TRACE ELEMENTS AND PROTEIN DETERMINATION IN SOME FOODSTUFFS

BY

KEHINDE OLOLADE OSIPITAN
B.Sc. (Hons) CHEMISTRY (IBADAN)

thesis submitted in partial fulfilment of
the requirements for the degree of

MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY
ZARIA - NIGERIA.

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 Information derived from the published or unpublished work of others has been acknowledged in the text.

K.O. OSIPITAN

9/2/82

Date

THESIS RECOMMENDATION

We hereby recommend that the thesis prepared by K.O. Osipitan entitled "Trace elements and protein determination in some vegetable samples" be accepted in partial fulfilment of the requirements for the degree of M.Sc. Analytical Chemistry.

INTERNAL EXAMINER
Supervisor
Julius Yinka Olayemi
B.Sc ; Ph.D. ; C Chem ; M.R.I.C.

18/8/82
Date

EXTERNAL EXAMINER

19.08.82
Date.

Praise the Lord, Oh my soul and all that is within me, praise His Holy name.

D E D I C A T I O N

To my Father, Mr. J.A. Osipitan and to the evergreen memory of my dearly beloved Mother, Mrs Modupe Osipitan.

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

I wish to express my profound gratitude to my supervisor, Dr. J.Y. Olayemi for his patience, easy accessibility, encouragement, forbearance, tolerance and cooperation. He also supplied some of the materials for analyses. I would also like to acknowledge my Head of Department, Dr. H. Bozimo and my course coordinator, Dr. K. Singh for making available the facilities within the department. I am highly appreciative of the assistance given me by Mr. Smith, the technical staff of the M.Sc. laboratory and the store personnel of the Chemistry department. Many thanks go to Alhaji Ajeigbe, Mr. S. Oyewo, Doris and the technical staff of the Biochemistry department.

I am grateful to Dr. Olanrewaju, the head of the Horticultural section of the Institute of Agricultural Research (IAR) for supplying some of the vegetable samples used in this study.
I am also grateful to Mr Ohaeri of the Biological Sciences department for identifying some of the samples.

My friends and classmates who contributed to the success of this course amd made the class quite interesting also should be thanked.

I am particularly grateful to my father Mr. J.A. Osipitan for affording me the opportunity to do the course, and also the members of my family for their encouragement.

Finally, I am grateful to God for seeing me through the course.

C O N T E N T S

	Page
Title	i
Declaration	ii
Thesis recommendation	iii
Dedication	iv
Aknowledgement	v
Table of contents	vi
List of Tables	vii
Abstract	viii
C H A P T E R I	
Introduction	1
C H A P T E R II	
EXPERIMENTAL	16
Sample collection and description	16
Sample preparation	16
Moisture content	21
Crude Protein	22
Preliminary Investigation of methods of digestion	25
Trace metal determination	43
Atomic Absorption Spectrophotometer	43

Iron - 45
Manganese - - - - - - - - - - - - - - - - - - 46
Lead - 47
Cobalt - - - - - - - - - - - - - - - - - - - 48
Chromium - - - - - - - - - - - - - - - - - - 49
Cadmium - - - - - - - - - - - - - - - - - - 50
Copper - - - - - - - - - - - - - - - - - - - 50
Zinc - 51

CHAPTER III

RESULTS AND DISCUSSION - - - - - - - - - - - - 53
Moisture content - - - - - - - - - - - - - - - 53
Crude Protein - - - - - - - - - - - - - - - - 58
Trace Element - - - - - - - - - - - - - - - - 60
Iron - 61
Manganese - - - - - - - - - - - - - - - - - - 62
Lead - 66
Cobalt - - - - - - - - - - - - - - - - - - - 67
Chromium - - - - - - - - - - - - - - - - - - 70
Cadmium - - - - - - - - - - - - - - - - - - - 70
Copper - - - - - - - - - - - - - - - - - - - 71
Zinc - 72
Essential trace elements - - - - - - - - - - - 76

Ratio of essential to non-essential trace elements ---- ---- ----- -- 80

Zinc/Cadmium ratio ---- ---- ---- 81

CONCLUSION AND RECOMMENDATION _____ 82

REFERENCES ___ _____ 87

APPENDIX ____ ____ _____ __

A B S T R A C T

Twenty six different vegetables were analysed for their moisture contents protein content and iron (Fe) manganese (Mn), cadmium (Cd), lead (Pb), cobalt (Co), copper (Cu), chromium (Cr) and zinc (Zn) as trace elements. The classes of vegetables analysed include leafy vegetables, okra, pepper, garden egg and onions. The best wet digestion methods for the different groups of vegetables were first determined. They were subsequently used in determining the trace elements.

The vegetables were found to have at least 55 percent water and up to 9 percent protein of dry weight of sample. Most vegetables contained over 80 percent water. There was variation in the protein content according to the class of vegetables.

Host of the vegetables were found to have up to lOmg/lOOg of iron of the dry weight of the samples. Garden egg and onions had values lower than this. With the exception of okra and onions, the vegetables had up to 2.00mg/100g of manganese of dry weight of samples; with the exception of a variety of pepper and a type of leafy vegetable (spinach), the vegetables had less than 2.00mg/100g of lead. They had less than 2.00mg/100g of cobalt. Chromium was found to be absent in most of the vegetables and very low levels of cadmium were observed. With the exception of onions which had lower values and pepper with higher values, the vegetables had

up to 0.85mg/100g of copper. They also had up to 2.50mg/100g of zinc with the exception of a few which had lower values.

