroscopicity, lightness, and fluffiness of the ash may sometimes present problems in determining precisely the total ash content or in

handling the mineral residue for subsequent analysis.

In wet oxidation or (wet ashing) relatively low temperatures and liquid conditions are maintained. The apparatus is simple and oxidation is rapid. On the other hand, the procedures require large amounts of corrosive reagents and correction from the reagents. Handling routinely large number of samples by wet digestion is difficult and time consuming.

## 2. MATERIALS AND METHODS

### 2.1. SAMPLE COLLECTION AND DESCRIPTION

#### 2.1.1. SAMPLE COLLECTION.

All the samples of Parkia Clappertoniana, Adansonia Digitata, and Tamarindus Indica were collected from markets in and around Zaria in Kaduna State, Nigeria. Pentaclethra mycrophylla was collected at Afor Obudi Agwa, Owerri, in Imo State, Nigeria. Different sets of the same sample from different sources and localities were mixed together and brought into the laboratory as experimental samples.

#### 2.1.2. SAMPLE DESCRIPTION

The ecological description of the trees of the seeds used in this study were abstracted mostly from the work of Dalziel (1944) in the Useful Plants of West Tropical Africa because, such information is not easily available in standard textbooks.

##### 2.1.2.1. PARKIA CLAPPERTONIANA

(DOROWA-HAUSA: AFRICAN LOCUST BEAN-ENGLISH)

The African locust bean tree is fairly widely distributed all over the natural grassland of the Northern States of Nigeria. It is a spreading tree of medium size, with compound leaves and numerous leaflets. The fruits are represented by bunches of pods which form the most nutritive part of the plant. Each pod which may vary between 15-25 cm in length according to soil fertility, contains

a yellow dry powdery pulp, inside which is embedded a number of dark brown or black seeds. The yellow meally pulp is sweet to the taste and is made into DOROWA, a valuable carbohydrate food usually taken by the Hausas along with ~~wereal~~ grains in meat, stew or soup. The seeds may sometimes be boiled for about 24 hours and are made into Daudawa cakes, another popular Hausa food. All parts of the fruit including the pod, the meally material and the seeds constitute valuable food for domestic animals. About 20% of the seed is made up of a semi-liquid oil and the meal is a satisfactory food for humans rich in easily assimilable carbohydrates and protein.

2.1.2.2. ADANSONIA DIGITATA

(KUKA-HAUSA; BAOBAB OR MONKEY BREAD-ENGLISH)

The Baobab is remarkable for its enormous trunk in comparison with the crown of foliage, the shape being similar even in the young state. Although common in the northern Savannah grassland, it is usually isolated or in groups. The bark is smooth, greyish with a purple reflection which differentiates it from others. The fruit is composed largely of a dry acid pulp, sometimes of a pinkish tinge in which the blackish seeds are embedded. Both pulp and seeds are edible, the pulp being made into a food called DANDARE in Hausa while the seeds serve as a valuable foodstuff and are similarly prepared into DAUDAWA KUKA. Also, the leaves, the white shoot from the germinating seed, as well as the tender root are edible to humans and livestock as well. The seed has

a comparatively thick shell difficult to separate from the kernel. The only 'useless' part of this tree is the wood which the Hausas epithetically call 'fanko', good-for-nothing, since the wood is not even good for fire-wood unless first very thoroughly dried.

2.1.2.3, TAMARINDUS INDICA

(TSAMIYA - HAUSA; INDIAN TAMARIND - ENGLISH)

The tamarind grows spontaneously throughout the Savannah grasslands, growing particularly well in the neighbourhood of towns and villages, but elsewhere, it is planted. It is probably originally African and introduced long ago into India, and not vice versa. The pulp (African variety) is rarely so well developed and juicy as that of the Indian variety showing that in the latter country, the tree must have been improved by selection.

It often forms a dense symmetrical crown with branches all round to (75-100 cm) from the ground; there is abundant leaf-fall and usually almost no undergrowth beneath it. In damp weather, the leaves are said to have a corrosive effect on fabrics on which they fall. This is probably due to the high content of tannin, about 7%, which though affords it an economic importance in tanning dyeing and dehairing.

The tamarind is frequently seen growing on or beside anthills, the structure of which is probably favourable to its root system.

The fruit is edible and sold in the markets in the form of cakes or balls of the pulp pressed together with the seeds-DAUDAWA TSAMIYA. This may be used without special preparation. The flowers are made into a sort of salad, FATEFATE (Hausa), while the leaves serve as vegetable in soups. The kernels of the seeds contain an oil and are edible, though not commonly eaten unless in times of scarcity. The hard shell must be removed after roasting or boiling, and the kernels made into meal mixed with cereals. Like parkia or adansonia, the tamarind seeds chiefly serve as seasoners and appetisers.

2.1.2.4. PENTACLETHRA MYCROPHYLLA

(UGBA - IBO; OIL BEANTREE-ENGLISH)

In Nigeria, Pentaclethra mycrophylla grows very commonly in the southern parts, especially in the Eastern states, where they are very often planted along roads as an evergreen shade tree, growing readily from seeds, and in such situations often branching low down. The timber shows a red-brown heartwood and a dirty-white sapwood, hard with large pores, durable and termite-proof; difficult to work, but finishes smoothly and takes a glossy look. No wonder it is used locally for making mortars and pestles. The wood and the pod husks are one of the best fuels. Like the Parkia, it is a spreading tree of medium size with compound leaves and numerous leaflets. Infact, in appearance, one can easily <sup>mistake</sup> the Parkia for Pentaclethra mycrophylla. Though, the pods are very large, hard and strongly

elastic; over 38 cm long and 8 cm wide, bursting violently, when matured and dried, to release the brownish beans. The beans yield some 44 - 45 % oil from the kernels after removal of the seed coats. They are rich in protein, exceeded only by Soja-beans, but poor in starch. The oil is non-drying and has a high melting point and has an unpleasant pungent odour. The oil is suitable for soap, candles and for lubrication. The residual cake is of high nutritive value with 30 - 35 % protein. The beans are commonly eaten after roasting but more as a condiment than a staple food. Usually the seeds are boiled for up to 24 hours in order to soften the seed-coats, split open with a knife to separate the two cotyledons, sliced into bits and finally fermented overnight, removed and washed to remove the bitter taste, which is due to a poisonous alkaloid, Paucine, according to Henry. The washed fermented slices are then wrapped in special leaves and sold as food condiments. It is usually added in soup as a condiment or prepared as salad alone or with stockfish and gardeneggs and a little vegetable, when it makes a good delicacy. The slices could also be pounded and made into cakes and serve as food flavourer and appetiser. Livestock relish this meal equally.

