STUDIES ON THE DETERMINATION 0F ESSENTIAL MINERALS IN SOME TROPICAL PLANT MATERIALS

BY

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D E C L A R A T I O N

"I hereby declare that all the work in this thesis is my own work and that it has not been presented elsewhere as a requirement for a degree."

M. E. Adiuku-Brown

January 1982
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THESIS RECOMMENDATION

We hereby recommend that the thesis prepared by Mrs M. E. Adiuku-Brown entitled "Studies on the Determination of Essential Minerals in some tropical plant materials" be accepted in partial fulfilment of the requirements for the degree of M.Sc. Analytical Chemistry.

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D E D I C A T I O N

TO MY HUSBAND, MY AUNTIE IMA, and TO MY CHILDREN CHIEDOCHIE, UZODIMA and NWAMAKA.

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

I would like to express my profound gratitude to my supervisor, Dr. S. A. Thomas for his guidance and cooperation.

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I am grateful to Dr. Obilanna of the Institute for Agricultural Research who supplied most of the samples used in this work. Also to Dr. and Mrs Umoh - area F 45 who supplied most of the vegetable samples, I extend my gratitude. Much thanks are also due to my brother inlaw Mr. Uchenna Adiuku for his contribution to the success of this work.

Finally I wish to thank my parents and parents inlaw for their moral support throughout the time I stayed here.

A B S T R A C T

Two varieties each of Vigna unguiculata , Arachis hypogaea, Pennisetum, Sorghum, Triticurum aestivum in addition to Voandzeia subterranea thouars, Brachystegia eurycoma., Xylopia acthiopiea; Heinsia Pulchella, Ocimum Viride willd, Curcubita linn and Telfaria occidentalis Hook ware analysed for their total calcium, copper, Iron, Manganese, Magnesium, potassium, sodium and Zinc contents.

The first eleven samples *were,* collected from the Institute for Agricultural research, Samaru Zaria. Brachystegia eurycoma and Xylopia aethiopica were brought from Samaru Zaria market. Heinsia pulchella was collected . . from a private garden in the Cross River State while Ocimum Viride willd, Curcubita Linn and Telfaria Occidentalis Hook were collected from a private garden in area F 45, A.B.U. Staff quarter, Samaru Zaria.

The different varieties of Vigna Unguiculata, Arachis hypogaea, Pennisetum, Sorghum and Triticum aestivum were found to have about the same composition of minerals.

The mineral composition of Brachystogia eurycoma did not seem to vary with the method of preparation of the sample except that sample L gave a higher value for potassium.

The leafy vegetables contained more minerals compared with the legumes, cereals and nuts.

Fairly high levels of potasium and magnesium were recorded, while calcium, iron, Copper, Zinc, sodium and manganese are present in low amounts. High amounts of calcium was however, recorded in the leafy vegetables.

Heinsia pulchclla showed a high level of manganese.

The literature values agreed substantially with the values obtained from the analyses, Significant differences compared with the literature were observed in some cases for calcium and sodium.

In the analyses using different digestion mixtures, the results obtained were virtually the same for most minerals. It was however noticed that the amounts of calcium and zinc obtained were slightly lowered in the digestion method using 10ml of sulphuric acid.

The addition of lanthanum was not beneficial for the release of calcium and magnesium.

Shaking of one grame of plant material for about five minutes with a mixture of Hel and water 1:1 is recommended as an accurate, safe and rapid extraction technique for the routine atomic-absorption decerminatior. of copper, manganese, zinc, sodium, potassium, calcium and magnesium in typical plant materials when dried and finely ground.

If however the state of a sample requires wet oxidation method, simple digestion using nitric acid only is recommended.

Potassium can be analysed in plant materials using either the atomic absorption spectophotometer or the flame photometer. For the analysis of sodium, the flame photometer is more sensitive.

Results obtained from the study of the effect of heating time on the amount of mineral released revealed that pro-'Ion heating was virtually unnecessary for the release of the minerals, calcium, manganese, zinc, and copper in Arachis hypogaea. However, eratic results were obtained for iron.

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RESEARCH OBJECTIVE

The minerals, potassium, sodium, manganese, calcium, iron, zinc, magnesium and copper are known to be essential to plant, animal and human physiology. Mineral composition tables of various food seeds and vegetables are therefore essential to the effective evaluation and improvement of world nutrition. In a developing country like Nigeria, unless the composition of local foods is known, calculation of the habitual diet and the introduction of complimentry foods to combat malnutrition cannot be done satisfactorily. The anallysis of minerals in these plant materials should also produce results which are equally valuable to industries engaged in the production of poultry feeds. However, the data available on the minerals composition of the Nigerian food-seeds and vegetables to be analysed are scanty.

Futhermore, although various methods are available for the determination of inorganic nutrients in plant mateials, many of them (particularly at the organic matrix destruction state), lack specificity and are vulnerable to errors and the commonly used wet digestion procedures involve the use of very concentrated acids.

In this work therefore, the levels of the above essential minerals in some food seeds and vegetables will be determined by an already documented wet digestion procedure

using atomic absorption spectrophotometry and flame photometry. The effects of various acids combinations and heating time during digestion on the level of minerals determined will also be studied, with a view to proposing an alternative wet digestion procedure for these plant materials. Independent tests will be made to detect chemical interference effect on calcium and magnesium and compare the efficiency of the flame photometer in the detection of sodium and pòtassium with the atomic absorption spectrophotometer.

Two varieties each of Vigna unguiculata, Arachis hypogaea, Pennisetum, Sorghum, Triticum aestivum. In addition to these, Voandzeia subterranea thouars, Brachystegia eurycoma seed, Xylopia aethiopica (fruit), Heinsia pulchella, Ocimum Viride willd, Curcubita Linn, Telfaria occidentalis Hook will be analysed.

CHAPTER I

I N T R O D U C T I O N

1.1 THE ESSENTIAL MINERALS

All forms of living matter require many minerals for their life processes. Many elements act alone or in conjuction with others as catalysts for essential enzymic processes in the body[1]. Each of the elements copper, Iron, Manganese, Zinc, Calcium, Sodium, Magnesium, and potassium are considered to be essential to animal and human physiology. The following daily requirement in food and water is given for man[2]:

Calcium is essential for the formation of bones and teeth, for blood clothing, nerve tissue regulation, cardiac control, regulation of muscular activity and regulation of body fluids.

In plants, calcium is essential for apical and root tip development and accumulates in cell walls as calcium pectate Coper is an excellent catalyst for oxidation-reduction systems, showing great versatility for an impressive variety of reactions, including the formation of water from oxygen and hydrogen at body temperatures; this reaction would be explosive without copper. It is a unique agent in biological systems.

TABLE I

The Daily Requirement of Essential Minerals in Food

Element	Amount in g
Calcium	1.1
Sodium	4.40
Potassium	3.30
Maganesium	0.31
Iron	0.013
Manganese	0.003
Zinc	0.013
Copper	0.005

Copper activates certain engyme systems in plants, especially those linked with oxidation processes. Copper in excess can be harmful to any living matter.

Iron is necessary for the carrying and exchange of oxygen in the blood of mammals, and for many systems involving oxidation and its converse, reduction. Most of the body's iron is outside the cells, but inside special blood cells - the red cells. It is a vital element in that without it one dies.

Iron occurs in the respiratory pigment of plants. The pigment is called porphyrin and is required in electron transfer processes.

Manganese is required by all living organisms, but its functions are not well known. It takes part in a number of enzymatic reactions. Deficiency in animals and birds affects bones, reproduction and brain, with abnormalities of bony growth, still births, early deaths sterility of the mother and convulsions. It can be a serious pollutant in some circumstances generally associated with iron, Steel, battery and coal burning processes.

Magnesium is said to be a component of soft tissues and bones. Correct calcium - magnesium balance is required for skeletal and nerve tissue function. It is an essential activator of phosphate transfering engymes in plants, magnesium is an essential component of chlorophylls.

Potassium helps in the regulation of body fuids, influences contractility of smooth sketetal and cardiac muscle, affects excitability of nerve and muscle tissue and intracellular - metabolism. Potassium is essential to all plants for cell synthesis. It appears to be the only major ion without a structural function in living organisms.

Sodium plays an important role in the regulation of body fluids. Excess loss of sodium in sweat, for example, results in muscular cramps. It is essential for animals although apparently not for plants.

Zinc appears to be necessary for the building of protein which makes up most of the solid matter of the cells. It is a component of metabolic enzymes, for example carbonic anhydrase and alkaline phosphatase. Almost all the Zinc is inside the cells, where it is more abundant than any other trace element. Human and animal sperm contain large amounts of zinc, up to 0.2

percent and the visual part of the eye contains up to four percent[2]. Zinc deficiency results in retarded growth and anaemia. It is present in adequate amounts in all foods except refined flour, sugar and fats. Zinc is freely available in plant over a wide P^H range but less so in very acid soils. Zinc can be a serious pollutant especially in areas close to industrial plants engaged on smelting, refining and galvanizing processes.

It has been suggested[2] that elemental variations in soil might be reflected in food crops and that deficiencies in man might result from foods grown on soils with very low amount of one or more trace elements.

1.2 NOTES ON THE FEEDING STUFF ANALYSED

Generally nineteen samples were used in this analyses, namely:

A Vigna Unguiculata (Ife Brown)

B Vigna Unguiculata (1696)

These samples are generally refered to as cowpea. The cowpea is indigenous to Africa[4,5] from where it was introduced into the West Indies, North America and other tropical and subtropical countries. It is a warmth-loving herbaceous annual, sensitive to cold and severe frost.

The bulk of the cowpeas in Nigeria is cultivated as a pulse for human consumption. The seeds form a valuable and the most prominent source of plant protein in the diet of Nigerians[4]. It is sometimes cooked and made into soup or it is mixed with rice, or some other cereal, It is often ground, mixed with fish, shrimps, pepper, onion, groundnut oil or palm oil, fried and taken as akara balls or it is boilod in tins and taken as moin-moin.

Cowpeas are high in protein and soluble carbohydrate, low in crude fibre and oil and contain a fair amount of minerals :

C Arachis hypogaea (M 25.68)

D Arachis hypogaea (MK 374)

Samples C and D are commonly called groundnut.

The groundnut plant, Arachis hypogaea, is an annual herb belonging to the papilionaceae division of the family leguminoseae.

Groundnut is one of the most important sources of edible vegetable oils and it is cultivated principally for this purpose. Its nutty agreeable flavour makes it a particularly suitable food when roasted. It is often

cooked and eaten mixed with maize, bananna and bread. Its high protein[2] content makes it especially valuable as a tissue builder. As a food groundnut is one of the most concentrated products, since one gram supplies 6.03 calories[4] and one pound furnishes a total of 1.4 pounds of digestible nutrients.

E Voandzeia subterranea thouars (AC 4406)

This sample commonly known as Bambara groundnut is a leguminous plant and it is native to Africa. It is widely grown in the drier part of Nigeria, and the Cameroons, for local consumption. The nut is a fair source of protein[4] and carbohydrate, suitable for incorporation in livestock ration.