From the results obtained, the total essential elements were calculated and chilli pepper was found to have the highest value due to its iron value which was conspicuously high. With the exception of the onions which had lower values, the others had values up to lO.Omg/lOOg. Apart from the chilli pepper, the leafy vegetable group had higher values than the rem aining vegetables. The ratio of the essential to the non-essential were found and all the vegetables had a value greater than 3.0. This meant that all contained more useful than harmful elements. The zinc/cadmium ratios were also found out. With the exception of a few, most of the vegetables had a value greater than 10.0. This showed that most of the vegetables had enough zinc to suppress the harmful effect of cadmium.

The results of the analyses were compared to that obtained in the literature. The values obtained agreed with that in the literature for protein and moisture content. Some differences were observed for some trace elements.

C H A P T E R I

INTRODUCTION

Food can be defined as all those materials which by their nature can contribute to the energy supply of an organism or provide the components necessary for the growth and repair of its body tissue[1]. The many food humans eat fall into a limited number of broad groups. The composition of the foods within these groups vary to some extent but each group has certain characteristics of its own which make it different from other groups. The groups are (i) Cereals (ii) Tubers and roots (iii) Legumes or pulses (iv) Oil seeds and nuts (v) Milk (vi) Meat (vii) Fish (viii) Eggs (ix) Vegetables (x) Fruits (xi) Visible fats and oils (xii) Sugars[2].

1.1 Vegetables

Botanically, a fruit is the ripened ovary of a plant together with any adjacent part that may be fused with it, whereas a vegetable may be defined as some other edible part of a plant[3]. In common practice however, this classification is not always applicable and some fruits are referred to as vegetables. Vegetables can be further classified from the standpoint of the keeping quality as perishable, semi perishable and staple. While the perishable ones have comparatively poor keeping quality even when stored in a refrigerator, the semi perishable will keep for some time in a

refrigerator while the staple may be kept without refrigeration[3]. Vegetables may also be classified from the physiological standpoint into (i) Leafy vegetables e.g. spinach and bitterleaf (ii) fruity vegetables e.g. okra and pepper and into (iii) root vegetables e.g. onion. Despite these different classification, most vegetables have fairly similar nutritional properties[2].

In the fresh state, vegetables contain a great deal of water and hence yield relatively few calories of energy per unit weight. Their protein content is low and they contain an indigestible matrix called roughage which enable the bowels to function regularly. Though human beings cannot live on them alone, vegetables are nevertheless valuable foods as they add to the flavour [2] and variety of diets. They are often a good source of certain vitamins and minerals particularly vitamin C, pro vitamin A, calcium and iron. The colour of which is either green, yellow or red depending upon the pigments that the vegetables contain. The green colour of most vegetables is due to the pigment chlorophyll.

For the formation of the substances which make up the living tissue and for their proper functioning, plants and animals require the supply of chemical elements[6]. A plant grows and develops normally only when certain essential elements are made available to it in the soil[5]. Some of these are required in greater quantities than the others. Those elements which are essential for health and are required in quantity are referred to

as 'bulk elements'. Other mineral elements occur in the living tissue in such small amounts that early workers were not able to determine their concentration precisely by the available analytical instruments. These were said to occur in traces and the term 'trace elements' were used to describe them. It is however difficult to draw a line of demarcation between these two groups [9].

1.2. Trace Elements

The definition given above for trace elements, can be broadened to include elements necessary for special purposes which provide perfect health but without which life can still exist at an unhealthy level[9]. Trace elements can be classified into two main groups (i) the ones which take part in necessary biochemical reactions and are referred to as 'essential trace elements' and (ii) those which do not take part in such processes.

The latter group can be further subdivided into two:

a) those elements that are harmless to living tissues and are referred to as non-toxic.

b) those that are harmful to living tissues and are called toxic.

A considerable number of the essential trace elements occupy the position in the periodic table of elements between atomic number 23 and 34[9]. The only similarity in these trace elements is that they normally occur and function in living tissues in low concentrations. Their concentrations are usually expressed in parts per million (ppm), microgram per gram (ug/g) or parts

per billion (ppb). These essential trace elements act primarily as catalyst in enzyme systems in the cells where they serve a wide range of functions[5].

The interest in trace elements in human nutrition has been heightened for several reasons among which are (i) the ever increasing rate of consumption of refined foods with reduced level of important micronutrients and (ii) the rapidly deteriorating environment which exposes the individual to certain heavy minerals for which no essential function is known[7]. There is plenty of these elements in sea water and sometimes on the earth's crust.

1.2.1 Trace elements and Homeostasis[9].

Homeostasis (same) is a condition of balance. When applied to elements, it means the amount taken in and amount passed out are equal. The body has a mechanism of maintaining the homeostasis of the elements, this is because accumulation of excesses are toxic and deficiences are unhealthy. Homeostasis is known to fail under the following conditions:

i) When the amount of element taken into the body daily is less than that excreted in faeces and urine so that the body operates on a negative balance and still maintain health. Eventually, a state of deficiency develops. Chronic disorder of the kidney, chronic diarrhoea and poor intestinal absorption and certain diseases are known to increase the excretion of trace metals, thus

increasing the need. For example, alcohol in continous amounts has been reported to mobilize zinc.

(ii) When the amount of the element is in excess of the intestine's ability to excrete, there will be an accumulation of that element in the body.

(iii) Hereditary diseases in which the transport mechanism for a trace metal are inefficient. For example, the intestinal rejection of metal allows for their accumulation and serious diseases occur. Such diseases are known for iron and copper. Here the gene carrying out the mechanism is damaged. Haemochromatosis is the outcome of the disorder for iron which slowly accumulates throughout life damaging the pancreas in which case diabetes results or in the liver where cirrhosis results[9]. Wilson's disease is the result of accumulation of copper. Copper becomes stored in the liver and in special parts of the brain, producing cirrhosis of the liver and a type of neurological disorder[9].