#### 2.2. GENERAL SAMPLE PREPARATION

Samples of the foodstuffs are made homogenous prior to analysis by a procedure that protects labile nutrients.

2.2.1. MATERIALS: The materials included Distilled water, sample bottles (Corkscrewed polythene), Hammer, Mincer, Waring Blender, Beakers, Drawing Paper and Trays.

2.2.2. PROCEDURE: Seeds of Parkia clappertoniana, Adansonia digitata, and Pentaclethra myriophylla were soaked in distilled water for a while in order to soften their seedcoats. The slimy brownish cover in the seeds of Parkia and Adansonia were removed by scrubbing the seeds together in the palm. Only the seedcoats of Parkia clappertoniana and Pentaclethra myriophylla got softened in the process and were separated from the tissue by peeling with a knife. The tissues were thereafter sun dried to normal texture. The seedcoat of Adansonia digitata was not softened by soaking, and so the seeds were sun dried to normal texture before being cracked with a hammer and scraping the endosperm (tissue) with a penknife or any other sharp object.

The seeds of Tamarindus indica were fried in an oven and then cracked with a hammer to separate the tissue from the seedcoat. For Adansonia pulp, the whole fruit was pounded in a mortar in order to separate the pulp from the seed. All the samples - tissue, testa and pulp were oven-dried to determine their moisture content, then ground in a PIFCO Seven Speed Waring Blender, sieved, as in the case of the pulp, and finally stored in screw-corked polythene bottles for subsequent analysis.

### 2.3. MOISTURE AND TOTAL SOLIDS

The sample is dried in an air-oven to constant weight according to the method described by Osborne et al. (1978) in the Analysis of Nutrients in Food.

2.3.1. MATERIALS: The materials used included an oven, which is temperature-controlled and electrically heated, with good natural ventilation, regulated so that the temperature of the air and of the shelves in the neighbourhood of the samples lies between 55<sup>0</sup>C and 60<sup>0</sup>C during the period of determination; dishes, in the form of 100 ml beakers, pyrex model; desiccators, containing an efficient desiccant such as phosphorus pentoxide, calcium chloride, granular silica gel or activated alumina, and provided with a metal plate which allows the dishes to cool rapidly.

2.3.2. PROCEDURE: The empty clean beakers were dried in the oven at 100 ± 5<sup>0</sup>C for about thirty minutes and then transferred to the desiccator to cool for another ten minutes.

Thereafter, the beakers were weighed to the nearest milligram, Mo. The prepared seed samples were thoroughly mixed and about 3-5 grams of it introduced into the beaker and both beaker and content weighed, M<sub>1</sub>. The beaker and its content were finally transferred into the oven, which had been adjusted to operate within a range of 55 - 60<sup>0</sup>C avoiding contact of the beaker with the walls of the oven. After about 8<sup>h</sup> hours, during which time, practically constant weight was achieved, the beaker was removed from the oven, cooled in a desiccator

and reweighed,  $M_2$ .

2.3.3. Expression and Calculation of Results.

Moisture and Total Solids contents were calculated as a percentage by mass of the samples, by means of the formulae:

$$\% \text{ Moisture Content} = \frac{M_1 - M_2}{M_1 - M_0} \times \frac{100}{1}$$

i.e.  $\frac{\text{Weight of moisture}}{\text{Weight of original sample}} \times 100$

$$\% \text{ Total solids} = \frac{M_2 - M_0}{M_1 - M_0} \times \frac{100}{1}$$

i.e.  $\frac{\text{Oven - dry weight}}{\text{Original weight}} \times \frac{100}{1}$

The arithmetic mean of three simultaneous determinations was taken as the result.

2.4. ASH AND ORGANIC MATTER

Organic matter is burnt off at as low a temperature as possible and the inorganic material remaining is cooled and weighed. Heating is carried out in stages - first to drive off the water, then to char the product thoroughly and finally to ash at a temperature of  $550^{\circ}\text{C}$  in a muffle furnace, according to the procedure outlined by Osborne et al. (1978).

2.4.1. MATERIALS: These included a muffle furnace, thermostatically controlled at 550°C; an electric hot plate, also with a thermostatic control; silica dishes, ideally 8 cm in diameter and 2.5 cm depth, which had previously been cleaned with 6 M hydrochloric acid and rinsed with water, and Desiccators, with efficient desiccants.

2.4.2. PROCEDURE: The requisite number of silica dishes were placed in the muffle furnace for about 15 minutes, then removed, cooled in a desiccator for at least one hour and then weighed,  $M_1$ , 1 gram of the powdered dry sample was accurately weighed into the dish,  $M_2$ . The samples were then charred in a hot plate placed under a fume cupboard, before transferring them to a muffle furnace (placed as near to the centre as possible) and ashed for at least 24 hours at a temperature of 550°C. After this period, the dishes were removed from the furnace, placed in desiccators and cooled for at least one hour. The ash should show a clean white appearance, otherwise continue ashing for a longer period. When cooled, the dishes and the ash were reweighed,  $M_3$ .

2.4.3. CALCULATION: By difference, the weight of ash and organic matter that burnt away were calculated and expressed as a percentage from:

$$\text{Ash (\%)} = \frac{M_3 - M_2}{M_2 - M_1} \times 100$$

$$\text{i.e. } \frac{\text{Weight of Ash}}{\text{Original weight of sample}} \times 100$$

$$\text{Organic Matter (\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

$$\text{i.e. } \frac{\text{Wt of sample burnt away}}{\text{Original weight of sample}} \times 100$$

Take the arithmetic mean of three simultaneous determinations as the result.

## 2.5. LIPID CONTENT

The fat is extracted with a solvent from the dried residue obtained after moisture determination. The solvent is thereafter removed by evaporation and the fat residue weighed. This is the process outlined by Osborne *et al* in Analysis of Nutrients in Food.