F Pennisetum (Ex Borno)

G Pennisetum (Nig Comp)

This is the staple cereal in those regions bordering on the sahara desert where the climate is too dry for the guinea corn harvest to be depended upon. It is drought resisting and will germinate with less moisture in the soil than almost any other crop. It is capable of growing and maturing quickly under short periods of rain-fall.

The grains are a fairly good source of protein, although poor in minerals.[4] They are slightly lower than guinea corn and "acha" in their content of these nutrients. Millet can be used as a cattle food.

H Sorghum (L 243)

I Sorghum (8K)

This is often refered to as Guinea Corn. It is a dry climate crop and does best in districts too dry for maize or upland rice but in areas where rainfall is higher than is required for growing bulrush millet.

The ash of guinea-corn, though rich in phosphorous is nevertheless deficient in calcium, sodium, potassium, chlorine and iron.[4] Feeding tests also seem to indicate that it is low in vitamin A and, after removing the bran, in members of the B complex. Chemical and nutritional tests of samples of short Kaduna variety grown at Samaru, Zaria, Northern Nigeria were carried out in U.S.A.[4] These tests indicate that the carotene content of the endosperm of the variety equals that of the best yellow maize produced in North America.

Guinea Corn is used in many forms as an important starchy food for human consumption, particularly in Northern Nigeria, and it is also used in feeding livestock. Guinea Corn is also used extensively in the pro-

duction of various alcoholic drinks.

J Triticum aestivum (Siette Cerros 1980-81)

K Triticum aestivum (Indus 6 6 1980-81)

English name - wheat.

Wheat is a temperate crop and does well in Nigeria only when it is grown during the harmattan period when night temperatures are low enough for adequate tillering.[6,7] The factor which prevents the extension of the crop southward in Nigeria is temperature. The main growing areas presently are Borno, Kano, Sokoto and Kaduna States where the major irrigation schemes are located.

Wheat is grown because it is a good cash grain crop. It may be used for grazing livestock, as a hay crop, or seeded to stabilize the soil and retard erosion. The grain is produced mainly for human food but may be fed successfully to all classes of farm animals. The crop fits well in many rotations and can be used as a companion crop under seeded to legumes and grasses. Wheat enters many food preparations. It is best known as the principal ingredient in many kinds of breads, cakes, cookies, crackers, breakfast cereals and macaroni. It is also an ingredient in pancakes, noodles, pie crust, puddings, ice cream cones and baby foods. Germ bran, and

malt are special portions of the wheat grain that appears in food items. Wheat is also used in gravies, sauces, soups, candies, and beverages. The stalks and heads often brightly coloured appear in floral bouguets.

L Brachystegia eurycoma seed (Peeled)

M Brachystegia eurycoma seed (unpeeled)

N Brachystegia eurycoma seed boiled for one hour and peeled.

Brachystegia eurycoma is called "Achi" in Ibo Language. The Efiks call it "Ukung".

The tree measures about 174ft in height, with girth 22½ft, above the butress and16ft from the ground. It reproduces itself readily from seed. The seed is used to thicken soups in the eastern parts of Nigeria.

O Xylopia aethiopica A (Fruit)

Also known as Ethiopian pepper,[6] spice tree or Uda in Ibo, Kimbaji in Hausa, atta in Efik. The tree is cultivated near villages, or protected in the forest in some of the coastal regions. The fruits are narrow, slightly torulose, dark brown or black about 2in long, borne many together on a stout peduncle.

They are used as a cough medicine, a carmitive, to some extent a purgative, and a stimultating addition to other medicines, e.g. to the "Yoruba agbo", of which

it is a very common ingredient. It is also used as a flavouer and spice; at one time similarly used in Great Britain but now probably an article of internal commerce. A fluid extract or a decoction of the fruit or bark, or both is useful in the treatment of bronchitis and dysenteric conditions. As a women's remedy it is taken to encourage fertility and in child birth. The crushed seeds are rubbed on the forehead for headache and neuralgia. An extract of the fruit is also taken as a vermifuge for roundworms, and as a vomitive for biliousness.

P Heinsia Pulchella (Leaves)

Efik - Atama

Yoruba - Tonoposho

The fruit (sometimes called a "bush apple")is a yellow or red berry, pleasantly acid, sweat when ripe. The leaves have odour like that of Anthoxanthum odoratum (Vernal grass), and are placed in huts or carned in the hand for the agreeable scent. Pulverised and mixed with white clay they form scented pommade used by women to conceal body odours.[6] They are rubbed on the forehead or applied as a poultice of the pounded dried leaves for - headache, and as a remedy for craw and for head lice in

..

children. Crushed and steeped in water they are applied to rheumatic stiff joints.

In Liberia the leaves are cooked with rice and given to children to reduce the common umblical hernia. In southern Nigeria a decoction of the leaves is given to horses suffering from lockjaw. In Efik markets the leaves are sold as a soup vegetable. As a women's remedy it is taken in child birth.

Ocimum Viride willd

Fever plant(of Sierra Leone)

Ghana - Onunum,

Efik - ntion

Yoruba - Efinrin

Ibo - Njuanwu

Its original home is doubtful, but is commonly found in native gardens and is sold in markets. It occurs in several varieties, sometimes classed under gratissimum.

A hot infusion of tea made from the leaves is a familiar febrifuge and diaphoretic, used also by Europeans, and sometimes taken as an ordinary tea with milk and sugar. It is also regarded as stomachic and laxative. In Ghana, Cameroons and Nigeria, the

application medicinally is often by rectal injection intended to act as a purge, or for fever and dysentery. If the patient is delirious, the leaves mixed with gunpowder, are rubbed on the face and body. The leaves are used in salads and pepper-soups in Calabar.

The essential oil which has been obtained in small proportion (0.35-1.2% from the dried leaves of west African samples), is orange yellow with thyme - like odour and pungent taste. It mixes with alcohol in all propertions, and containing Thymol in amount varying from 32-65%, it may be regarded as highly antiseptic and the plant itself a possible Source of this drug.[6] Internally the oil is supposed to be a laxative, followed by an astringent effect.[6] In Nigeria, this plant leaves is especially used by women before and after parturition or as emmenagogue. They are also put in soup or boiled with meat or used as stuffing for fowls to disquise odours, e.g. of fish.

R Curcubita Linn

English - Pumpkin

Efik - Ndiṣẹ

Yoruba - Elegede

Ibo - Ugbogulu.

The plants are coarse ground trailers with harsh foliage and stems and rather large golden-yellow flowers. The young leaves and shoots, sometimes with the flowers, are used as pot herbs.

S Telfaria occidentalis Hook (Leaves)
(Fluted pumpkin).

The leaves of fluted pumpkin are well known in Eastern Nigeria for their pleasant taste. They are often picked while the fruit is developing for inclusion, as a vegetable, in the preparation of soup or stew. They are much sought after by sheep and goats and as a result are closely guarded in backyard gardens and on farms during growth. The leaves are rich in protein oil and minerals.[5]

1.3 OXIDATION PROCEDURES

When determining the levels of inorganic nutrient in plant materials, the first objective is to obtain the minerals in concentrated form separated from as many sources of interference as possible. This is invariably achieved by destruction of the organic food matrix by wet oxidation or dry ashing[1,3] techniques.

Various reagents have been reported for the wet digestion of plant materials. These include, nitric-sulphuric acid[1,3,8,9]

nitric-perchloric acids[3,10] nitric acid,[11-13] nitric acid - Hydrogen peroxide,[7,14] Hydrochloric acid.[15] The conditions are usually chosen so as to minimise loses and obtain the elements that require measuring in the most readily handled form for subsequent determination.

The second objective is the determination of individual elements. This may be achieved by electrochemical analysis, atomic spectroscopy, Colorimetric/spectrophotometric, Chromatographic, complexometric, volumetric and gravimetric techniques. There is thus a wide choice of procedures for determining each element.

Total in-organic matter is determined by dry ashing of the food samples.[1] The residual ash is then digested in hydrochloric acid, and the metal chlorides are taken up into approporiate volume of dilute acid and determined using atomic spectrophotometry.

The method of dry ashing has many advocates[3] and is attractive because large samples can readily be handled and, reagent blanks are generally low so that it is advantageous for trace element work[3]. Nevertheless many workers have reported low recoveries of specific elements[3]

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 structure of the crucible. Certain metals are said to be lost by volatilization, notably mercury and selenium for which dry ashing is completely unsuitable.[15]

For some metals such as lead, volatilization losses may occur according to the composition of the sample. A high chloride content is suspect in this respect although it has been shown[16] that losses differed according to the type of chloride. Volatilization losses are aggravated by violent deflagration with local overheating which must be avoided. Samples should preferably by placed in the muffle cold and the temperature allowed to rise slowly. The use of oxidative additive such as nitric acid (to facilitate oxidation) or magnesium nitrate (to raise base status) should be cut to a minimum.

Losses by retention[3] have long been suspected and are usually attributed to the formation of complex silicates. This may arise through combination with the silica wall of the crucible and or the siliceous residue of the sample itself[3]. It seems trace elements, particularly

Copper and Zinc together with iron, are most likely to be affected in this way[3] as some workers have reported unexpected increases for specific elements including iron aluminium and boron. This has been attributed to contamination from the lining of the muffle furnance and so this possibility should not be overlooked.

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 material.

All acid digestion procedures incorporate oxidizing reagents to break down the organic matter.[3] They have a number of advntages compared with the dry ashing procedures including:

a. No volatilisation Losses of metallic elements or phosphorous
b. The procedure is fairly rapid and is easily adapted for routine use.
c. A larger number of nutrient elements can be determined in one digest solution.

Against these advantages must be set certain defects. Perhaps the one which causes most concern is the danger of explosion through using strong oxidizing agents such as perchloric acid or hydrogen peroxide. Provided certain precautions are taken, the acid can be used safely for digestion of ecological materials.

Some of these precautions include:

a. Working on as small scale as possible
b. Stopping the digestion immediately chairing occurs.
c. Not digesting materials high in fat, or oily substances or anything containing hydroxy groups.

1.4 FLAME SPECTROSCOPY

The development of flame techniques, using emission spectra and more recently absorption spectra has made sensitive and highly specific methods available to the analyst. Many elements in particular the alkali and alkaline earth elements can now be estimated in a few minutes[3] providing certain precautions are taken regarding interferences and flame conditions.

Atomic absorption has now almost replaced direct emission for the analysis of most elements except perhaps sodium and potassium.

1.4.1 FLAME EMISSION

When atoms of an element are heated in a flame, some of the heat energy is absorbed by a few of the atoms which become excited, i.e. there is a transition by one or more electrons from the ground state to higher energy levels. On reverting to the ground state the electrons lose this energy which is emitted as electro-magnetic radiation. The wave length of the emitted radiation is governed by the energy change involved in the transition. For each element there are certain permitted shifts giving rise to a series of lines, each series being characteristic of the element in the flame at any one instant. If operating conditions are kept constant the intensity of radiation will be a measure of this number. This is the basis of quantitative flame photometry.