1.2.2. Principal functions of some trace metals.

Iron: Iron is an essential element in the nutrition of both plants and animals. The amounts required are not large though always greater than mere trace quantities. Though it is not a constituent of the cholorophyll molecule, it is reported to be necessary in the culture medium if the plant is to form this pigment[1]. In animals, it is a component of the haemoglobin molecule and a supply of the metal is essential for the formation of that

pigment too. The chief function of haemoglobin is that it is necessary for the carrying and exchange of oxygen in the blood of mammals and also in many systems involving oxidation and reduction. It is a vital element.

Copper: Copper is an excellent catalyst for oxidation reduction systems and is involved in a variety of reactions including the formation of water from oxygen and hydrogen at body temperature[9]. This reaction could be explosive without copper. It is thus a unique agent in biological systems and all living things require it.

Manganese: It is required by all living organisms. Its functions are not well known. It takes part in a number of enzymatic reactions[5]. The most important functions of manganese are as an essential constituent for bone structure, for reproduction and for the normal functioning of the nervous system[8].

Chromium: It is necessary for the maintenance of normal cholesterol metabolism and normal sugar metabolism. It increases growth[9].

Zinc: Zinc is an essential nutrient for plants and man. One of its major functions is as a constituent of the enzymes involved in protein and carbohydrate metabolism and also for (RNA) synthesis. Protein makes up most of the solid matter of the cells. It is also a constituent of the enzyme which releases carbon dioxide from bicarbonate, of the enzyme which begins the oxidation of alcohol and other natural substances and of the splitting off

portions of protein.

Cobalt: Cobalt in small quantities is essential in the nutrition of animals. Its principal function is as a necessary constituent of vitamin B_{12} which is manufactured by bacteria. Vitamin B_{12} is required in the formation of red blood cells[1]. All the cobalt required is obtained from plants. Humans obtain their cobalt from animal sources.

1.2.3. Trace element deficiency and disease.

A trace element necessary for the growth of plants must be present in the soil or the plant would not grow and produce seeds, root and fruits; so also a trace element necessary for the animal must be available in its diet. The absence of the trace element leads to deficiency in both plants and animals and a disease could result. Below are a few of the results of deficiencies as reported for some trace elements.

Iron: Inadequate supply of iron in plants leads to chlorosis[1], the result of deficiency in animal is a disease called anaemia. (chronic loss of blood). A decrease in the amount of dietary iron leads to less available iron in the body and results in deficiency. Iron deficiency is most commonly found among women during reproductive years. Blood losses from menstruation and greater nutritive demands during childbearing increases iron needs[7]. Certain drugs cause alimentary bleeding and thus loss of

blood[7]. The richest sources of iron are liver, organ meat, egg yolk, cocoa, dried legumes and green vegetables.

Copper: Deficiency could result in anaemia, skeletal defects, defects in pigmentation, reproductive failure and alteration of the structure and colour of hair. However, deficiency of copper is reported to be rare in man[3]. The richest sources of copper include crab, liver, nuts and seeds, coffee and tea.

Manganese: Natural deficiency occurs in a number of food plants which grow poorly and show chlorosis or bleaching of the leaves[1]. Deficiency of manganese has been observed in man in association with vitamin K deficiency[5]. The main manifestation of deficiency in animals and birds are skeletal abnormalities, depressed reproductive function, stillbirths and sterility of mother. The major food high in manganese are nuts, whole cereals and dried fruits[5].

Chromium: Atherosclerosis or hardening of the arteries is a common disease of civilised man. This is a disease of the arteries characterised by fatty deposits under the innermost layer of the arteries. It results in irregularities in arteries and affect the smooth flow of blood. All the tissues need plenty of oxygen carrying blood and when the artery becomes narrow due to atherosclerosis, less blood reaches the part it supplies and in some cases none reaches the parts. That part of the organ can die. The damage caused by this disease depends on the organ affected[9],

viz, if the heart, coronary occlusion or heart attack results; if the brain, a stroke, dizziness, ringing in the ears, loss of speech or loss of vision is certain; if the kidney, high blood pressure results. For several years, high dietary intake of cholesterol was said to be responsible for the disease, but recently, this view has been discarded with as it does not apply to every area of the world. In recent times, chromium deficiency is being proved to be a probable cause of atherosclerosis[9]. Several experiments have been performed on rats to prove this. Unrefined flour and unrefined sugar are known to be a high source of chromium. Chromium is usually lost on refining[9]. Countries where the disease is rare are those where major calories come from refined flours.

Zinc: Zinc deficiency results in retarded growth and anaemia. The best sources of zinc in foods are meat, whole grain cereals and seeds, vegetables and eggs.

Cobalt: Cobalt deficiency in grass could lead to vitamin B_{12} deficiency in the grazing animal. Vitamin B_{12} is necessary for the growth of animals. Cobalt deficiency is reported to be rare in man[9]. Cobalt is widely distributed among foods, hence it is available to man.

1.2.4. The non-Essential trace elements

Any substance which is toxic to reproduction adversely affects

the health of the species. Cadmium, lead and mercury are widespread contaminants. They accumulate in mammalian tissues with age under continous exposure. Pollution with metals arise from industrialisation, refining and the burning of coal and petroleum energy. Metals which go up in the smoke come down with soot or rain and enter the soil and plants.

1.2.4.1. Cadmium

No evidence is available that cadmium is essential for man, rather the effects of an accumulation of this trace element is undesirable. Cadmium accumulates in both human and rat kidney, arteries and liver where it interferes with certain enzyme system requiring zinc. It has a greater affinity for certain kidney tissues than zinc therefore it displaces zinc in some zinc-dependent reactions[9].