2.5.1. MATERIALS: The materials employed in the process included: Petroleum ether, 40-60°C; sets of Soxhlet Extractor, including the flasks; Oven, Heating Mantle (Set of Six-in-one) and sizeable Extraction Thimbles.

2.5.2. PROCEDURE: 1 gram of the dry powdered sample was accurately weighed into the thimble which thereafter was slotted into the extractor and then connected to a weighed flask containing 100 cm<sup>3</sup> of petroleum ether. The flask and extractor were finally connected to a reflux condenser and the sample extracted under reflux, on the heating mantle, for three hours. At the end of the extraction the solvent was evaporated and the flask containing

the fat residue dried in an oven at 100°C for just five minutes; cooled in the desiccator and reweighed.

2.5.3. CALCULATION OF RESULT

$$\begin{aligned}\text{Let weight of dry sample} &= M_1 \text{ g} \\ \text{weight of flask alone} &= M_2 \text{ g} \\ \text{weight of flask + oil} &= M_3 \text{ g}\end{aligned}$$

Then

$$\text{Extractible fat (\%)} = \frac{M_3 - M_2}{M_1} \times 100$$

$$\text{i.e. } \frac{\text{Weight of fat Extracted}}{\text{Weight of original sample}} \times 100$$

The extractible fat could also be calculated from the loss in weight of the thimble divided by the original weight of the sample and multiplied by one hundred. In this case the dried thimble was initially weighed alone, and then reweighed with the sample before and after extraction. As usual, the mean of three simultaneous extractions was taken as the result.

2.6. NITROGEN AND CRUDE PROTEIN CONTENT

The sample is digested with concentrated sulphuric acid using a mixed catalyst of potassium sulphate, copper sulphate and selenium powder, to convert organic nitrogen to ammonium ions. Alkali is then added and the liberated ammonia is distilled into an acid solution. The distillate is titrated with an acid solution to determine the amount of ammonia absorbed by the acid-as outlined in the

Analysis of Nutrients in Food.

2.6.1\ MATERIALS:

- (i) Sulphuric acid: Concentrated; Sp.Gr. 1.84; Nitrogen-free.
- (ii) Hydrochloric acid: 0.01M, standardised
- (iii) Boric acid: 2% solution, prepared by dissolving 2 grams of boric acid  $H_3BO_3$  in water and making up to 100  $cm^3$ .
- (iv) Sodium hydroxide solution, 50%, prepared by dissolving 50 g of carbonate-free sodium hydroxide pellets in distilled water and making up to 100  $cm^3$ .
- (v) Mixed catalyst, made up of:-  
160 g Potassium sulphate, 10 grams copper sulphate pentahydrate, and 3 g selenium powder, ground and mixed thoroughly in a mortar.
- (vi) Mixed indicator, prepared by mixing together 99 mg of bromocresol green, 66 mg of methyl red and 11 mg of thymol blue and then dissolved in 10  $cm^3$  of ethanol.  
Other materials employed included:
  - (vii) Kjeldahl flask, capacity 50  $cm^3$ ;
  - (viii) Distillation apparatus; steam; heating device, so that the flask can be heated in an inclined position and that the source of heat only touches that part of the flask wall which is below the liquid level.

2.6.2. PROCEDURE: The process involved three steps of Digestion, Distillation and Titration.

2.6.2.1. DIGESTION: 0.1 g of the dry sample was accurately weighed into the kjeldahl flask. A few boiling regulators and about 1 g of the mixed catalyst were added to it. This was followed by the addition of 5 cm<sup>3</sup> of the concentrated sulphuric acid which mixed well by boiling <sup>the contents of</sup> the flask gently. With the flask on the heating device, it was heated gently until frothing subsided and the contents completely gone into solution. Then digested by boiling vigorously, occasionally rotating the flask, until the liquid had become completely clear or had a light blue-green colour. Making sure there was no overheating (which evaporates some of the sulphuric acid leading to low nitrogen recovery) the liquid was kept boiling for the next one hour bringing the total digestion time to at least two hours. At the end of the digestion, the flask was cooled, 15 cm<sup>3</sup> of distilled water cautiously added to it, mixed, allowed to cool again, before transferring it quantitatively into a 50 cm<sup>3</sup> volumetric flask. The digest was completely rinsed out with distilled water before the flask was made to mark.

2.6.2.2. STEAM DISTILLATION: 5 cm<sup>3</sup> of the 2% boric acid solution was added into a 50 cm<sup>3</sup> conical flask, and was followed by two drops of the mixed indicator solution. The mixture turned violet in colour. The flask was placed under the condenser of the distillation apparatus, so that the outlet of the adapter dipped

into the liquid, in order to avoid loss of ammonia. With the aid of a pipette, 10 cm<sup>3</sup> of the digest was transferred into the Markham semimicro nitrogen still, followed, cautiously, by 10 cm<sup>3</sup> of the 50 % sodium hydroxide solution. The ensuing reaction liberated ammonia which was steam distilled into the 5 cm<sup>3</sup> boric acid in the conical flask, whereupon the indicator turned green and distillation was continued for the next two minutes. The conical flask was lowered, before terminating the distillation. Before removing the distillate, the outlet of the adapter was rinsed with distilled water both internally and externally.

2.6.2.3. TITRATION: The contents of the conical flask were titrated with the 0.01 molar hydrochloric acid, the end point being reached when the indicator changed from green through grey to definite pink or violet.

Finally, a blank was conducted following the instructions from digestion, through distillation to titration, the difference being that no actual sample was added for the digestion.

2.6.2.4. CALCULATION:

Let weight (g) of test portion = W  
volume (cm<sup>3</sup>) of hydrochloric  
acid solution required for the  
blank titration = V<sub>1</sub>  
Volume (cm<sup>3</sup>) of the hydrochloric  
acid solution required for the 10 cm<sup>3</sup> test portion = V<sub>2</sub>

Molarity of the hydrochloric acid solution = M

Then,

$$\% \text{ total nitrogen} = \frac{V_2 - V_1 \times M \times 5 \times 14 \times 100}{W \times 1000}$$

Since 1 cm<sup>3</sup> of 1 Molar acid neutralised by the ammoniacal mixture collected corresponds to 0.014 g N, and 10 cm<sup>3</sup> out of 50 cm<sup>3</sup> digestate was used.