Instruments used for flame emission consist of three main parts, the burner-sample system, a means of isolating the required emission line and the detection-measuring system.

The design of the aspiration system which introduces the sample into the flame has been an important factor in the success of flame instruments. Most widely used is the nebulizer-spray chamber system. In this the

flow of oxidizing gas through a tube draws the solution through a concentric inner capillary. The solution leaving the inlet jet is partly disintegrated into a fine spray by the gas stream. This process is called nebulization and the term atomization which was formerly used is now restricted to the reduction of elements to the atomic state. Only a small, proportion of the solution aspirated passes into the flame and the remainder condenses in the spray chamber and is drained away. Both the size of this proportion and the overall rate of aspiration are related factors which must be controlled to achieve maximum sensitivity. The emission line can be isolated by a filter, prism or grating. Simple flame photometers employ a filter.

A number of interferences occur which are associated with atomization conditions. These can usually be reduced by careful control of the flame condition or solution preparation.

The important classes of interferences are given below:

a. Radiation effects resulting from band spectra of other elements or molecules and also from intense adjacent emission lines.

b. Self absorption: This is an effect whereby unexcited atoms reabsorb emitted radiation, resulting in a reduced sensitivity.

c. Formation of refractory molecules:

This reduces the number of atoms available for excitation. An example of this is the interferance of aluminium and phosphate on calcium emission.[3] It may be suppressed by the addition of lanthanum or strontium as a releasing agent. The formation of oxides in lower temperature flames is another example of this effect, The use of nitrous oxide flame often overcomes this difficulty.

d. Ionization: This occurs most readily with the alkali metals in hotter flames and is significant because ionized atoms do not emit radiation at the wavelength normally selected for the analysis. The effect increases with temperature and is greater for a metal present in low concentrations. However, when other ionizing species are present, these furnish electrons which tend to inhibit ionization. An example of this is the enhancement of alkali metals by other alkalis.[3]

The concentration at which inter-element interferences occur differ with each type of instrument and should be checked before bringing the instrument into routine use.

In general the spectral line chosen for the determination of an element is that which gives the greatest sensitivity and will usually be the most intense line. However, if there are interferences with this line, it may be necessary to choose another.

There are great differences in the case with which various elements are excited in a flame, sodium and potassium are more readily excited than calcium and magnesium. For emission in particular the available energy is therefore important and varies from the very hot cyanogen oxygen flame (4550C) to the relatively cool propane air system (1920c). Metals such as calcium which tend to form heat stable oxides in the flame are better determined at the higher temperatures obtained with air or oxy-aeetylene flames.

It is essential to maintain stable flame conditions. Small changes in temperature can lead to considerable instrumental instability. The flame and burner temperatures should be allowed to come to equilibrum by aspirating the solvent into the flame for a few minutes before taking any readings. The flow rates of the fuel and oxidant gases are extremely important in this respect and should be carefully set at the beginning and maintained

throughout the run to achieve maximum sensitivity and stability. The height of the burner is a contributing factor which must also be regulated. Sudden changes in gas pressure must be avoided.[3] A large fall in air pressure may result in a minor explosion caused by the flame sucking back into the chamber. When using acetylene the cylinder pressure should not be allowed to fall too low or the acetone vapour from the solvent in the cylinders may contaminate the acetylene and affect the readings.

1.4:2 ATOMIC ABSORPTION

PRINCIPLES: The use of flame absorption spectra was first proposed and developed by Walsh (1955). Atomic absorption spectra are formed by the absorption of radiations of certain wavelengths by atoms whose electrons are in the ground state. On absorbing this energy the atoms become excited. The extent of absorption is dependent on the number of atoms in the ground state in the path of the radiation beam at any one time and can thus be used as a quantitative method of determing this number.

In practice a solution of the element is sprayed into a relatively cool flame in which the atoms tend to

remain in the ground state. Radiation of a characteristic wavelength from a hollow cathode discarge lamp is passed through the flame and the decrease in intensity is measured using a monochromator and detector system. This decrease is related to the concentration of the element in solution.

Atomic absorption instruments are basically spectrophotometers with a burner compartment instead of a cell compartment. They consist of a source of radiation, burner plus sample compartment, monochromator, and a detection and measurement system.

Light sources such as tungsten filament and hydrogen discharge lamps give a continuous spectra and so are unsuitable for atomic absorption work. The usual source is a hollow cylindrical cathode made from a material which contains a substantial proportion of the element to be measured. The radiation produced corresponds to the emission spectrum of that element and so the required line may be readily isolated by the nonochromator. Individual lamps are now available for a large number of elements. Lamp turrets which allow advance warming-up to take place are a feature on most instruments.

Many of the points dealt with under flame emission concerning nebuliser design, flame conditions and interferences also apply to atomic absorption.

After the elimination of flame and Chemical interferences the most importnat causes of error in atomic absorption are:-

a. Nebuliser blockage;

b. changes in air and fuel flow rate;

c. wavelength drifting off peak;

d. very low acetylene cylinder pressure; and

e hollow cathode lamp drift.

Changes in burner height over a period may also cause difficulty. By using an external burner or having a water cooled enclosure around the burner, this difficulty can be overcome.

Calibration with standard solutions to produce a graph is generally used to determine the concentration of the test solution both in atomic absorption and flame photometry.

CHAPTER II

E X P E R I M E N T A L

2.1 APPARATUS: Atomic absorption Spectrophotometer - A.A.S. (Unicam SP 1900 with digital readout) equiped with the standard wide slot air-acetylene burner head.

Digestion apparatus:- 500ml conical flask (pyrex), electric heating block.

2.2 REAGENTS:

Unless otherwise stated, all the reagents were of analytical reagent grade.

Nitric acid (69-71% $^{m}/_{m}$)

Sulphuric acid (98% $^{m}/_{m}$) B.D:H. Chemicals.

Perchloric acid (60-62% $^{m}/_{m}$).

Hydrochloric acid (35.4% $^{m}/_{m}$)

Calcium Carbonate (99.5% $^{m}/_{m}$)

Copper Sulphate (98% $^{m}/_{m}$)

Ferric Chloride 99%

Magnesium Sulphate 99.5%

Manganous Sulphate (98-101% $^{m}/_{m}$)

Potassium Chloride (99.8% $^{m}/_{m}$)

Sodium Chloride (99.9% $^{m}/_{m}$)

Zinc Nitrate (98% $^{m}/_{m}$)

Lanthanum Oxide

Acid washing liquid for glassware:

Prepared by diluting concentrated nitric acid 1 to 100 with distilled water.

2.3 COLLECTION AND PREPARATION OF SAMPLES

The samples and their sources are as shown in the table below:

2.4 DETERMINATION OF % DRY MASS[3]

The moisture content of foods is a measure of yield and total solids and for this reason it has become customary to report the results of proximate analysis on a dry weight basis in tables of food composition. The method generally used for the determination of percent dry mass in plant material and which has been used in this work, is the measurement of the loss in weight due to drying at a temperature of about 80^{c}.

One draw back with this method is that some of the water is held in tightly bound forms in the tissue, and can only be removed by increasing the temperature. This leads to loss of volatile components.

Inspite of this drawback the overall simplicity of this method is a great advantage and under controlled conditions, gives satisfactory results.

TABLE 2

SAMPLE DESIGNATION, DESCRIPTION AND SOURCE

Sample Designation	Sample Description	Source
A	Vigna Unguiculata (Ife Brown)	Institute for Agricultural Research, Samaru-Zaria
B	Vigna Unguiculata (1696)	"
C	Arachis hypogaea (M. 25.68)	"
D	Arachis hypogaea (MK. 374)	"
E	Voandzeia Subterranea thouars (AC 4406)	"
F	Pennisetum (EX-Borno)	"
G	Pennisetum (NIG Comp)	"
H	Sorghum (L 243)	"
I	Sorghum (8K)	"
J	Triticum aestivum (Siette Cerros 1980-81)	"

TABLE 2 (Contd)

Sample Designation	Sample Description	Source
K	Triticum aestivum)Indus 66 1980-81)	Institute for Agricultural Research Samaru Zaria
L	Brachystegia eurycoma Seed (Pealed with knife)	Purchased from Samaru Zaria Market
M	Brachystegia eurycoma Seed (unpealed)	" "
N	Brachystegia eurycoma (seed)(boiled for one hour then pealed)	Purchased from Samaru Zaria Market
O	Xylopia aethiopica (fruit)	" "
P	Heinsia Pulchella willd (Leaves)	Private garden Abak, C.R.S.
Q	Ocimum Viride Willd (leaves)	Area F.45 A.B.U. Zaria F. 45, A.B.U. Zaria.
R	Curcubita Linn (Leaves)	" "
S	Telfaria Occidentalis (Leaves)	" "

2.4.1 METHOD[3]

About 5g of the samples in clean dried petridishes were heated in an air circulating oven at 80^{C} for 24 hours.

The loss in weight of each sample on heating was determined and from it, the percent dry mass was calculated as follows:

$$\text{Dry mass \%} = \frac{\text{Oven dry weight (g)} \times 100}{\text{Initial Sample weight (g)}}$$

2.5 PREPARATION OF SOLUTIONS FOR CALIBRATION

Materials:

(i) Hydrochloric acid 3M: Prepared by diluting 93cm^3 of concentrated hydrochloric acid (35.4%^{m}/m) to one dm^3 with distilled water.

(ii) 5% Lanthanum solution: This was prepared by dissolving 117.3g of Lanthanum Oxide in 350ml of concentrated hydrochloric acid, transfering the solution to 1dm^3 volumetric flask and making up the volume with distilled water.

(iii) Potassium diluting solution: This was prepared by dissolving 3.8g of potassium chloride in distilled water and diluting to 1dm^3.

NOTE:- All glassware were washed with the acid washing liquid before use.

2.5.1 STANDARD CALCIUM SOLUTION A

2.497g of Calcium carbonate was transfered to a one liter volumetric flask using 100ml of 3M hydrochloric acid. The mixture was diluted to mark with distilled water (Iml ≡ Img Ca)

Standard working solutions were then prepared as follows:-10ml of solution A was diluted to 100ml (Iml ≡ 100mg Ca) B. To each of six volumetric flasks, 0, 5, 10, 15, 18 and 29ml of B was added 20ml 5% lanthanum solution was added to each of the flasks and diluted to volume. These solutions contained 0, 5, 10, 15, 18, and 20 ug Ca ml respectively.

2.5.2 STANDARD COPPER SOLUTION A

3.93g of C S04.5$H_2$0 was dissolved in 100ml 3M Hcl. The solution was transfered to a one lite flask and the volume made up.

For standard Copper working solution B; 10ml of the stock solution was diluted to 100ml (Im ≡ 100ug Cu) B. To each of seven volumetric flasks, 0.0,1 0.2,0.4, 0.6, 0.8 and 1.0ml of B was added and diluted to volume using distilled water. These solutions contained 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0ug Cu/ml respectively.