From many experiments, a conclusion has been drawn that cadmium in kidneys in relation to zinc is a contributing, if not the whole, cause of high blood pressure. High blood pressure or hypertension is one of the most common chronic diseases of civilised countries[9]. It appears to be linked to environment but not to race, and usually found in people of above 40 years of age.

In areas where the zinc/cadmium ratio is low, the incidence of hypertension is high and vice versa. It was found that injecting some rats with zinc thus removing cadmium, hypertension is cured overnight[9]. Excesses of zinc prevent accumulation of cadmium and

deficiency allows it. The natural sources of cadmium are food, water and air.

Superphosphate fertilisers are rich in cadmium and were believed to be a source of cadmium in vegetables. However, this was proved to be wrong[9]. Refining of foods (e.g. refining wheat) removes zinc and not cadmium, thus resulting in cadmium accumulation. Cadmium enters the biosphere through its increasing use in plastics, in paints as pigments and in cadmium batteries. The amount inhaled from air is insignificant compared with that ingested with the food.

Oysters, kidneys and the edible portion of some fruits and vegetables contain some cadmium.

1.2.4.2. Lead

Lead is one of the most abundant pollutants of the human environment and contaminant of the body. Its principal source is from tetraethyl lead, an anti knock chemical added to petrol and this comes out of the tail pipes of motor cars to pollute the air, food and water[9].

Natural lead occurs in low concentration (10 ppm) in the earth's crust. A grass or plant grown near a well-used road would likely contain so much lead as to poison an animal feeding on it exclusively[9]. Leaves absorb lead from air and rain. The level of airborne lead in cities has been known to be proportional to the consumption of petrol. The only solution to the lead problem

apparently is the removal or severe reduction of lead additives in petrol. Biological interest in lead has centered on the fact that it is a highly toxic cumulative poison in both man and animal[5].

1.2.5 Trace element and the soil

Plants are dependent on the local soil and the soil is ultimately the primary natural source of trace elements in animals and man[5]. Plant materials provide the main source of minerals to animals and to most members of the human race. It is therefore essential to appreciate the factors that control trace element content and availability in soils.

A soil is formed from a parent material derived from a rock. When the nature of the parent material of a soil is known, it is possible to assess the probable contents of the different trace elements. This however gives little information of the amount available in a form capable of being taken up by plants. The extent to which a trace element can be mobilized and stored in an available form is significant. As a result of the weathering of a mineral e.g. ferromagnesia which contain manganese, zinc, cobalt, molybdenum, nickel and lead, these elements become mobilized and available for the plant[10]. Thus one of the factors affecting the availability of the mineral in the soil is the form in which the element is available.[10].

Another factor affecting availability is the soil pH[10].

The uptake of some elements by plants can be modified by changes in soil pH. Such elements most affected include cobalt, nickel, zinc, manganese and molybdenum.

The application of certain fertilisers can induce trace element deficiency e.g. regular addition of nitrogen to some soils can bring about copper deficiency in cereal while overgenerous application of phosphate may give rise to zinc deficiency[10]. Environmental pollution also cannot be excluded as a possible factor.

The plant root draws its supply of nutrients from the soil zone in its immediate neighbourhood.

1.2.6 Factors Affecting The Trace Element Content of Plants

The factors affecting the trace elements in plants are the major determinants of dietary intakes of these elements. The concentration of all minerals in plant depend on:

(i) Genus, species or strain of plant.

(ii) The type of soil on which the plant is grown.

(iii) The climatic or seasonal conditions during growth.

The relative impact of these factors depend on the element and also on the use of fertilisers, irrigation etc[5].

Genetic differences: Some plant species have the ability to accumulate high concentration of particular elements than other species[5]. Even within the same species, different strains could have different accumulating tendencies. When grown on the same soil and at the same stage of maturity, leguminous plants commonly

contain higher concentration of cobalt, nickel, iron copper and zinc than grass or cereals. It has been reported that several species grown on strontium-rich soils in England contained as high as 26,000ppm of strontium while others contained 100 - 200ppm[10].

Soil and fertiliser effects: Introduction of a high concentration of an element into the soil through fertiliser increases the plant availability of the element. The plant responds by either increasing their growth or increasing the concentration of that element in the tissue. The reverse happens when concentration of an element is low in the soil. The extent to which the plant responds varies among different trace elements and different plant species[5]. The soil composition is however the basic determinant of the level of trace elements in plants.

Influence of season and stage of maturity:

Plants mature both in response to internal factors and external factors like climatic and seasonal effects. The concentration of trace elements in a plant may increase, decrease or show no consistent change with the stage of growth depending on the element, plant species and soil or seasonal conditions. Most workers observed a rise in concentration of chromium with advancing maturity but a fall in the level of copper, zinc, cobalt, nickel, molybdenum, iron and manganese[5].

1.3. Crude Protein

Proteins play a crucial role in all biological processes. All enzymes that take part in chemical reactions in biological systems contain proteins. Hence proteins determine the pattern of chemical transformation in cells.

Crude protein is the figure obtained by multiplying the total nitrogen content of the food by 6.25. The factor 6.25 owes its origin to the assumption that proteins from foods and feed contain about 16 percent nitrogen and that all the nitrogen in food is from the protein molecule. These two assumptions are however not entirely valid. Thus the term 'crude' is used to denote the protein so calculated. The proteins of foodstuff vary from 13 - 18 percent so that in many cases a factor other than 6.25 is used for multiplying. In the case of milk, 6.38 is used; 5.40 is used for oil seed protein; 5.9 for cereal protein and 6.6 for plant leaf protein. Varying amounts of nitrogen in a feeding stuff are actually present as the true protein.