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

#### 2.7. MINERALS:

Mineral determination was made with the Atomic Absorption (Pye Unicam SP. 1900 model) and Corning-Eel Flame Emission Spectrophotometers. The Atomic Absorption Spectrophotometer (AAS) is mainly applicable to the determination of calcium, copper, iron, magnesium, manganese, and zinc, for the purposes of this work, while the Flame Emission (FES) is chiefly applicable to the alkaline metals, and for this work, sodium and potassium, for which they are most sensitive.

After the removal of the organic material by either dry or wet ashing, the digestate was made up to solution volume. The metal to be investigated was then determined using either the Absorption or Emission Spectrometer.

In the Atomic Absorption Spectrometer, the solution is sprayed into the flame of the Spectrophotometer. Radiation of characteristic wavelength from a 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 intensity of the (cathode) radiation is measured using a monochromator and a 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 is thus a function of the concentration of the atoms of the element in the sprayed solution.

In the case of the PES, after spraying the investigated solution into the flame of the photometer, the atoms of the metal under investigation become excited with the absorption of radiant energy. On returning to the ground state, they emit the absorbed radiation. The intensity of this emitted radiation is also directly proportional to the concentration of the element.

#### 2.7.1. SAMPLE PREPARATION

##### 2.7.1.1. TREATMENT OF THE ASH OBTAINED FROM DRY ASHING.

The ash was treated with 5 - 10 cm<sup>3</sup> of 6M hydrochloric acid to wet it completely and carefully taken to dryness on a low temperature hot plate. Then 15 cm<sup>3</sup> of 3M hydrochloric acid was added and the dish heated until the solution just boiled. It was then cooled and filtered through a filter paper into a 100 cm<sup>3</sup> graduated flask, retaining as much of the solids (if any) in the dish. Another 10 cm<sup>3</sup> of 3M hydrochloric acid was added to the dish and heated to just boiling; cooled and filtered again into the flask. The dish and filter paper were thoroughly washed with distilled water into the graduated flask. Then 5 cm<sup>3</sup> of Lanthanum chloride solution

was added per 100 cm<sup>3</sup> of digestate, to prevent interference in calcium determination. The flask was finally cooled and volume made up to mark with distilled water. A blank solution was finally prepared by taking the same amount of reagents through the whole process.

2.7.1.2. WET DIGESTION:

Organic matter is destructed by and oxidised by the action of boiling mixed acids (Sulphuric, nitric and perchloric) as outlined by Allen et al in the Chemical Analysis of Ecological Materials.

2.7.1.3. MATERIALS: Perchloric acid 62 % V/V, Nitric acid 69 % (conc) and Sulphuric acid 98 % concentrated,

Acid washing liquid - prepared by diluting 1 cm<sup>3</sup> of concentrated nitric acid to 100 cm<sup>3</sup> with distilled water - and used to wash all glasswares.

2.7.1.4. PROCEDURE: 0.5 g of the dried sample was weighed into a 100 cm<sup>3</sup> kjeldahl's flask. 20 cm<sup>3</sup> of the acid mixture (prepared by mixing 80 cm<sup>3</sup> of perchloric acid, 650 cm<sup>3</sup> of nitric acid and 20 cm<sup>3</sup> of sulphuric acid) was added, the flask gently swirled and digested at increasing temperature for ten to fifteen minutes, after the appearance of white fumes. On cooling, the digestate was poured into a 50 cm<sup>3</sup> volumetric flask and made up to volume after thoroughly washing the digestion flask. A blank digestion was carried out as usual. All operations were carried out in a

properly ventilated fume cupboard.

2.7.2. PREPARATION OF STANDARD CURVES.

2.7.2.1. MATERIALS: The materials needed for this operation included:- Hydrochloric acid 6M, 3M and 0.3M - prepared by dissolving 186 cm<sup>3</sup>, 93 cm<sup>3</sup> and 9.3 cm<sup>3</sup> of concentrated hydrochloric acid (Sp. Gr. 1.18) per dm<sup>3</sup> respectively; Lanthanum Chloride, 10% W/V, prepared by dissolving 10 g of Lanthanum Chloride in distilled water and making up to 100 cm<sup>3</sup>; Potassium diluting solution - 1000 µg/cm<sup>3</sup> - prepared by dissolving 3.8 g of Potassium Chloride per 1 dm<sup>3</sup>; Sodium diluting solution - 1000 µg/cm<sup>3</sup> - prepared by dissolving 3g of Sodium chloride per 1 dm<sup>3</sup>; Distilled/Deionised water; Filter paper - 3g of Whatmann 541 - washed before use with 3M hydrochloric acid to remove trace elements. Other reagents required included the Stock Standard solution - 1000 ppm i.e. 1000 mg/dm<sup>3</sup> - prepared by weighing out the quantities of the A.R. grade of reagents as given in table IV, and dissolving in 100 cm<sup>3</sup> of 3M hydrochloric acid and then diluting to 1 dm<sup>3</sup> with distilled water. To ensure their strength, the stock Solutions were standardised with EDTA using Eriochrome Black T indicator. Working standard solution; obtained by diluting the Stock Standard Solutions with water (if wet digestion is used) or with 0.3M hydrochloric acid (if dry ashing is applied) to concentrations that fall within the working range as shown in Table V.

The dilutions were to ensure that concentrations equivalent to 0 ppm, 0.5 ppm, 1, 1.5, 2 ppm etc. could be obtained.

(For example, a stock standard solution (1000 ppm), diluted 1000 times gives 1 ppm. If we diluted it 500 times it would give 2 ppm. If further, the 1 ppm was diluted 20 times, it would give 0.05 ppm, 10 times dilution would give 0.1 ppm while 2 times dilution would give 0.5 ppm).

Atomic Absorption Spectrometer: Pye Unicam SP 1900 model with digital readout.

Flame Emission Photometer, and special glassware for trace metal analyses - all washed with 1 % dilute nitric acid solution before use.