2.5.3 STANDARD IRON STOCK SOLUTION A

This was prepared by dissolving 4.84g of Ferric chloride hexa hydrate ($FeCl_3 .6H_2O$) in $100cm^3$ of 3M Hcl. The solution was transfered to Idm^3 volumetric flask and the volume made up with distilled water (Iml ≡ Img Iron).

Standard Iron working solutions were then prepared as follows: $10cm^3$ of A was transfered to $100Cm^3$ volumetric flask and the volume made up with distilled water (Iml ≡ 100ugIron) B. 0, 0.5, 1.0, 2.0, 4.0 and $5.0cm^3$ of this solution was transfered to each of six volumetric flasks and the volume made up with distilled water. These solutions contained 0, 0.5, 1.0, 2.0, 4.0 & 5.0ug Iron/ml respectively.

2.5.4 STANDARD MAGNESIUM SOLUTION A

This solution was prepared by dissolving 10.12g of $Mg\ SO4.7H_2O$ in $100cm^3$ of 3M Hel. The solution was transfered to Idm^3 volumetric flask and diluted to volume (Iml ≡ Img magnesium)

Standard Magnesium working solution B was then prepared by transfering $10cm^3$ of solution A to a 100ml volumetric flask and making up the volume (Iml ≡ 100ug Mg). To each of six volumetric flasks, 0, 0.2, 0.4, 0.6, 0.8 & $1.0cm^3$ of solution B was added.

20ml of 5% lanthanum solution was added to each of the flasks and diluted to volume. These solutions contained 0, 0.2, 0.4, 0.6, 0.8 & 1.0ug Mg/ml respectively.

2.5.5 STANDAR. MANGANESE SOLUTION A

The solution was prepared by dissolving 4.06g of $MnSO4.4H_2O$ in 100ml 3M Hel and making up to volume in a Idm^3 volumetric flask. (Iml ≡ Iml Mn).

Standard Manganese working solution B was prepared by transfering 10ml of A to a 100ml volumetric flask and making up the volume. (Iml ≡ 100ug Mn). To each of six volumetric flasks, 0, 0.5, 1.5, & 2.0ml of solution B was added and the volumes made up. These contained 0, 0.5, 1.0, 1.5, & 2.0ug Mn/ml respectively.

2.5.6 STANDARD POTASSIUM SOLUTION:

This was prepared by dissolving 1.907g of oven dried potassium chloride in distilled water and making up the volume to Idm^3 (Iml ≡ Img K.)

Standard potassium working solution B was prepared by transfering 10ml of A to a 100ml volumetric flask and making up the volume (Iml ≡ 100ug K). To each of six volumetric flasks, 0, 0.5, 1.0, 2.0, 3.0 & 4.0ml of solution B was added and the volume made up. These contained 0, 0.5, 1.0, 2.0, 3.0 & 4.0ug of K/ml respectively.

2.5.7 STANDARD SODIUM STOCK SOLUTION A

This was prepared by dissolving 2.542g of Oven dried sodium chloride in distilled water and making up to Idm^3. (Iml ≡ Img Na).

Standard working solutions were then prepared as follows:- 10ml of solution A was transfered to a 100ml volumetric flask and the volume made up. (1ml ≡ 100ug). To each of seven volumetric flasks 0, 0.5, 1.0, 1.5, 20, 3.0 & 4.0ml of solution B was added and the volumes made up using distilled water. These solutions contained 0, 0.5, 1.0, 2.0, 3.0 & 4.0ug Na/ml.

2.5.8 STANDARD ZINC STOCK SOLUTION A

4.6941g of Zinc nitrate hexahydrate($Zn\ NO_3\ .6H_2O$) was dissolved in 100ml of 3M Hel and diluted to volume.

Standard Zinc working solution were then prepared as follows: 10ml of A was transfered to a 100ml volumetric flask and the volume made up. (Iml ≡ 100ug). To each of six volumetric flasks, 0, 0.1, 0.5, 1.0, 1.5 and 2.0ml of the solution was added and the volume made up. These solutions contained 0, 0.1, 0.5, 1.0, 1.5 & 2.0ug Zn/ml respectively.

2.6 WET DIGESTION OF SAMPLES

About 2g of sample (2 ± 0.0005g) was accurately weighed into a 500cm^3 clean dry conical flask, 10cm^3 of concentrated sulphuric acid (98%) and 5cm of concentrated nitric acid (69.71%) were added and the mixture heated until most of the brown fumes were removed.

Concentrated nitric acid was added dropwise until all organic matter was removed (i.e. the mixture became a faint yellow solution). Heating was continued until white fumes of sulphuric acid were evolved. The digestion flask was cooled and the contents transfered quantitatively to a 100cm^3 volumetric flask. 20ml 5% lanthanum solution was added and the volume made up with distilled water.

Appropriate dilutions of this Stock sample solution were made for the analyses of the various elements. Blank solutions were prepared to contain the same amounts of reagents mentioned above.

The minerals were determined using atomic absorption spectrophotometer (AAS)

2.7 THE DETERMINATION OF MINERALS

Instrumental setting:- The atomic absorption spectrophotometer was set up with the following instrumental operating conditions:

TABLE 3

OPERATING PARAMETERS

Element	Wavelength	Flame	Range ug/ml
Calcium	422.7	Rich Air-C_2H_2	5-20
Copper	324.7	Air-C_2H_2	0.2-1.0
Iron	248.3	Rich Air-C_2H_2	1-5
Magnesium	285.2	Rich Air-C_2H_2	0.2-2
Manganese	279.5	Air-C_2H_2	1-5
Potassium	766.5	Rich Air-C_2H_2	0.5-4
Sodium	589.0	Air-C_2H_2	0.5-4
Zinc.	213.8	Air-C_2H_2	0.1-2.0

2.8 THE DETERMINATION OF CALCIUM

Water was sprayed into the flame and the instrument was brought to Zero reading. The atomic absorption spectrophotometer was set up with a hollow cathode lamp using the line 422.7nm[17] and a fuel rich flame.

The standard solution for calcium, sample, and blank were sprayed successively. The instrument was washed through with water between each spraying. Using the same standard solutions, the instrument was also calibrated at the end of the determinations. This was necessary because calcium requires fuel rich flame and a fall in gas pressure greatly affects the absorbance values.

A mean of three readings for each standard solution was plotted against the concentration, The mineral contents of the samples were assessed (allowing for that of the blank) from the standard curves, using the formula:-

$$\text{Metal content (mg/100g)} = \frac{\text{Concentration ug/ml of sample X 100}}{2 \text{ X } 10}$$

The same procedure was used in obtaining the concentrations of the other minerals analysed in this work, and for each mineral using the appropriate instrumental operating condition.

2.9a DETERMINATION OF SODIUM AND POTASSIUM USING FLAME EMISSION METHOD[3]

Atomic absorption is also suitable for sodium but it does not have any marked advantage over emission, and the equipment is initially more expensive.[3] Sodium is most conveniently determined by flame photometry.[10]

Except when large amounts are present the classical gravimetric methods for estimating potassium in foods have now been replaced by more rapid techniques employing flame photometry or atomic absorption spectrophotometry.[10]

2.9b. DETERMINATION OF SODIUM USING FLAME PHOTOMETER

PROCEDURE:- The standard solutions were prepared as described for AAS. Gas pressure, slit width and other settings were selected as recommended for the instrument.

A calibration was prepared from the standard range after setting the top standard to a suitable scale deflection and the zero ppm standard to zero.

The smaple solution was aspirated into the flame under the same conditions as the standards. The top zero and an intermediate standards were checked frequently. The instrument atomizer and burner was flushed frequently with water particularly at the end of the run. The calibration curve was used to determine concentration of the mineral. Blank determinations were carried out and subtractions made where necessary. The concentration in mg/100g

of dry sample was calculated using the formula

$$\text{Metal content (mg/100g)} = \frac{\text{Concentration ug/ml of sample} \times 100}{2 \times 10}$$

2.9c DETERMINATION OF POTASSIUM USING FLAME PHOTOMETER:-

The determination was carried out as described for sodium but using a sodium filter. 200 fold dilution was necessary in this case.

2.10 COMPARISON OF VARIOUS DIGESTION PROCEDURES

Five different wet digestion procedures were evaluated using five different samples, namely:-

Sample A representing the grain legumes

Sample D to represent the Nuts

Sample K to represent the cereals

Sample O to represent the fruits and

Sample P to represent the leafy vegetables

From an initial study it was concluded that these groups contained essentially similar amounts of the minerals studied.

The procedures used were

1. Addition of 20ml nitric acid (69-71%)
2. 5ml nitric acid (69.71%) and 10ml sulphuric acid(98%)
3. 20ml nitric acid (69.71%) and 10ml sulphuric acid(98%)
4. 20ml nitric acid (69.71%) and 2ml sulphuric acid (98%)
5. 2ml nitric acid, 6ml perchloric acid and 20ml Hcl 1:1

2.10 PROCEDURE 1

About 2g of sample (2 ± 0.0005g) was accurately weighed into a 500cm^3 clean dry conical flask. 20cm^3 of cone nitric acid(69-71%)was added and the mixture was allowed to stand for about one minute to allow any initial vigorous reaction to take place. The mixture was then heated on an aluminium hot plate until evolution of brown fumes ceased. The flask was allowed to cool down and the digest transfered to 100ml volumetric flask. 20ml of 5% lanthanum solution was added and the volume made up. The solution was filtered before determinations were carried out. Blank solutions containing the same reagents were prepared. The samples were digested in triplicates.

The minerals were determined using atomic absorption spectrophotometer.

2.10b PROCEDURE 2

As in 2.6.

2.10c PROCEDURE 3

About 2g of sample (2 ± 0.0005g) was transfered into a 500cm^3 conical flask. 20ml of concentrated nitric acid (69 - 71% $^m/_m$) was added follwed by 10ml of concentrated sulphuric acid. The mixture was allowed to stand for about one minute to allow any initial vigorous reaction to take

place. The mixture was then heated on an aluminium hot plate until evolution of white fumes of sulphuric acid. The flask was cooled and the contents treated as in 2.10.

2.10d PROCEDURE 4

Procedure 4 is similar to procedure 3 but 2ml of concentrated sulphuric acid was added instead of 10ml of the acid. Filtration was carried out where necessary.

2.10e PROCEDURE 5

About 2g of sample was trransfered to a clean dry 500ml conical flask 20ml of nitric acid(69-71 $^{m}/m$) was added and the sample allowed to soak thoroughly. 6.0ml of perchloric acid (60% $^{m}/m$) was added and the mixtures heated on a hot plate, slowly at first, until frothing ceased. Heating was continued until the Nitric acid was almost evaporated. The mixture waw then heated until white fumes of perchloric acid was evolved. The flask was cooled. 20ml hydrochloric acid $^{1:1}$ was added and content transfered to a 100ml volumetric flask. 20ml of 5% lanthanum solution was added and the volume made up.