C H A P T E R II

EXPERIMENTAL

2.1. Materials: Sample collection, description and preparation.

Most of the vegetables analysed were collected from the Horticulture Department of the Institute of Agricultural Research (IAR) Samaru, attached to the Ahmadu Bello University, Samaru, Zaria. Some of the samples however were collected from Dr. J.Y. Olayemi's backyard garden in area A_1 at the Ahmadu Bello University, Samaru, Zaria. The onion samples were collected from different plots located at different areas between Zaria and Sokoto through the Horticulture Department of IAR. All the samples analysed may therefore be said to have been grown in the northern part of Nigeria. The samples were collected between July and September 1981 and could be said to have been collected during the rainy season.

2.1.1. Sample description

Sample	Description
S_1	Local or English name - Spinach (green)
	Botanical name - *Amaranthus viridis*
	Location - Samaru (area A)

Sample	Description	
S_2	Local or English name	Spinach (purple)
	Botanical name	- Amaranthus tricolor
	Location	- Samaru (area A)
S_3	Local or English name	- Eku (Yoruba)
	Botanical name	- Sesamum radiatum
	Location	- Samaru (area A)
S_4	Local or English name	- Amukan (Yoruba)
	Botanical name	- Hibiscus articulalus
	Location	- Samaru (area A)
S_5	Local or English name	- Ewedu (Yoruba) (striated edge)
	Botanical name	- Corchorus family
	Location	- Samaru (IAR)
S_6	Local or English name	- Bitterleaf
	Botanical name	- Vernonia amygdalina
	Location	- Samaru (area A)
S_7	Local or English name	- Ewedu (Yoruba)(small leaf)
	Botanical name	- Corchorus olitorius
	Location	- Samaru (area A)
S_8	Local or English name	- Ewedu (Yoruba)(long leaf)
	Botanical name	- Corchorus tridens
	Location	- Samaru (area A)

Sample	Description
S_9	Local or English name - Okro(without fertiliser)
	Botanical name - Hibiscus esculentus L
	Location - Samaru (area A)
S_{10}	Local or English name - Okro (with fertiliser)
	Botanical name - Hibiscus esculentus L
	Location - Samaru (area A)
S_{11}	Local or English name - Okro (white velvet)
	Botanical name - Hibiscus esculentus L
	Location - Samaru (IAR)
S_{12}	Local or English name - Cayene long red pepper (Hausa)
	Botanical name - Capsicum annum L.
	Location - Samaru (IAR)
S_{13}	Local or English name - Cubanella tatasai (Hausa)
	Botanical name - Capsicum annum L.
	Location - Samaru (IAR)
S_{14}	Local or English name - Bellboy sweet pepper
	Botanical name - Capsicum annum L.
	Location - Samaru (IAR)
S_{15}	Local or English name - Chilli pepper
	Botanical name - Capsicum frutescens L.
	Location - Samaru (IAR)

S_{16}	Local or English name	-	Gauta yalo (Hausa)
			(Samaru sweet white)
	Botanical name	-	Solanum gilo
	Location	-	Samaru (IAR)
S_{17}	Local or English name	-	Gauta yalo (Hausa)
			(Long stripped type.)
	Botanical name	-	Solanum gilo
	Location	-	Samaru (IAR)
S_{18}	Local or English name	-	Gauta yalo (Hausa)
			(green round type)
	Botanical name	-	Solanum gilo
	Location	-	Samaru (IAR)
S_{19}	Local or English name	-	Gauta yalo (Hausa)
			(large green type)
	Botanical name	-	Solanum melongena
S_{20}	Local or English name	-	Gauta dashi (Hausa)
	Botanical name	-	Solanum melongena
	Location	-	Samaru (IAR)
S_{21}	Local or English name	-	Onion
			(small white type)
	Botanical name	-	Allium sativum
	Location	-	Samaru (area A)

Sample			Description
S_{22}	Local or English name	-	Onion
	Botanical name	-	Allium cepa L.
	Location	-	W80/B7 Plot 119 TY (IAR)
S_{23}	Local or English name	-	Onion
	Botanical name	-	Allium cepa L.
	Location	-	Comp 2 Plot 116 TVIC (III) (IAR).
S_{24}	Local or English name	-	Onion
	Botanical name	-	Allium cepa L
	Location	-	Ex Worno Plot 113 TL 5 (IAR)
S_{25}	Local or English name	-	Onion
	Botanical name	-	Allium cepa L
	Location	-	Samaru (IAR)
S_{26}	Local or English name	-	Onion
	Botanical name	-	Allium cepa L
	Location	-	Samaru (IAR)

2.1.2 Sample preparation

The primary aim of preparing a sample for analysis is to ensure homogeneity. In this way the sample is considered to be a representative of a defined whole. The fresh samples were

washed thoroughly in distilled water and dried. When it was necessary, as was in the case of onions, the skin was removed and the onion cut into pieces. Samples were taken from this preparation to determine the moisture content. The remainder of each sample was dried in an air oven at about 60°C as recommended by Oyenuga[23]. Drying was completed within 4 - 5 days. The dried samples were ground to powder in a waring blender and stored in air tight screw-capped bottles. These bottles were kept in a dessicator until required for use.

2.2. Determination of moisture content

The moisture content determination is important and is widely used in processing and testing of food products. The moisture content of foods is somewhat a measure of the ability of foods to store well. It has been reported that foods containing more than fifteen percent moisture cannot be stored in bulk[14]. Several methods are available for the determination of moisture in foods. These include the reflux distillation [14] of the food with an immiscible solvent having a higher boiling point and a lower specific gravity than water e.g. toluene, heptane. Other methods include the electrical method and the Karl Fischer method[14].