Table IV: Element - Reagent Standard Preparations\*

Metal	Reagent	Weight Reagent/dm <sup>3</sup>
Calcium	CaCO <sub>3</sub> (dry)	2.5020
Copper	Cu(NO <sub>3</sub> ) <sub>2</sub> ·3H <sub>2</sub> O	3.7982
Iron	FeCl <sub>3</sub> ·6H <sub>2</sub> O	4.8403
Magnesium	Mg SO <sub>4</sub> ·7H <sub>2</sub> O	10.1400
Manganese	Mn SO <sub>4</sub> ·4H <sub>2</sub> O	4.0604
Potassium	KCl (dry)	1.5040
Sodium	NaCl (dry)	2.4440
Zinc	ZnO	1.2446

\*Osborne et al. 1978.

Table V. Recommended Conditions For Metal Analyses Using the AAS. OR the FES\*

Element	Wavelength	Absorption A or Emission E	Max Current ( $\mu\text{l}$ )	Limit of Detection $\mu\text{g metal}$ $\text{cm}^{-1}$	Working Range $\mu\text{g metal}$ $\text{cm}^{-1}$	Possible Interference	Control by addition of:
Calcium	422.7	A	6	0.01	0.05-5	$\text{Al}^{3+}$ , $\text{S}^{2+}$ , $\text{SO}_4^{2-}$	$\text{LaCl}_3$
Copper	324.8	A	6	0.005	0.05-5		
Iron	248.3	A	15	0.03	0.05-5		
Magnesium	285.2	A	6	0.001	0.02-5	$\text{Al}^{3+}$ , $\text{S}^{2+}$ , $\text{SO}_4^{2-}$	$\text{LaCl}_3$
Manganese	279.5	A	12	0.005	0.02-3		
Potassium	-	E	-	0.002	1-20	$\text{Na}^+$	1000 $\mu\text{g Na}^+$
Sodium	-	E	-	0.002	1-20	$\text{K}^+$	1000 $\mu\text{g K}^+$
Zinc	213.9	A	10	0.004	0.1-1		

\*Osborne et al., 1978.

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

2.7.2.2. PROCEDURE: Having prepared the sample solution, either by dry ashing or wet digestion the calibration solutions, which must be within the working range (Table V) were also prepared. The instruments were also set according to the recommended conditions (Table V). Then the calibration solutions as well as their blanks were measured, followed by the investigating samples, which were diluted, if need be. Assurance was made that the calibration values were constant by periodical checks. Releasing agents were added to check any interferences (Table V). Finally, a calibration curve was prepared for each metal investigated, by plotting a graph of absorption or emission against the metal concentrations in parts per million ( $\mu\text{g}/\text{cm}^3$ ).

2.7.2.3. CALCULATION: The metal concentrations of the metals that corresponded to the samples and blanks were read from the graphs; so that if :

Weight (g) of the sample = W

Volume ( $\text{cm}^3$ ) of Digestate = V

Concentration (ppm) of sample solution = a

Concentration (ppm) of blank solution = b

Then, metal content (mg/100 g, dry sample)

$$= \frac{(a - b) \times V}{10 W}$$

OR

$$\text{mg}/1000\text{g} = \frac{(a - b) \times V}{W}$$

Alternatively, the metal concentration could be calculated as a percentage from the formula:

% Metal Content

$$= \frac{C(\text{ppm}) \times \text{Solution volume (cm}^3\text{)}}{10^4 \times \text{Sample weight (g)}}$$

where C is the concentration in ppm ( $\mu\text{g/cm}^3$ ) of the sample after taking care of the blank concentration.

### 2.7.3. PHOSPHORUS:

Hanson (1950) has proposed a simple colorimetric method for the determination of phosphate based on Mission's reaction. The acid solution (digest) containing orthophosphate is treated with an acid reagent containing molybdic acid and vanadic acid and a stable orange yellow coloured complex of vanadi-molybdiphosphoric acid ( $\text{H}_3\text{PO}_4$ ,  $\text{WO}_3$ ,  $11\text{MoO}_3$ ,  $\text{NH}_2\text{O}$ ) is formed. The maximum absorption is at 330 nm, but Donald et al (1956) have reported that satisfactory results can be obtained in the region of 420-480 nm, provided the light is monochromatic. The colour development is not markedly affected by the presence of hydrochloric, sulphuric, acetic or citric acid or even by fluorides provided they are not present in relatively large quantities.

#### 2.7.3.1. MATERIALS: (1) Vanadate-molybdate composite reagents:

Prepared by dissolving 20 g of ammonium molybdate in  $400\text{cm}^3$  of warm water; 1 g of ammonium vanadate in another  $300\text{cm}^3$  of boiling distilled water, and cooling.  $140\text{cm}^3$  of concentrated perchloric

(preferably) or nitric acid was gradually added, with stirring to the vanadate solution. Finally, the molybdate solution was gradually added to the acid vanadate solution, with stirring, and then diluted to 1 dm<sup>3</sup> with distilled water.

Stock phosphate solution; obtained by dissolving 4.394 g of Potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) per dm<sup>3</sup>, that is, 1000mg P/dm<sup>3</sup>. Standard working phosphate solution, obtained by diluting, for example, 25 cm<sup>3</sup> of the stock solution to 250 cm<sup>3</sup> with distilled water to give a 100 mg P per 1 dm<sup>3</sup> of solution. Thereafter, concentrations equivalent to 0,5,10,15,20 ppm P were prepared.

1:2 perchloric/Nitric acid, prepared by diluting, say, 50 cm<sup>3</sup> of the acid to 100 cm<sup>3</sup> with distilled water; and Ammonia solution (0.88).

2.7.3.2. PROCEDURE: 5 cm<sup>3</sup> of each ppm concentration was put in a 50 cm<sup>3</sup> volumetric flask, and diluted to about 20 cm<sup>3</sup> with distilled water. (In this perchloric acid modification, the addition of few drops of ammonia solution, and then just making acid with perchloric acid again was not necessary). Then 10 cm<sup>3</sup> of the vanadate-molybdate reagent was added before mixing and making volume to mark. After ten minutes, a yellow colour developed and their optical densities were measured at 470 nm on a Unicam Sp 8000 U.V. Recording Spectrophotometer and used to plot a calibration curve. The investigating sample solutions were similarly treated and their optical densities determined. From the curves, the phosphorus

concentrations in the samples were calculated.