The determination of the minerals was carried out as previously described.

2.11 RECOVERY EXPERIMENT

In the analysis using procedure one and four, seemingly higher values were obtained for calcium and Zinc in the five samples used in the anlysis. A recovery experiment was therefore carried out to see if this procedure was more efficient than the others.

2.11a METHOD

200ug of calcium and Zinc was added in the same flask to 2g of sample in a clean dry conical flask. 20ml of concentrated nitric acid (69-71% $^{m}/m$) was added, followed by 2ml of concentrated sulphuric acid 98%$^{m}/m$. The mixture was digested until white fumes of sulphuric acid was evolved. The flask was cooled and the mixture transfered to a 100ml volumetric flask. 20ml of 5% lanthanum solution was added and the volume made up. A blank was prepared along with the samples. Three determinations were carried out. The minerals calcium and Zinc were analysed using atomic absorption spectrophotometer (AAS.)

The recovery was calculated by subtracting the concentrations of calcium due to the sample from the observed concentration due to samples and blank. The difference was regarded as the concentration due to the standard added.

The recovery was calculated from the formula below:

$$\frac{\text{Concn. Observed - Concn. due to sample X 100}}{\text{Concn. of metal added}}$$

2.12 EXTRACTION OF SAMPLES USING $HCl^{\frac{1}{2}}$ /H_2O MIXTURE (1 + 1)

About 1g of sample was transfered to a clean dry 500ml conical flask. 30ml HCl (1 + 1) was added and the mixture shaken for about five minutes. The content of the flask was transfered quantitatively to a 100ml volumetric flask, 20ml of 5% lanthanum solution was added and the volume made up. The solution was filtered using glass wool previously washed in an acid washing liquid and dried. Blanks were prepared along with the samples and the minerals were determined using atomic absorption spectrophotometer.

2.13 EFFECT OF DIGESTION TIME AT CONSTANT TEMPERATURE ON THE AMOUNTS OF MINERALS RELEASED FROM SAMPLE D

Sample D is rich in fat-44.8 grams of fat in 100 grams of edible portion.[18] The sample took longer time to digest. Even at the white fume stage using the procedures described above, it was discovered that most of the fats could not be digested.

The time study was performed on the sample to see if the decomposition of the lipid was necessary for a complete release of the minerals. There was also the need to try and determine the optimum digestion time for the sample.

2.13a METHOD

To each of twelve conical flasks 2g of the sample was added. 20ml of conc nitric acid (69-71%) was added to each of the flasks followed by 2ml of conc sulphuric acid (98%). The flasks were allowed to stand for about one minute to allow any initial vigorous reaction to take place. The flasks were then placed on a Heatrae electrothermal plate and the temperature set at three, on the temperature scale of the plate.

Three blanks were also put on the hot plate along with the samples.

Replicate samples (3 each) were removed along with a blank after 10, 20, 30 & 40 minutes. The samples were cooled and transfered to a 100ml volumetric flask. 20ml 5% lanthanum solution was added to each of the flasks and the volume made up.

The procedure was repeated with the timing increased to 50, 60, 90 & 120 minutes.

The determination of the minerals was carried out using Atomic Absorption spectrophotometer.

2.14 ANALYSIS OF SAMPLE D USING THE DRY ASHING METHOD

2g of sample was accurately weighed into glaced, high-form procelain crucibles. The crucibles were placed

on a hot plate under a fume-hood and the temperature was slowly increased until smoking ceased and the samples became thoroughly charred.

The crucibles were then placed in a muffle furnance and the samples were ashed for six hours at 500°c. The dishes were removed from the furnance and allowed to cool. The ash white and clean was moistened with ten drops of water. 5ml of nitric acid (1 + 1) was added and excess nitric acid was evaporated on a hot plate set at about 100-120°c. The crucibles were cooled and the ash dissolved in 20ml hydrochloric acid (1 +1). The mixture was transfered to a 100ml volumetric flask quantitatively. 20ml 5% lanthanum solution was added and the volume made up. The minerals were analysed using atomic absorption spectrophotometer.

INTERFERENCE CONSIDERATIONS

The biggest drawback in the application of flame methods for Calcium has been the interference from other elements.[3] The most important in biological materials particularly vegetation, are phosphorous and aluminium both of which depress the absorption or emission. The most suitable procedure for a wide variety of biological materials involves using a releasing agent as suggested

by Yofe and Finklestein (1958)[19] and Dinin (1960)[20]. For flame emission Dinin found that both strontium and lanthanum gave full release from the effects of aluminium, phosphorous and sulphur under his experimental conditions. An alternative to a releasing agent is to use nitrous Oxide acetylene flame.[3]

Certain elements interfere with the absorption of magnesium in the flame and present problems similar to those described for calcium.[3] With sulphuric acid (1% $^{v}/v$) and an air acetylene flame, it was found that 400ppm lanthanum will release 3ppm magnesium from the effects of at-least 100pp - calcium. 3ppm aluminium, 10ppm phosphorous and is suitable for plant materials and waters[3]. If significant Chemical interferences were taking place, the addition of lanthanum to the digests should minimise this interference.[21-22]

Lanthanum is adopted below because strontium precipitates with sulphuric acid.[3]

2.15a METHOD

An independent test was carried out ot detect chemical interference effect.

Samples $A_1D_1K_1O_1P_1Q$ and S were digested as in method two and the volume made up without the addition of lanthanum. The results were compared with the results obtained in the presence of Lanthanum releasing agent.

CHAPTER III

RESULTS AND DISCUSSION

For the purpose of comparison, the samples have been divided into four groups.

GROUP ONE:- The legumes; comprising of samples A, B, C, & D

GROUP TWO:- The cereals, comprising of samples F, G, H, I, J, and K

GROUP THREE:- Nuts and seeds, comprising of samples E, L, M, and N

GROUP FOUR:- Fruit and the leafy vegetables, comprising of Samples Q, R, S, and T.

3.1 CALCIUM CONTENT

The calcium contents of the samples are given in table 5.

In group one, sample C gave the lowest value (33.25mg/100g) while sample A gave the highest value (51.0mg/100g)

In group two, sample K gave the highest value (25.61mg/100g) while sample I gave the lowest value (4.04mg/100g).

In group three, sample M gave the lowest value (29.88mg/100g) while sample N gave the highest value (71.22mg/100g). No marked difference was observed between samples L, M and N.

TABLE 4

% DRY MASS OF SAMPLES ANALYSED

SAMPLE	DRY MATTER %
A	89.9 ± 0.00
B	89.8 ± 0.20
C	92.3 ± 0.00
D	92.1 ± 0.00
E	94.4 ± 0.01
F	87.1 ± 0.06
G	87.8 ± 0.00
H	84.4 ± 0.5
I	87.9 ± 0.06
J	89.6 ± 0.10
K	87.3 ± 0.00
L	85.4 ± 0.42
M	95.0 ± 0.19
N	56.4 ± 0.00
O	90.2 ± 0.00
P	13.8 ± 0.00
Q	13.2 ± 0.00
R	11.1 ± 0.07
S	9.6 ± 0.00

TABLE 5

CALCIUM CONTENT OF SAMPLES ANALYSED

SAMPLE	CALCIUM (mg/100g of the Dry wt)
A	51.00 ± 0.26
B	50.78 ± 0.22
C	33.25 ± 0.13
D	46.51 ± 0.47
E	29.88 ± 0.34
F	8.54 ± 0.34
G	6.74 ± 0.13
H	5.62 ± 0.00
I	4.04 ± 0.00
J	23.59 ± 0.13
K	25.61 ± 0.13
L	70.10 ± 0.13
M	69.87 ± 0.22
N	71.22 ± 0.34
O	247.13 ± 0.70
P	907.00 ± 0.22
Q	1440.00 ± 46.40
R	2480.00 ± 96.00
S	3040.00 ± 46.40

Concentration ranges generally encountered in plant materials:- 300-2500mg/100g dry weight[3].

In group four, the lowest value was given by sample O (247.13mg /100g) while the highest value was given by sample S (3040.00mg/100g).

Generally samples in group four gave higher values for Calcium. It was also observed that samples A to O gave concentrations of calcium lower than that which is commonly encountered in plant materials[3].

3.2 COPPER CONTENT

The copper contents of the samples analysed are given in table 6.

In group one, the lowest value was recorded for sample A (2.50mg/100g) while the highest value was given by sample D (3.46mg/100g).

In group two, the lowest value was given by sample 1 (1.86mg/100g) while sample J gave the highest value (3.46mg/100g)

In group three, the lowest value was given by sample M (2.67mg/100g) while sample E gave the highest value (7.23mg/100g) slight variations were observed between samples L, M, and N. It was however difficult to give any reason for these variations.

TABLE 6

COPPER CONTENT OF SAMPLES ANALYSED

Sample	Copper (mg/100g of the dry weight)
A	2.50 ± 0.19
B	2.67 ± 0.09
C	2.83 ± 0.09
D	3.46 ± 0.09
E	7.23 ± 0.00
F	2.99 ± 0.33
G	1.73 ± 0.00
H	1.89 ± 0.09
I	1.86 ± 0.09
J	3.46 ± 0.09
K	2.04 ± 0.09
L	3.30 ± 0.16
M	2.67 ± 0.19
N	4.09 ± 0.09
O	4.56 ± 0.09
P	6.29 ± 0.09
Q	2.51 ± 0.16
R	2.83 ± 0.46
S	1.86 ± 0.16

Concentration ranges generally encountered in plant materials 0.25-2.5mg/$100_{g\ dry}$ weight.[3]

In group four, the lowest value was given by sample S (1.86mg/100g) while sample P gave the highest value (6.29mg/100).

Intragroup variations do not appear to be significant. However some results, especially E and P are particularly out of the reported range.[3]

Generally the concentration of copper fell within the range of concentrations commonly encountered in plant materials.

IRON CONTENT

The iron contents of the samples analysed are given in table 7.

In group one sample C gave the lowest value (5.67mg/100g) while sample B gave the highest value (10.00g /100g).

In group two, sample I gave the lowest value (5.67mg/100g) while sample F gave the highest value (9.33mg/100g).

In group three sample M gave the lowest value (6.33mg/100g) while sample E gave the highest value (9.00mg/100g). No marked difference was noticed between samples L, M, and N.

TABLE 7

IRON CONTENTS OF SAMPLES ANALYSED

Sample	Iron (mg/100g of the dry weight)
A	7.00 ± 0.19
B	10.00 ± 0.67
C	5.67 ± 0.47
D	7.33 ± 0.00
E	9.00 ± 0.19
F	9.33 ± 0.19
G	6.33 ± 0.50
H	6.33 ± 0.00
I	5.67 ± 0.33
J	6.00 ± 0.36
K	7.33 ± 0.19
L	6.67 ± 0.19
M	6.33 ± 0.19
N	6.33 ± 0.19
O	11.00 ± 0.19
P	14.00 ± 0.16
Q	8.33 ± 0.33
R	9.00 ± 0.40
S	9.33 ± 0.19

Concentration ranges generally encountered in plant materials 4.0-5.0mg/100g dry weight.[3]

In group four, sample O gave the lowest value (8.33mg/100g) while sample P gave the highest value (14.00mg/100g).