The method generally used for determination of moisture in plant materials is measurement of the loss in weight of a sample

as a result of oven drying at a temperature just over 100°C. For many biological samples, such drying result in loss of volatile oils and some sugars decompose above 70°C. Drying at lower temperatures until a constant weight is reached is often used. Suitable drying temperatures and time are predetermined to obtain a constant dry weight of the sample.

The result of proximate analysis are usually reported in terms of the dry weight of the sample.

2.2.1. Method

Wet samples which had not been heated at any stage were accurately weighed in clean, dry petridishes. They were heated at 60°C in the air oven to a constant weight.

The loss in weight of each sample was determined and the moisture content calculated using the formula:

$$\text{Moisture content (\%)} = \frac{\text{loss in weight on drying(g)} \times 100}{\text{sample weight (g)}}$$

This determination was carried out in duplicates.

2.3. Determination of Crude Protein

Crude protein is obtained by multiplying the nitrogen content of the food by a factor 6.25. For the estimation of the total nitrogen in biological materials, Kjeldahl method is used. The sample is digested with sulphuric acid containing potassium or sodium sulphate to raise the digestion temperature.

A catalyst (e.g. selenium) is required. The digestion stage converts organic nitrogen to ammonium nitrogen. The classical method for estimating ammonium nitrogen is by distillation. In this method, free ammonia is liberated from the diluted digest by steam distillation in the presence of excess alkali. The distillate is collected in a receiver containing excess boric acid combined with an indicator. The ammonia is then titrated with standard hydrochloric acid. This method though it is reproducible and invaluable for routine analysis, has its limitations. The method does not measure protein directly but uses an emperical factor to convert nitrogen to protein. It thus assumes that all the nitrogen is from protein . This assumption is inaccurate although the results from it might be meaningful.

Materials

(i) Concentrated sulphuric acid (sp.gr 1.84)

(ii) Boric acid solution (2%): prepared by dissolving 20g of boric acid in water and diluting to $1dm^3$.

(iii) Sodium hydroxide solution (40%): prepared by dissolving 400g of sodium hydroxide pellets in $1dm^3$ of distilled water.

(iv) Hydrochloric acid, 0.02M, standardized.

(v) Kjeldahl mixed catalyst tablet.

(vi) Mixed indicator solution: prepared by dissolving 0.20g of methyl and 0.1g of methylene blue in ethanol and making it up to $100cm^3$.

(vii) Glass beads.

Method

(a) Digestion : About 0.2g of the sample was accurately weighed into a clean, dry 50cm^3 Kjeldahl flask. One tablet of the mixed catalyst was added. 6cm^3 of concentrated sulphuric acid and a glass bead were also added. The mixture was heated slowly at first, then more strongly until a clear colourless solution was obtained. It was allowed to cool and the solution quantitatively transferred to a 100cm^3 volumetric flask and made up to mark with distilled water.

(b) Distillation: 10cm^3 of the above solution was transferred to the micro Kjeldahl distillation flask. 10cm^3 of 40% sodium hydroxide solution was added to the digest and the ammonia liberated was steam distilled into 10cm^3 boric acid containing 2 drops of the mixed indicator.

(c) Titration : The ammonia collected in the boric acid was titrated with 0.01M hydrochloric acid.

A blank was run using the same amount of reagents.

Calculation

1 cm^3 of 0.01M acid neutralised by the ammoniacal mixture corresponds to 0.00014g of nitrogen.

i.e. 1 cm^3 of 1M acid $\equiv$ 0.014g of nitrogen.

Let the titre value be Fcm^3 of 0.01M hydrochloric acid.

$\therefore$ F cm^3 of 1M acid $\equiv$ 0.014xg of nitrogen.

Let the molarity of HCL used = M

Weight of sample = W

$$\text{Total nitrogen (\%)} = 10 \times \frac{F \times M \times 0.014 \times 100}{W}$$

$$\text{Crude protein (\%)} = 10 \times \frac{F \times M \times 1.4}{W} \times 6.25$$

2.4. Preliminary investigation of methods of digestion

The decomposition and dissolution of a biological material for the determination of the trace metals (elements) is an important part of any chemical analysis done on it. No single method has been found which is equally good for all types of materials[12]. For determination in organic samples, the element desired must be separated and recovered quantitatively without introducing any contamination. It must also be in an easily measurable form. A lot of information is available on the various methods for the destruction of organic matter and preparation of biological materials for trace metal analysis. Much of these information from the literature are conflicting as each worker describes the method that has proved to be most satisfactory in his laboratory. Middleton and Stuckey[15], in one of their papers, presented a critical review of the various methods of digestion and proposed a general method for biological materials. It has however been reported[11] that though reasonable results were obtained for most of the trace metals using this method, the recoveries

were said to be completely lost when this method was used. The method has also been proved to be tedious and time consuming.

Moss[18] indicates that the type of sample under investigation is of major importance in its preparation for spectrophotometric analysis and suggests that the easiest and simplest method of attack is the best one. He however concluded that wet ashing was the best for biological materials. A worker in one of his papers reported that dry ashing procedure is most successful in his laboratory. Heanes[16] in his article wrote that using either potassium bisulphate or nitric acid or sulphuric acids as ashing aids, (dry ashing method) the rate of oxidation and extraction of trace metals were improved. He reported that in the absence of chemical aids, some dark ashes were observed which indicated that the ashing was imcomplete for these samples. The concentration of the trace elements were also found to be lower than was anticipated. The accuracy of his method compared very well with that of a standard wet digestion method.