2.7.3.3. CALCULATION: The percentage phosphorus content was calculated by the formula:

$$\% P = \frac{(\text{ppm} \times \text{Solution Volume (Cm}^3))}{10 \times \text{aliquot volume} \times \text{Sample weight}}$$

Or by proportion:

$$5 \text{ cm}^3 \text{ aliquot} \equiv Y \text{ ppm P}$$

$$\therefore 100 \text{ cm}^3 \text{ (original sample dilution)} \equiv \frac{100Y}{5} \text{ ppm}$$

$$\equiv 20Y \mu\text{g P}$$

$$\text{OR } \frac{20Y}{10^6} \text{ g P}$$

$$\therefore \% P = \frac{20Y \times 100}{10^6 \times \text{Sample weight}}$$

## 2.8. FIBRE CONTENT.

The sample is successively treated with dilute acid and dilute alkali in which fibre is not hydrolyzable. The residue left after this treatment is incinerated in the muffle furnace and the loss in weight calculated as the fibre content.

2.8.1. MATERIALS. (1) Hydrochloric acid, 1 % W/V, prepared by diluting 10 cm<sup>3</sup> of concentrated hydrochloric acid (Sp. Gr. 1.18) to 100 cm<sup>3</sup> with distilled water.

(ii) Sulphuric acid Stock Solution, 10 % W/V, prepared by diluting 275 cm<sup>3</sup> of concentrated sulphuric acid (Sp.Gr.1.86) to 5000 cm<sup>3</sup> (5 dm<sup>3</sup>) with distilled water.

- (iii) Sulphuric acid working solution, 1.25 %, prepared by diluting 625 cm<sup>3</sup> of the above stock solution to 5 dm<sup>3</sup> with distilled water.
- (iv) Sodium Hydroxide Stock Solution, 10 % W/V, prepared by dissolving 500 g of sodium hydroxide in and diluting to 5 dm<sup>3</sup> with distilled water.
- (v) Sodium hydroxide working solution, 1.25 %, prepared by diluting 625 cm<sup>3</sup> of stock solution to 5 dm<sup>3</sup> with distilled water.
- (vi) Alcohol (Industrial methylated spirit) 95-96 % V/V.
- (vii) Diethyl ether.

2.8.2. PROCEDURE: 2 g of the defatted sample was weighed and transferred quantitatively into a 1 dm<sup>3</sup> conical flask. 200 cm<sup>3</sup> of 1.25 % sulphuric acid, which has been brought to boiling point was added to the flask, and the mixture boiled under reflux for 30 minutes. The hot mixture was filtered quickly under suction. The insoluble matter was washed free of acid with water (use litmus paper) and then washed back into the original flask with 200 cm<sup>3</sup> of 1.25 % sodium hydroxide solution which has been brought to boiling point. The mixture was again refluxed for another 30 minutes, after which it was again quickly filtered under suction (always use 541 filter paper). The insoluble material was washed first with boiling water, then with 1 % hydrochloric acid and finally with boiling water until free from acid. It was then washed twice with alcohol, and three times with ether, dried at 100°C at a constant weight and finally ashed

for two hours in a muffle furnace set at 550°C. The loss in weight on ashing represented the fibre.

2.8.3. CALCULATION: The fibre content percent was calculated from;

$$\text{Percent Fibre Content} = \frac{\text{Loss in weight on ashing}}{\text{Original weight of sample}} \times 100$$

In the instrumental method, the Fibertec System, Model 1010 Heat Extractor, was used at the National Animal Production Research Institute, Shika. The chemical procedure was similar to the laboratory manual.

3. RESULTS3.1. PROXIMATE COMPOSITION

The results of the proximate composition of the nuts and testas are shown in table VII.

The values of moisture content ranged from  $5.18 \pm 0.02$  % for ADN to  $13.35 \pm 0.20$  % for PMN, while the testa values ranged from  $7.66 \pm 0.15$  % for AD to  $21.63 \pm 0.45$  % for PM. The ash values ranged from  $2.06 \pm 0.50$  % for PMN to  $5.53 \pm 0.18$  % for ADN; from  $0.94 \pm 0.45$  % for TTP to  $2.32 \pm 0.27$  % for PCT. The lipid content of the nuts ranged from  $12.90 \pm 2.10$  % for TI to  $52.09 \pm 4.80$  % for PM; from  $1.62 \pm 0.01$  % for ADT to  $3.88 \pm 0.01$  % for PMT. For crude protein, the values were from  $0.44 \pm 0.02$  % for ADP to  $40.17 \pm 0.32$  % for PCN;  $0.24 \pm 0.07$  % for ADT and  $4.55 \pm 0.01$  % for PMT. Total carbohydrate ranged from 8.36 % for PMN to 75.89 % for ADP; from 68.04 % for PMT to 88.69 % for ADT. The levels of fibre content rose from 1.24 % for PCN to 8.95 % for ADP; and from 12.97 % for PMT to 39.86 % for ADT. calorific values similarly ranged from 409.00 calories for ADP to 598.81 calories for PMN, and from 325.24 calories for PMT to 370.26 calories for ADT. Nitrogen free extract (NFE) ranged from 3.57% for PMN to 66.94 % for ADP and from 48.82 % for ADT to 55.06 % for PMT. From the above, it is observed that Pentaclethra mycrophylla had higher values of proximate composition than Adansonia digitata, Parkia clappertoniana or Tamarindus indica.

### 3.2 MINERALS.

The results of the mineral composition of the nuts and testas are shown in Table VIII. The wet and dry ashing values are shown side by side. The amount of sodium ranged from 0.02% for ADP to 0.09% for PMN, and from 0.02% for TIT to 0.06% for PCT. Potassium values ranged from 1.21% for PMN to 4.10% for ADP; from 0.07% for TIT to 1.17% for ADT. For calcium, the values ranged from 0.04% for TIN to 0.13% for PCN, from 0.04% for ADT to 0.10% for PCT. Magnesium levels ranged from 0.30% for ADP to 1.22% for ADN; 0.10% for PMT and 0.36% for PCT. The values of phosphorus were from 0.02% for ADP to 0.17% for ADN, while all the testas gave the same value of 0.01%.