Generally there was no marked variations in iron content between similar samples of different varieties except for sample A (7.00mg/100g) and sample B (10.00mg/100g). None of the groups seemed to show a marked difference in iron content also.

On the whole the amounts of iron obtained from the samples fell within the range of concentration of iron commonly encountered in plant materials.

3.4 MAGNESIUM CONTENT

The magnesium content of the samples are given in table 8.

In group one, the lowest value was obtained for sample B (213.8mg/100g), while sample C gave the highest value (258.36mg/100).

In group two sample G gave the lowest value (124.73mg/100g) while sample J gave the highest value (221.40mg/100g).

In group three, sample M gave the lowest value (194.73mg/100g) while sample L gave the highest value (300.00mg/100). No marked variations was observed in the magnesium content of samples L, M, and N.

TABLE 8

MAGNESIUM CONTENT OF SAMPLES ANALYSED

Sample	Magnesium (mg/100g of the dry weight)
A	220.18 ± 0.73
B	213.80 ± 2.60
C	258.36 ± 1.30
D	239.3 ± 0.74
E	194.73 ± 0.74
F	137.5 ± 0.00
G	124.73 ± 0.74
H	203.64 ± 3.30
I	221.40 ± 3.30
J	197.27 ± 0.00
K	204.91 ± 1.00
L	300.00 ± 1.30
M	292.70 ± 0.00
N	353.80 ± 1.30
O	180.20 ± 3.20
P	379.90 ± 2.30
Q	197.50 ± 3.95
R	241.70 ± 5.16
S	293.30 ± 4.27

Concentration ranges generally encountered in plant materials 100-500mg/100 of dry weight.[3]

In group four, th lowest value was obtained for sample O (180.2mg/100g) while the highest value was obtained for sample P (379.90mg/100g).

The amounts of magnesium obtained from the samples fell within the range of concentrations of magnesium commonly encountered in plant materials.[3]

Magnesium content of the samples was generally high. This is probably because magnesium is an important component of plant chlorophyll. It would be expected that the leafy vegetables will contain more magnesium, but this was not the case. Magnesium is probably present in equally high amounts in other parts of the plant e.g. the seeds and cereals because it activates many engyme systems in plants.

3.5 MANGANESE CONTENT

The results obtained from the analysis of the samples for manganese are given in table 9. The results show that:- In group one, sample B gave the lowest value (1.45mg/100g) while sample D gave the highest value (2.36mg/100g).

In group two, sample F gave the lowest value (0.99mg/100g) while sample J gave the highest value (4.03mg/100g).

TABLE 9

MANGANESE CONTENTS OF SAMPLES ANALYSED

Sample	Manganese (mg/100g of the dry weight)
A	1.75 ± 0.13
B	1.45 ± 0.13
C	1.60 ± 0.13
D	2.36 ± 0.13
E	1.29 ± 0.13
F	0.99 ± 0.13
G	1.29 ± 0.13
H	2.28 ± 0.09
I	1.52 ± 0.11
J	4.03 ± 0.08
K	3.88 ± 0.13
L	9.74 ± 0.09
M	10.04 ± 0.13
N	9.82 ± 0.13
O	2.74 ± 0.13
P	95.90 ± 0.44
Q	3.50 ± 0.55
R	2.77 ± 0.18
S	14.50 ± 0.00

Concentration range in plant material 5.0-100.0mg/100g dry weight.

In group three, sample L gave the lowest value (1.29mg/100g) while sample M gave the highest value (10.04mg/100g).

The results obatined for samples L, M, and N were appreciably higher than the results obtained for samples in groups one and two. There was however no marked difference in manganese content of samples L, M, and N.

In group four, sample O gave the lowest result (2.74mg/100g) while sample P gave the highest value (95.90mg/100g). The amount of manganese obtained for sample P was very high compared with values obtained in other samples. The soil type may have contributed to this high amount of manganese.

Generally, values obtained from this analysis fell within the range of amount of manganese commonly encountered in plant materials.

3.6 POTASSIUM CONTENT

The postassium contents of the samples are given in table 10.

In group one, sample D gave the lowest value (880.31mg/100g)while sample B gave the highest value (1568.49mg/100g).

In group two, sample J gave the lowest value (358.98mg/100g while sample H gave the highest value

TABLE 10

POTASSIUM CONTENTS OF SAMPLES ANALYSED

SAMPLE	POTASSIUM (mg/100g of the Dry Weight)
A	1504.40 ± 53.8
B	1568.49 ± 32.31
C	1000.00 ± 67.95
D	880.31 ± 82.05
E	1290.53 ± 14.87
F	410.32 ± 25.68
G	393.10 ± 39.23
H	487.19 ± 25.64
I	453.60 ± 26.64
J	358.98 ± 12.82
K	410.30 ± 25.64
L	649.50 ± 14.87
M	461.47 ± 14.87
N	470.00 ± 13.87
O	1777.70 ± 14.87
P	2562.30 ± 14.87
Q	2900.00 ± 40.94
R	4700.20 ± 0.00
S	2831.70 ± 98.94

Concentration ranges generally encountered in plant materials:- 500-3000mg/100g dry weight.

(487.19mg/100g)

In group three, sample M gave the lowest value (461.47mg/100g) while sample E gave the highest value (1,290mg/100g). Sample L gave a higher value compared with samples M and N. Probably some potassium was lost from sample N during boiling.

Also the cotyledon probably contains greater amount of potassium than the husk. In sample M the seed was ground with the husk. It may be that the amount of cotyledon contained in the 2g of sample used during the analysis was smaller compared with the amount present in sample L.

In group four, sample O gave the lowest value (1,777.70mg/100g) while sample R gave the highest value (4708.20mg/100).

Except for sample E, samples in group four showed higher potassium content compared with samples in all other groups.

Generally the plant materials analysed contained high amounts of potassium. This is because potassium is essential to all plants for cell synthesis. The values obtained fell within the range commonly encountered in plant materials.

3.7 SODIUM CONTENT

The sodium contents of the samples are given in table 11.

In group one, the lowest value was given by sample C (4.00mg/100g) while the highest value was given by sample B (8.83mg/100g).

In group two, sample I gave the lowest value (3.34mg/100g) while samples F and K gave the highest value (4.17mg/100g).

In group three sample N gave the lowest value (3.67mg/100g) while sample E gave the highest value (4.84mg/100g). No marked difference was obtained in the Sodium contents of samples L, M, and N.

In group four, sample P gave the lowest value (18.17mg/100g) while sample R gave the highest value (24.00mg/100g).

Generally, samples in groups one to three showed low level of sodium compared with the range commonly encountered in plant materials whereas samples in group three showed relatively higher amounts of sodium some of which could fall within the range commonly encountered in plant materials. Low sodium content is expected in plant materials because sodium is considered

TABLE 11

SODIUM CONTENTS OF THE SAMPLES ANALYSED

Sample	Sodium (mg/100g of the dry weight)
A	8.17 ± 0.28
B	8.83 ± 0.28
C	4.00 ± 0.49
D	5.17 ± 0.57
E	4.84 ± 0.28
F	4.17 ± 0.75
G	3.84 ± 0.28
H	3.67 ± 0.56
I	3.34 ± 0.28
J	3.84 ± 0.75
K	4.17 ± 0.57
L	4.67 ± 0.75
M	4.00 ± 0.98
N	3.67 ± 0.56
O	21.84 ± 0.98
P	18.17 ± 0.72
Q	19.00 ± 1.00
R	24.00 ± 1.20
S	21.00 ± 0.00

Concentration ranges generally encountered in plant materials:- 20-300mg/100g dry weight.

a non essential mineral for plants.[3] The soil type could also be a contributing factor.

3.8 ZINC CONTENT

The Zinc contents of the samples are given on table 12.

In group one, the lowest value was given by sample D (1.15mg/100g) while the highest value was given by sample B (3.86mg/100g).

In group two, the lowest value was given by sample K (0.80mg/100g) while the highest value was given by sample G (2.81mg/100g).

In group three, the lowest value was given by sample N (1.91mg/100g) while the highest value was given by sample E (3.16mg/100g), Sample N contained a lower amount of Zinc compared with samples L and M. This can be attributed to the fact that some Zinc was lost in the course of boiling.

In group 3, the lowest result was given by, sample O, (0.16mg/100g). Intragroup variations appear insignificant. Except for sample O, the amount of Zinc obtained from the analysis fell within the range commonly encountered in plant materials.

TABLE 12

ZINC CONTENTS OF SAMPLES ANALYSED

Sample	Zinc (mg/100g of the dry weight)
A	3.35 ± 0.11
B	3.86 ± 0.18
C	1.56 ± 0.05
D	1.15 ± 0.23
E	3.16 ± 0.22
F	1.59 ± 0.14
G	2.81 ± 0.28
H	1.82 ± 0.20
I	1.79 ± 0.25
J	1.60 ± 0.13
K	0.80 ± 0.11
L	2.55 ± 0.09
M	2.23 ± 0.07
N	1.91 ± 0.10
O	0.16 ± 0.13
P	2.11 ± 0.30
Q	2.10 ± 0.16
R	5.55 ± 0.17
S	3.19 ± 0.14

Concentration ranges generally encountered in plant materials: 1.5-10.0mg/100g dry weight.

COMPARISON OF THE VARIOUS DIGESTION PROCEDURES USED

Tables 13 to 20 show results obtained from the analysis of Samples A, D, K, O and P for the minerals, calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc, using various digestion procedures. All the digestion methods gave similar results for the minerals, copper, magnesium, manganese, potassium, sodium and iron. However, the procedure using simple nitric acid and 2ml of concentrated sulphuric acid seemed to give significantly higher results for calcium (19-48%) and zinc (18.6-65%) compared with the method using a mixture of 10ml of concentrated sulphuric acid and nitric acid. This may be attributed to the formation of stable sulphate compounds using the later method. This probably was not a problem with the simple nitric acid digestion alone or nitric acid digestion in the presence of 2ml of concentrated sulphuric acid.

Multi element (calcium and zinc) standard carried through the procedure showed complete recovery (± 4%) except in the case of calcium in sample D with a recovery error of + 14% possibly due to some interference.

However, a more complete digestion of the oily components of the samples was observed using a mixture containing 10ml of concentrated sulphuric acid compared with any other digestion procedure, but the results did not show a correspondingly higher amount of minerals.