A study of the recovery of trace metals in organic matter was made by Gorsuch[17]. Using the radiochemical techniques to study both wet and dry oxidation, specific information on many elements were obtained. It was observed that the dry ashing method was affected by temperature, the sample and by the trace elements determined while the wet digestion was less affected. It was concluded that no simple procedure could be postulated for

the determination of the trace elements. However, the wet oxidation using nitric acid and perchloric acid was found to give the best recovery of trace metals in samples of cocoa.

From the above, it would be seen that, to accurately determine the level of trace metals in a biological material or in a group or type of biological materials, it would be necessary to try a series of digestion methods and from the recoveries the best method is employed for that group. In this study, wet digestion has been employed. This has certain advantages over the dry ashing method. The advantages include:

(i) Non-volatilization of metallic elements or phosphorus.

(ii) A larger number of nutrient elements can be determined in one digest solution.

(iii) Lower working temperatures are used.

(iv) The maintenance of liquid conditions which reduces the chances of retention of trace elements on solid material[6].

(v) Simple apparatus is required.

(vi) A faster rate of oxidation[6].

The dry ashing method also has some advantages over the wet ashing method.

These include :

(a) No reagents are needed. Consequently the risk of increased blank values is eliminated.

(ii) Larger samples can be handled easily[17].

(iii) The operation is simple.

Six methods of wet digestion were tried on two classes of vegetables so as to determine the best method of digestion which would give the maximum reproducible recovery of the elements to be determined. The vegetables used were: spinach, representing the class of leafy vegetables; and okra, for the class of fruity vegetables.

The procedures for the digestions are as follows.

2.4.1 Method A[21].

About one gram of the dried material was accurately weighed into a 100cm^3 Kjeldahl flask and to it was added 16cm^3 of concentrated nitric acid (sp.gr 1.42). The flasks were allowed to stand for one minute to allow any vigorous reaction to take place and subside. The mixture in flask was then digested. The samples were allowed to reflux gently and when the digestion was completed,the solution was allowed to cool. It was then quantitatively transferred to a 50cm^3 volumetric flask and diluted to the mark. Three more digestions were carried out as described. A blank was also prepared using the same amount of reagents. The absorbance readings were read on an SP 1900 Pye Unicam Atomic Absorption Spectrophotometer.

2.4.2. Method B[19].

About one gram of the dried sample was accurately weighed into a 100cm^3 Kjeldahl flask and to it was added 13cm^3 of concentrated nitric acid (sp. gr 1.42). The mixture was boiled slowly for 30 minutes. It was allowed to cool and 7.5cm^3 of perchloric acid (62% w/w) was added and the solution boiled very gently until it was colourless or nearly so, and dense white fumes appeared. When the digestion was completed, the mixture was allowed to cool and 5cm^3 of deionised water was added, followed by warming. The mixture was quantitatively transferred to a 50cm^3 volumetric flask and diluted to volume. Three more digestions were carried out as described. A blank was also prepared using the same amount of reagents. The absorbance readings were read on an SP 1900 Pye Unicam Atomic Absorption Spectrophotometer.

2.4.3. Method C[17].

About one gram of the dried sample was accurately weighed into a 100cm^3 Kjeldahl flask and 13cm^3 of concentrated nitric acid (sp. gr. 1.42) was added. The mixture was boiled slowly for 30 minutes and allowed to cool. 7.5cm^3 of perchloric acid (62% w/w) was added and the solution boiled gently until it was colourless or nearly so, and dense white fumes appeared in the flask. This latter boiling took nearly an hour. The contents

of the flask were not allowed to get dry. The digest was quantitatively transferred to a 50cm^3 volumetric flask and diluted to volume. Replicates were prepared. A blank was also prepared using the same amount of reagents. The absorbance readings were read on an SP 1900 Pye Unicam Atomic Absorption Spectrophotometer.

2.4.4 Method D[14]

About one gram of the homogenised dried sample was accurately weighed into a 100cm^3 Kjeldahl flask and to it was added 10cm^3 of concentrated sulphuric acid (Sp. gr. 1.18), followed by a rigorous shaking to ensure that no dry lumps remained. 5cm^3 of concentrated nitric acid (Sp. gr. 1.42) was added and mixed. The mixture was heated cautiously until the initial vigorous reaction had subsided. It was heated more strongly until most of the nitrous fumes were removed and white fumes of sulphuric acid evolved. The digest was quantitatively transferred to a 50cm^3 volumetric flask and made up to mark. Replicates were prepared. A blank was also prepared using the same amount of reagents. The absorbance readings were read on an SP 1900 Pye Unicam Atomic Absorption Spectrophotometer.

2.4.5. Method E[22].

About one gram of the dried sample was accurately weighed into a 100cm^3 Kjeldahl flask. 10cm^3 of concentrated nitric acid (sp. gr. 1.42) was added to soak the sample thoroughly. 3cm^3 of perchloric acid (62% w/w) was added and the mixture heated slowly at first until frothing ceased. It was heated more strongly until the nitric acid was almost evaporated. Whenever charring occured, more concentrated nitric acid was added and heating continued until white fumes of perchloric acid were observed. The mixture was cooled and 10cm^3 of (1 + 1) HCl/water mixture was added. 10cm^3 of water was added followed by warming. The mixture was quantitatively transferred to a 50cm^3 volumetric flask and made up to mark. Replicates were prepared. A blank was also prepared using the same amount of reagents. The absorbance readings were read on an SP 1900 Pye Unicam Atomic Absorption Spectrophotometer.