For the trace elements, the amount of iron ranged from 53.60 micrograms per gram for PMN to 96.50  $\mu\text{g/g}$  for ADP, while the testa values were from 57.70  $\mu\text{g/g}$  for AD to 159.70  $\mu\text{g/g}$  for TI. Values of copper ranged from 1.52  $\mu\text{g/g}$  for TIN to 2.10  $\mu\text{g/g}$  for ADI; 1.02  $\mu\text{g/g}$  for TIT and 4.10  $\mu\text{g/g}$  for ADT. Zinc values ranged from 14.70  $\mu\text{g/g}$  for TIN to 27.70  $\mu\text{g/g}$  for ADN, while the testa values were from 9.47  $\mu\text{g/g}$  for AD to 54.70  $\mu\text{g/g}$  for PC. The levels of manganese were from 0.44  $\mu\text{g/g}$  for ADP to 1.87  $\mu\text{g/g}$  for PCN; and from 0.26  $\mu\text{g/g}$  for PCT to 12.50  $\mu\text{g/g}$  for TIT.

From the above, it is observed that the mineral values were higher for Adansonia digitata than for Parkia clappertoniana, Pentaclethra mycrophylla or Tamarindus indica.

TABLE VI: PROXIMATE COMPOSITION OF SOME OF THE SAVANNA SEEDS IN PER CENT DRY MATTER.

ITEM	MOISTURE CONTENT	DRY MATTER	ASH	ORGANIC MATTER	CRUDE LIPID	CRUDE PROTEIN	TOTAL CARBOH- YDRATES	NITRO- GEN FREE EXPR- ACT.	CRUDE FIBRE	FIBRE	CALORIFIC VALUE CALORIES
PCN	7.5 ± 0.01	92.50 ± 0.01	4.11 ± 0.31	95.89 ± 0.31	23.46 ± 1.12	40.17 ± 0.32	24.76	23.52	b*	o*	470.86
ADN	5.18 ± 0.02	94.82 ± 0.02	5.53 ± 0.18	94.47 ± 0.18	24.66 ± 2.60	38.38 ± 0.00	26.25	23.19	4.30	3.06	480.46
ADP	7.04 ± 0.11	92.96 ± 0.11	5.11 ± 0.68	94.89 ± 0.68	11.52 ± 1.83	0.44 ± 0.02	75.89	66.94	10.13	8.95	409.00
TIN	5.59 ± 0.02	94.41 ± 0.02	2.45 ± 0.14	97.55 ± 0.14	12.90 ± 2.10	37.91 ± 0.05	41.15	38.23	2.20	2.92	432.34
PMN	13.35 ± 0.20	86.65 ± 0.20	2.06 ± 0.50	97.94 ± 0.50	52.09 ± 4.8	24.14 ± 0.02	8.36	3.57	6.64	4.79	598.81
PCT	10.80 ± 0.03	89.20 ± 0.03	2.32 ± 0.27	97.68 ± 0.27	1.91 ± 0.00	3.20 ± 0.04	81.77	53.10	27.06	28.67	357.07
ADT	7.66 ± 0.15	92.34 ± 0.15	1.80 ± 0.18	98.20 ± 0.18	1.62 ± 0.01	0.24 ± 0.07	88.69	48.82	39.69	39.86	370.26
TIT	9.52 ± 0.13	90.48 ± 0.13	0.94 ± 0.45	99.06 ± 0.45	1.89 ± 0.01	3.90 ± 0.01	83.75	60.51	52.31	23.24	367.61
PMT	21.63 ± 0.45	78.37 ± 0.45	1.91 ± 0.39	98.09 ± 0.39	3.88 ± 0.01	4.55 ± 0.01	68.04	55.06	14.86	12.97	325.24

PCN Parkia clappertoniana, nut  
 ADN Adansonia digitata, nut  
 ADP Adansonia digitata, pulp  
 TIN Tamarindus indica, nut  
 PMN Pentaclethra mycrophylla, nut

PCT Parkia clappertoniana, testa b\* Manual  
 ADT Adansonia digitata, testa  
 TIT Tamarindus indica, testa c\* Instrumental  
 PMT Pentaclethra mycrophylla, testa

TABLE VII. PROXIMATE COMPOSITION OF SOME OF THE SAVANNA SEEDS BY VARIOUS WORKERS: % DRY MATTER.

ITEM	Calorific Value Cals.	Moisture Content	Dry Matter	Crude Protein	Crude Fat	Total Carb	Crude Fibre	Ash	Nitrogen Free Ext.	ADCP
<u>Parkia clappertoniana</u> (whole seed) a*	-	-	-	56.20	-	-	-	-	-	50.03
<u>Parkia filicoidea</u> (whole seed) b*	444.70	-	94.33	30.38	20.30	-	80.82	5.38	35.12	-
<u>Parkia Spp.</u> (Seed, dried) c*	426.00	7.00	-	32.30	19.50	37.10	4.10	4.10	-	-
<u>Adansonia digitata</u> (whole seed) a*	-	-	-	21.40	-	-	-	-	-	16.73
<u>Adansonia digitata</u> (seed, dried) c*	452.00	7.80	-	30.00	29.60	24.70	3.20	7.90	-	-
<u>Adansonia digitata</u> (pulp) c*	290.00	16.00	-	2.20	0.80	76.70	6.80	4.30	-	-
<u>Tamarindus indica</u> (whole seed) a*	-	-	-	10.90	-	-	-	-	-	6.68
<u>Tamarindus indica</u> (Fruit, dried) c*	270.00	21.30	-	5.00	0.60	70.70	18.30	2.40	-	-
<u>Pentaclethra mycrophylla</u> (Seeds, dried, shelled) c*	558.00	6.20	-	22.60	46.30	22.60	2.50	2.30	-	-

a\* Kapu, M.M. (1980), Unpublished data

b\* Oyenuga, V.A. (1968), Nigeria's Foods and Feedingstuff.

c\* Woot-Tsuen (1968), Food Composition Table for Use in Africa.

TABLE VIII: MINERAL COMPOSITION OF SOME OF THE SAVANNA SEEDS, PER DRY MATTER.