TABLE 13

CALCIUM CONTENT OF SAMPLES ANALYSED USING VARIOUS ACID DIGESTION PROCEDURES

SAMPLE	5ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 2ml H_2SO4	20ml HNO_3	Added metal Recovered %	6ml $HClO_4$ + 20ml HNO_3	1:1 $HCL:H_2O$
A	43.40 ± 0.13	56.22 ± 0.00	84.87 ± 0.27	74.39 ± 0.31	114	63.60 ± 0.94	56.52 ± 0.16
D	37.50 ± 0.34	50.11 ± 0.63	81.98 ± 0.00	73.01 ± 0.00	100	62.00 ± 0.17	41.70 ± 0.44
K	24.00 ± 0.00	29.70 ± 0.32	48.79 ± 0.27	42.00 ± 0.27	96	32.10 ± 0.34	52.20 ± 0.30
O	144.00 ± 0.00	176.48 ± 0.00	126.00 ±0.00	178.37 ± 0.45	-	138.78 ±0.26	139.89 ± 0.00
P	647.00 ± 0.00	727.91 ± 0.00	916.65 ± 0.00	912.12 ± 2.60	-	952.60 ± 0.00	952.63 ± 0.15

N.B. Tables 13-20 show mineral contents of samples A, D, K, O, and P using various acid digestion Procedures.

TABLE 14

COPPER CONTENT OF SAMPLES ANALYSED
USING VARIOUS ACID DIGESTION PROCEDURES

SAMPLE	5ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 2ml H_2SO4	20ml HNO_3	6ml $HClO_4$ + 20ml HNO_3	1:1 HCl:H_2O
A	2.20 ± 0.24	2.20 ± 0.30	2.49 ± 0.63	2.50 ± 0.03	2.42 ± 0.50	1.6 ± 0.48
D	2.53 ± 0.00	3.30 ± 0.18	4.40 ± 0.00	3.91 ± 0.05	2.76 ± 0.19	3.29 ± 0.05
K	1.30 ± 0.19	1.76 ± 0.33	1.59 ± 0.44	1.31 ± 0.03	1.40 ± 0.26	1.30 ± 0.10
O	4.60 ± 0.00	4.10 ± 0.29	3.49 ± 0.24	3.57 ± 0.07	3.50 ± 0.41	3.90 ± 0.12
P	5.72 ± 0.24	6.00 ± 0.33	7.11 ± 0.33	6.00 ± 0.25	6.92 ± 0.00	3.80 ± 0.12

TABLE 15

IRON CONTENT OF SAMPLES ANALYSED
USING VARIOUS ACID DIGESTION PROCEDURES

SAMPLE	5ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 2ml H_2SO4	20ml HNO_3	6ml $HClO4$ + 20ml HNO_3	1:1 $HCl:H_2O$
A	10.00 ± 0.47	7.10 ± 0.33	8.00 ± 0.33	8.00 ± 0.50	8.25 ± 0.32	6.65 ± 0.15
D	3.29 ± 0.00	7.05 ± 0.30	6.69 ± 0.13	11.30 ± 0.36	8.00 ± 0.13	0.00 ± 0.00
K	10.31 ± 0.36	7.00 ± 0.93	8.00 ± 0.76	4.00 ± 0.56	8.75 ± 0.49	6.70 ± 0.33
O	16.31 ± 0.00	11.00 ± 0.19	10.00 ± 0.06	6.72 ± 0.33	8.25 ± 0.33	7.10 ± 0.54
P	17.70 ± 0.40	14.67 ± 0.67	15.31 ± 0.67	16.30 ± 0.33	17.00 ± 0.33	6.65 ± 0.25

TABLE 16

MAGNESIUM CONTENT OF SAMPLES ANALYSED USING VARIOUS ACID DIGESTION PROCEDURES

SAMPLE	5ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 2ml H_2SO4	20ml HNO_3	6ml $HClO4$ + 20ml HNO_3	1:1 HCI:H_2O
A	168.00 ± 0.77	157.80 ± 0.36	168.12 ± 0.00	161.59 ± 0.29	156.60 ± 0.50	165.00 ± 0.60
D	196.02 ± 0.74	190.91 ± 0.80	193.54 ± 0.50	194.73 ± 0.43	196.00 ± 0.66	206.23 ± 0.52
K	128.62 ± 0.89	128.56 ± 0.80	136.20 ± 0.16	131.11 ± 0.38	132.40 ± 0.50	138.50 ± 0.90
O	184.50 ± 0.32	183.32 ± 0.70	192.21 ± 0.20	190.89 ± 0.59	185.30 ± 0.86	188.25 ± 0.00
P	440.39 ± 2.30	439.00 ± 1.90	441.00 ± 0.86	436.51 ± 0.00	481.10 ± 0.55	468.41 ± 0.42

TABLE 17

MANGANESE CONTENT OF SAMPLES ANALYSED USING VARIOUS ACID DIGESTION PROCEDURES

SAMPLE	5ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 2ml H_2SO4	20ml HNO_3	6ml $HClO4$ + 20ml HNO_3	1:1 $HCl:H_2O$
A	1.75 ± 0.13	1.75 ± 0.13	1.90 ± 0.09	1.90 ± 0.09	1.83 ± 0.19	1.45 ± 0.13
D	2.28 ± 0.04	2.36 ± 0.03	2.43 ± 0.00	2.51 ± 0.11	2.28 ± 0.19	1.96 ± 0.09
K	3.90 ± 0.04	3.90 ± 0.13	4.00 ± 0.04	4.02 ± 0.11	3.80 ± 0.24	3.35 ± 0.12
O	2.65 ± 0.00	2.80 ± 0.13	2.81 ± 0.04	3.11 ± 0.04	3.00 ± 0.04	2.70 ± 0.00
P	94.44 ± 0.00	95.89 ± 0.88	91.31 ± 0.88	95.90 ± 0.88	95.93 ± 0.84	94.34 ± 0.52

TABLE 18

POTASSIUM CONTENT OF SAMPLES ANALYSED
USING VARIOUS ACID DIGESTION PROCEDURES

SAMPLE	5ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 2ml H_2SO4	20ml HNO_3	6ml $HClO4$ + 20ml HNO_3	1:1 $HCl:H_2O$
A	1461.50 ± 14.87	1512.80 ± 15.84	1538.49 ± 0.00	1435.92 ± 15.28	1461.48 ± 18.23	1500.13 ± 19.00
D	871.80 ± 30.77	871.82 ± 18.21	948.75 ± 38.46	975.00 ± 14.62	923.10 ± 15.28	1207.54 ± 20.00
K	410.33 ± 38.47	410.33 ± 28.21	410.30 ± 14.6	461.52 ± 0.00	437.21 ± 0.00	375.00 ± 15.90
O	1743.60 ± 14.87	1769.21 ± 14.87	1717.88 ± 14.87	1717.90 ± 35.90	1743.60 ± 14.80	1810.00 ± 14.00
P	2487.25 ± 0.01	2561.50 ± 0.06	2871.80 ± 0.02	2615.32 ± 0.10	2564.10 ± 0.07	2925.00 ± 0.00

TABLE 19

SODIUM CONTENT OF SAMPLES ANALYSED
USING VARIOUS ACID DIGESTION PROCEDURES

SAMPLE	5ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 2ml H_2SO4	20ml HNO_3	6ml $HClO4$ + 20ml HNO_3	$HCl:H_2O$ 1:1
A	8.01 ± 0.00	3.20 ± 0.29	10.50 ± 0.25	10.00 ± 0.00	6.85 ± 0.29	14.00 ± 0.00
D	4.00 ± 0.12	5.20 ± 0.25	7.00 ± 0.55	6.52 ± 0.00	5.00 ± 0.00	7.00 ± 0.43
K	3.12 ± 0.00	4.17 ± 0.25	4.50 ± 0.50	4.55 ± 0.50	2.50 ± 0.25	3.59 ± 0.15
O	19.43 ± 0.00	23.00 ± 0.00	28.51 ± 0.29	29.53 ± 0.15	24.11 ± 0.35	24.90 ± 0.00
P	17.50 ± 0.75	18.20 ± 0.77	30.53 ± 0.58	26.90 ± 1.05	20.35 ± 0.58	28.00 ± 0.58

TABLE 20

ZINC CONTENT OF SAMPLES ANALYSED
USING VARIOUS ACID DIGESTION PROCEDURES

SAMPLE	5ml NHO_3 + 10ml H_2SO4	20ml HNO_3 + 10ml H_2SO4	20ml HNO_3 + 2ml H_2SO4	Added Metal Recovered %	20ml HNO_3 HNO_3	6ml $HClO4$ 20ml HNO_3	1:1 $HCl:H_2O$
A	3.45 ± 0.02	3.41 ± 0.45	4.15 ± 0.00	104	4.24 ± 0.48	4.95 ± 0.95	5.30 ± 0.00
D	1.34 ± 0.04	1.31 ± 0.05	2.94 ± 0.02	96	2.94 ± 0.02	3.19 ± 0.09	2.31 ± 0.04
K	0.73 ± 0.00	0.73 ± 0.11	1.24 ± 0.00	102	1.34 ± 0.04	1.34 ± 0.05	1.33 ± 0.02
O	0.22 ± 0.04	0.22 ± 0.05	0.61 ± 0.05	96	0.64 ± 0.02	0.89 ± 0.03	1.46 ± 0.09
P	2.40 ± 0.29	2.40 ± 0.24	3.10 ± 0.00	104	3.50 ± 0.00	3.40 ± 0.04	2.50 ± 0.01

A simple digestion using concentrated nitric acid (69-71% $^{m}/_{m}$) was seen to be adequate for the routine determination of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc in plant materials compared with the standard A.O.A.C. method. The addition of perchloric acid during the nitric acid digestion did not appear to be beneficial for the analysis of dried plant materials.

The use of this reagent caused the formation of perchlorates which only went into solution on the addition of HCl-water mixture (1:1). It is possible that the perchlorates containing the element being measured were not all broken down into individual atoms at the temperature of the flame being used, as the amounts of minerals obtained using this technique were slightly lower in almost all cases except for Zinc where this method gave the highest values. However, white fume stage was reached earlier using this method.

10 EXTRACTION USING 1:1 HCl-WATER MIXTURE IN THE COLD

The results are as shown in the last columns of tables 13 to 20. From the results it was observed that comparable amounts of Sodium, Potassium, Manganese, Zinc, Calcium and Copper were obtained using this extraction procedure compared with digestion method using perchloric acid or concentrated sulphuric acid-nitric acid procedures. In the analysis for iron using this technique, comparable results were obtained for samples A, K, and O.

Probably the minerals are not present in any complexed form. The HCl was probably able to extract the metals from suspended solid material and also standardized the acidity of the prepared solution. About 50% of Iron was obtained for sample F compared with techniques using conventional digestion procedures. This result was difficult to explain. In the analysis of sample D for Iron using this techniques, zero iron-content was observed. Probably there was an interference in the air acetylene flame between Iron and the organic constituents of sample D.

Sodium, Calcium, Magnesium and Manganese were extracted quantitatively in the cold when, dried ground samples were shaken in N-hydrochloric acid for 24 hours, but under these conditions it was claimed[23] that only 60-70% of Copper, Zinc, Iron and Chromium were extracted. Premi and Cornfield[24] showed that trace amounts of copper, zinc, iron, manganese and chromium can be extracted quantitatively from plant material and organic residue by boiling with hydrochloric acid. Price and Roos[25] showed that, for orange and pineaple juices, correct results are obtained by acidifying the juices and separating suspended matter by centrifugation.