ITEM	% SODIUM		% POTASSIUM		% CALCIUM		% MAGNESSIUM		% PHOSPHORUS		$\mu\text{g/g}$ IRON		$\mu\text{g/g}$ COPPER		$\mu\text{g/g}$ ZINC		$\mu\text{g/g}$ MANGANESE	
	a*	b*	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
PCN	0.02	0.03	1.15	2.35	0.08	0.13	0.17	1.15	0.03	0.08	49.40	79.00	1.45	1.65	13.75	19.70	1.47	1.87
ADN	0.02	0.04	1.67	2.96	0.04	0.07	0.17	1.22	0.04	0.17	43.40	80.40	1.98	2.10	22.00	27.70	1.19	1.30
ADP	0.01	0.02	1.62	4.10	0.07	0.09	0.22	0.30	0.01	0.02	85.00	96.50	1.10	1.63	12.30	22.30	0.41	0.44
TIN	0.01	0.05	0.99	1.41	0.03	0.04	0.14	0.36	0.02	0.06	51.97	68.20	1.32	1.52	10.00	14.70	0.71	0.82
PMN	0.01	0.09	0.49	1.21	0.03	0.05	0.18	0.47	0.02	0.05	41.33	53.60	1.40	1.60	12.67	17.70	0.79	1.40
PCT	0.04	0.06	0.54	0.87	0.08	0.10	0.29	0.36	0.01	0.01	56.13	67.00	0.80	1.20	30.20	54.70	0.20	0.26
ADT	0.02	0.03	0.79	1.17	0.04	0.04	0.18	0.32	0.01	0.01	38.80	57.70	0.85	4.10	6.93	9.47	0.45	0.49
TIT	0.01	0.02	0.05	0.07	0.05	0.07	0.30	0.32	0.01	0.01	97.33	159.70	0.30	1.02	16.50	17.00	11.86	12.50
PMT	0.03	0.04	0.72	0.81	0.05	0.07	0.09	0.10	0.01	0.01	54.50	66.70	0.95	1.52	10.83	38.30	1.70	1.80

\* a Dry Ashing

\* b Wet Digestion

\* To convert % mineral to  $\text{mg}/100\text{g}$ , multiply by 1000To convert "  $\text{mg}/100\text{g}$  to  $\mu\text{g}/\text{g}$ , multiply by 10.

Divide by these figures if conversion is reverse.

TABLE IX: MINERAL COMPOSITION OF SOME SAVANNA SEEDS BY VARIOUS WORKERS.

Item	Sodium %	Potassium %	Calcium %	Magn. %	Phosphorus %	Iron ppm	Copper ppm	Zinc ppm
<u>Parkia clappertoniana</u> (whole seed) <sup>a*</sup>	0.035	0.13	0.69	0.35	0.02	410	8	13.2
<u>Parkia filicoidea</u> (whole seed) <sup>b*</sup>	-	-	310	-	260	-	-	-
<u>Parkia Spp</u> (seed, dried) <sup>c*</sup>	-	-	291	-	384	33.2?	-	--
<u>Adansonia digitata</u> (whole seed) <sup>a*</sup>	0.033	0.15	0.15	0.09	0.31	112.5	-	111
<u>Adansonia digitata</u> (seed, dried) <sup>c*</sup>	-	-	263	-	1,494	13.9	-	-
<u>Adansonia digitata</u> (pulp) <sup>c*</sup>	-	-	284	-	118	7.40	-	-
<u>Tamarindus indica</u> (whole seed) <sup>a*</sup>	0.02	0.15	0.16	0.34	0.05	135	6	60
<u>Tamarindus indica</u> (Fruit, dried) <sup>c*</sup>	-	-	166	-	190	2.2	-	-
<u>Pentaclethra mycrophylla</u> (seed , dried, shelled) <sup>c*</sup>	-	-	190	-	172	16	-	-

<sup>a\*</sup> Kapu, M.M. (1980) (in %, ppm)

<sup>b\*</sup> Oyenuga, V.A. (1968) (mg/100g sample)

<sup>c\*</sup> Woot-Tsuen (1968) (mg/100g sample)

TABLE X: PLANT-LIFE MINERAL RANGES\*

<u>MINERAL</u>	<u>RANGE</u>
<u>Sodium</u>	0.02 - 0.3 %
<u>Potassium</u>	0.5 - 3.0 %
<u>Calcium</u>	0.3 - 2.5 %
<u>Magnesium</u>	0.1 - 0.5 %
<u>Phosphorus</u>	0.05 - 0.3 %
<u>Iron</u>	40 - 500 $\mu\text{g/g}$
<u>Copper</u>	2.5 - 25 $\mu\text{g/g}$
<u>Zinc</u>	15 - 100 $\mu\text{g/g}$
<u>Manganese</u>	50 - 1000 $\mu\text{g/g}$

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

#### 4. DISCUSSION

The tendency of the testa to show higher values of crude fibre and carbohydrate might be explained by the fact that biologically, it consists mainly of cellulose and lignin. These materials decrease the digestibility of the materials in which they occur, thereby rendering the nutritional values low (Pomeranz and Meloan). The levels of mineral composition obtained by dry ashing were lower than those obtained by wet digestion as a result of the losses due to volatilization. The high values of minerals in Adansonia digitata corresponded with its high ash content which is an index of the mineral content.

The high values of dry matter and the low values of moisture content indicated the Over-all food content and long shelf-life of the seeds. The higher moisture content of PMN agreed with the fact that lipid-rich materials contained substantial amounts of moisture. Consequently PMN had a lower value of protein since most of the protein must have been bound as lipoprotein. The 'availability' of the carbohydrates was indicated by the high values of nitrogen free extracts compared to the total carbohydrate content.

The values of proximate composition obtained in this, investigation were slightly higher than literature values (Table VII). This was also true for most of the minerals (Table IX). These differences could be attributed to factors such as moisture content (which fluctuates with season, maturity at harvest and method of storage,

as well as the degree of exposure in the open market), methods of analytical procedures employed, variety of specimens analysed, parts of the materials analysed and differences in soil conditions.

It is suggested that because of their high protein contents, the seeds investigated could be used as protein concentrates for both humans and livestock. Subsequent work on these seeds should include the fermented seeds, because this is the state in which they are usually taken by humans. Wet digestion methods are preferable when determining the mineral composition of the seeds and similar materials because of the losses associated with dry ashing.

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