The method was extended to other metals[26] in juices- Calcium, magnesium and potassium.

It is interesting to note that copper, zinc, manganese, calcium, sodium, potassium and magnesium can be extracted quantitatively from plant materials and organic residue simply by shaking dried ground sample in cold (1:1) HCl-water. Complete destruction of the organic matter is therefore not very important. When prepared in the way described above (2:12), the analysis solution becomes ready in a much shorter time than by either dry or wet ashing. The use of harzadous reagent may also be avoided. In addition to these, concentrated acids are not wasted. If the method is used in the routine analysis for other elements or for different types of material, it would be advisable to check initially that quantitative extraction is obtained.

3.11 ADDITION OF LANTHANUM

Tables 21 A & B show that almost all of the results obtained in the presence of lanthanum show essentially no difference for calcium and magnesium compared with the results obtained in the absence of lanthanum.

TABLE 21[A]

RESULTS SHOWING EFFECT OF LANTHANUM ON THE RELEASE OF CALCIUM

Sample	Conc Ca(mg/100g) in the presence of lanthanum	Concn Ca(mg/100g) in the absence of lanthanum
A	84.29 ± 0.81	83.60 ± 0.27
D	63.82 ± 0.16	64.01 ± 0.22
K	39.29 ± 0.18	39.50 ± 0.36
P	948.00 ± 0.26	948.21 ± 0.22
Q	1440.00 ± 0.13	1440.00 ± 0.00
R	2480.26 ± 0.26	2640.00 ± 0.00

TABLE 21B

EFFECT OF LANTHANUM ON THE RELEASE OF MAGNESIUM

Sample	Concn Magnesium (mg/100g) in the presence of lanthanum	Concn Magnesium (mg/100g) in the absence of lanthanum
A	161.50 ± 1.03	215.73 ± 0.84
D	193.51 ± 0.90	224.32 ± 0.19
K	136.22 ± 0.00	162.10 ± 0.00
P	441.00 ± 2.30	405.00 ± 2.51
Q	197.01 ± 0.76	194.50 ± 1.25
R	241.76 ± 0.30	244.71 ± 1.00

It was concluded that the addition of lanthanum was not beneficial for the release of calcium and magnesium. This probably suggests that calcium and magnesium do not suffer from any kind of interference in this matrix.

3.12 THE A.A.S. AND FLAME PHOTOMETER IN THE ANALYSIS OF SODIUM AND POTASSIUM

Table 22A gives the results obtained from the analysis of samples A, D, K, O, P, Q, R, S, for sodium using the Atomic absorption spectrophotometer (absorption technique) and a flame photometer (flame emission technique). The results revealed significantly lower results for sodium using the A.A.S. compared with the results obtained using the emission technique. This may be attributed to the fact that sodium can form refractory compounds during digestion and may also ionise in air-acetylene flame.[15] It is suggested that absorption methods may not be an alternative method for the analysis of sodium in plant materials; flame emission methods is more specific for sodium.

Table 22B shows that both the A.A.S. and Flame photometer can be used for the analysis of potassium. Potassium is determined from dilute solutions using

TABLE 22A

CONCENTRATION OF SODIUM USING ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS) COMPARED WITH CONCENTRATION OBTAINED USING FLAME PHOTOMETER

Sample	Concn Na (mg/100g) Using A.A.S.	Concn Na (mg/100g) using Flame photometer
A	5.15 ± 0.09	10.50 ± 0.08
D	2.88 ± 0.01	7.10 ± 0.16
K	1.64 ± 0.01	4.50 ± 0.00
O	18.68 ± 0.22	28.51 ± 0.20
P	14.00 ± 0.60	18.20 ± 0.46
Q	4.61 ± 0.16	19.00 ± 0.17
R	4.78 ± 0.00	24.00 ± 0.12
S	3.00 ± 0.00	21.00 ± 0.00

TABLE 22B

CONCENTRATION OF POTASSIUM USING A.A.S. COMPARED WITH VALUES OBTAINED USING FLAME PHOTOMETER

Sample	Concn K (mg/100g) Using A.A.S.	Concn K (mg/100g) Using Flame Photometer
A	1920.00 ± 29.95	1504.00 ± 0.04
D	1021.32 ± 28.59	880.30 ± 0.04
K	394.90 ± 42.20	410.31 ± 0.00
O	2600.92 ± 23.90	1977.70 ± 0.09
P	2028.90 ± 24.34	2562.30 ± 0.00
Q	2900.00 ± 40.94	3000.00 ± 27.5
R	4708.21 ± 0.00	4275.00 ± 0.00
S	2831.76 ± 78.94	2700.00 ± 0.00

both techniques and as a result, formation of refratory molecules may be less in this case.

EFFECT OF DIGESTION TIME ON THE RELEASE OF MINERALS FROM SAMPLE D

Table 23 shows the results obtained from a study of effect of digestion time at a prechoosen heating level on the release of the minerals, calcium, copper, iron, manganese, and zinc from sample D. From the results it was observed that 100% of copper, 87.7% of zinc, 93.42% of manganese, 49.8% iron were released compared with the amounts released at the white fume stage (60 minutes). The values obtained for iron and copper tended to reach a maximum after heating for fourty minutes, followed by a decrease. Probably iron and copper were lost in the course of the heating either due to complex formation or interference between the two minerals and the organic constituents. For calcium, it was observed that slightly higher value was obtained after heating for ten minutes. At this time, the flask was not quite warm and the sample solution was filtered before aspiration into the instrument. The slight decrease in the amounts of calcium released as the time of digestion was increased may be due to the formation

TABLE 23

EFFECT OF DIGESTION AT CONSTANT TEMPERATURE ON THE RELEASE OF THE LISTED ELEMENTS

Time (Minutes)	Conc Calcium (mg/100g)	Concn. Copper (mg/100g	Concn Iron (mg/100g)	Concn. Manganese (mg/100g)	Concn. Zinc (mg/100g)
10.0	86.00 ± 2.63	2.20 ± 0.09	1.33 ± 0.19	2.13 ± 0.00	2.71 ± 0.08
20.0	79.40 ± 2.58	2.36 ± 0.09	2.33 ± 0.56	2.28 ± 0.00	2.84 ± 0.17
30.0	71.40 ± 2.46	2.51 ± 0.00	2.67 ± 0.56	2.28 ± 0.08	3.03 ± 0.09
40.0	69.90 ± 3.79	2.82 ± 0.26	6.67 ± 2.32	2.28 ± 0.04	2.87 ± 0.16
50.0	70.22 ± 3.04	2.99 ± 0.09	6.67 ± 1.03	2.28 ± 0.00	3.09 ± 0.07
60.0	65.73 ± 4.05	2.20 ± 0.09	2.67 ± 1.33	2.28 ± 0.00	2.60 ± 0.04
90.0	69.31 ± 6.18	2.20 ± 0.09	2.33 ± 1.39	2.21 ± 0.08	2.61 ± 0.08
120.0	65.16 ± 5.4	2.20 ± 0.09	3.0 ± 3.09	2.28 ± 0.00	3.00 ± 0.14

insoluble compounds or interference between the calcium and the organic constitutents of the sample.

On the whole the results seem to agree with the observation made earlier that a simple nitric acid digestion is quite sufficient for plant materials and the fear of the flask drying up should be ruled out since these minerals are released within 30--40 minutes of heating.

These results also agree with suggestion that for the sample used, complete destruction of the organic matter is not important. It is the believe of the author that the minerals, magnesium, sodium and potassium will be fully released within about twenty minutes of heating. This is because these minerals were fully released on extraction of samples A.B.K.O. & P with HCl / water mixture(1:1).

3.14 DRY ASHING ANALYSIS OF SAMPLE D

Table 24 shows the results obtained from the analysis of sample D for calcium, copper, iron, manganese, and zinc using the dry ashing method. The results obtained was comparable with the result obtained using the wet digestion methods 5, except in the case of

TABLE 24

RESULTS FROM ANALYSIS OF SAMPLE D USING DRY ASHING METHOD

ELEMENT	IRON	MANGANESE	ZINC	COPPER	CALCIUM
CONCN. mg/100g	4.22 ± 0.60	2.03 ± 0.00	2.71 ± 0.05	2.24 ± 0.00	41.69 ± 1.26

calcium where there was a 42.21% decrease in the amount obtained using the dry ashing method. The digest solution did not contain any undigested oily particles just as was found in the procedures whereby 10^{ml} of concentrated sulphuric acid was included in the wet digestion mixture.

The complete digestion of the organic matter in sample D was almost always accompanied by a decrease in the amount of calcium. Probably there are some components of the organic matrix which form stable compounds with the calcium in the final digest solution, preventing it from being broken down into individual atoms for analysis at the temperature of the flame being used.

CHAPTER IV

CONCLUSION AND SUGGESTIONS FOR FURTHER WORKS

From the results it can be concluded that the cereals have low content of the minerals analysed while the leafy vegetables and fruit are rich in minerals. It is also concluded that Brachstegia eurycoma is a better source of minerals compared with the cereals and legumes. The method of processing the seed before using it in the thickening of soups has no effect on the mineral content except that sample L which was peeled with knife before grinding contained more potassium than samples M and N.

Heinsia pulchella may be recommended as a good source of manganese but before this conclusion is drawn, this vegetable should be collected from other parts of the country and analysed for manganese to see if actually the sample is rich in manganese or the high manganese content was due to the soil on which the plant was grown.

From the observations during the experiement, it can be concluded that the atomic absorption spectrophotometer is as good as the flame photmeter in the analysis of potassium. In the case of sodium, the flame photometer is more sensitive.

For the determination of minerals in dried ground plant materials, complete destruction of the organic matter may not be very important. As a result, a simple digestion using concentrated nitric acid alone is recommended as an effective method of digestion. The addition of concentrated sulphuric acid may cause the formation of insoluble compounds. It is harzadous to use hydrogen peroxide or perchloric acid yet their addition is of no particular advantage in the analysis. As an extension of this work, it is recommended that investigation be carried out using animal material. This will help to test the efficiency of the simple nitric acid digestion on such samples.

From the results, it can also be concluded that the addition of lanthanum as a releasing agent was not necessary for the matrix used. The values obtained for calcium and magnesium in the presence of lanthanum releasing agent were quite close to those obtained in the absence of lanthanum releasing agent.

Shaking one grame of dried ground plant material for about five minutes in 30ml of a mixture of concentrated hydrochloric acid and water (1:1) is recommended as a fast and accurate technique for the routine deter-

mination of calcium, copper manganese, magnesium, potassium, sodium and zinc in typical plant materials.

The results obtained for the analysis of sample D using dry ashing technique seemed to show a lowering of the calcium content compared with results obtained from the wet digestion methods. It is therefore suggested that this experiment be carried out on various plant materials. If the above observation persists, then it may be concluded that the dry ashing technique is not a sufficiently good method for the analysis of calcium in plant materials.

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