2.4.6. Method F[19]

About one gram of the dried sample was accurately weighed and transferred to a 100cm^3 Kjeldahl flask and to it was added 10cm^3 of concentrated nitric acid (sp.gr. 1.42). The flask was gently warmed until solution was complete and 10cm^3 of oxidising agent (a solution of 50g of ammonium nitrate and 25g of nitric acid (sp. gr. 1.42), made up to 100cm^3 with deionised water)

was added. It was heated gently to expel water so that oxidation (indicated by effervescence) proceeded in a melt of ammonium nitrate. From time to time, more reagent was added ($5cm^3$ of oxidising reagent) until no browning of the solution was observed and a clear melt was obtained. When oxidation was complete, the clear melt was heated more strongly to volatilize the excess of ammonium nitrate. The flask was held over a free flame to expel salt subliming on the side. The residue was dissolved in $2cm^3$ of concentrated hydrocloric acid (Sp.gr. 1.18) and evaporated to dryness. The residue was fused in the flask so as to remove all nitric acid. It was dissolved in about $10cm^3$ of 1M HCl and evaporated to a small bulk to ensure conversion of any metaphosphate to orthophosphate. It was evaporated to dryness and the residue was dissolved in a few millilitres of warm water and a few drops of 1M HCl. It was diluted to $50cm^3$ with deionised water. Replicates were prepared. A blank was also prepared using the same amount of reagents. The absorbance readings were read on an SP 1900 Pye Unicam Atomic Absorption Spectrophotometer.

2.4.7 Observation

The observations are presented in tables.

Table 2.1

Spinach

Digestion Method

Observation	A	B	C	D	E	F
Speed	Fast	very fast	very fast	Fast	very fast	very slow
Condition of Experiment	Safe	Safe	Safe	Safe	Safe	Safe
Mixture after digestion	White powder remained, small in quantity, insoluble in acid, alkali, alcohol and acetone, insoluble on warming.	White beautiful crystals, soluble on adding water and warming. Small amount of white powder remained. Insoluble in available reagents.	Solution with some crystalline particles.	White powder. After some days, more white precipitate obtained. Insoluble in available reagents.	White crystalline solid, soluble on adding water and warming. White powder insoluble in available reagents remained.	Brown precipitate, soluble in 1M hydrochlorid acid.

Table 2.2

Okra

Digestion Methods

Observation	A	B	C	D	E	F
Speed	Fast	Fast	Fast	Fast	Fast	Very slow
Mixture after digestion	Clear, colourless solution.	White crystals. Soluble on adding water and warming. Colourless solution.	White crystals. Colourless solution.	Clear, colourless solution.	White crystals. Soluble on adding water and warming. Colourless solution.	Brown precipitate. Insoluble in 1M HCl.

Table 2.3. Chemistry of the methods.

Digestion Method	Chemistry
A	Nitric acid oxidised the organic part of the sample and the inorganic part formed soluble nitrates and remained in solution.
B	Treatment with a mixture of nitric acid and perchlorates destroyed the organic part of the material and formed soluble nitrates of the metals. Some crystalline perchlorates were also formed which dissolved on addition of water and subsequent warming.
C	Treatment with a mixture of nitric acid and perchloric acid destroyed the organic part of the material and formed soluble nitrates of the metals. Some crystalline perchlorates were also formed.
D	Treatment with a mixture of nitric acid and sulphuric acid destroyed the organic part of the material and formed soluble nitrates of the metals. In the presence of alkaline earth metals e.g. magnesium and calcium, insoluble

D	sulphates could be formed. The insoluble sulphates might absorb a considerable proportion of trace metals, particularly lead. This would reduce the recovery of lead and other adsorbed metals in the digest.
E	Treatment with a mixture of nitric acid and perchloric acid destroyed the organic part of the material. Insoluble perchlorates were formed which on adding water and warming dissolved. In the presence of hydrochloric acid, all the iron adsorbed on the white powder (in spinach and suspected to be silica) were returned to the solution as ferric chloride[13].
F	The solution of the mixture of ammonium nitrate and nitric acid destroyed the organic part of the sample. Excess of this solution was removed by volatilization. Addition of hydrochloric acid gave soluble ions.

2.4.8 Results and Discussion

The results from the six methods of wet digestion used are discussed. Blank corrections were made for each of the methods.

Interference of iron from chromium was eliminated by following the procedure described in section 2.5.1. Calibration curves were plotted for each of the elements by following the procedures described in section 2.5.3 - 2.5.10. The absorbance readings were made to reflect one gram of the dry weight of sample and from the calibration curves, the concentrations were read off. Table 2.4 shows the results obtained for Amaranthus tricolor (spinach). This is sample two in the analysis.

The best digestion method for spinach was determined on the following basis:

(i) Must be highly reproducible.

(ii) Should have the highest mean for all the values obtained from the different methods.

(iii) Should have the lowest value for reagent blank.

Using these criteria, all the six methods were considered suitable for the determination of manganese (Mn); A,B,C and E for iron (Fe); E for lead (Pb); A and E for cobalt (Co); C,D,E and F for chromium (Cr); A for cadmium (Cd); A,E and F for copper (Cu); A, E and F for zinc (Zn).

For the ability to estimate the eight elements reliably, a digestion method is scored eight points as a maximum. Table 2.5 summarises the scores for the six digestion methods employed for estimating manganese, iron, lead, cobalt, chromium, cadmium, copper and zinc in spinach sample.

TABLE 2.4

Digestion Methods (concentration in ppm)

Trace Element	Determinations	A	B	C	D	E	F
Mn	I	0.74	0.77	0.72	0.65	0.72	0.75
	II	0.75	0.72	0.71	0.68	0.69	0.77
	III	0.67	0.74	0.72	0.65	0.72	0.75
Fe	I	8.36	7.50	8.57	6.79	8.14	6.29
	II	8.14	6.86	7.36	7.57	7.79	4.07
	III	8.71	7.86	7.45	6.90	7.90	6.10
Pb	I	0.25	0.25	0.25	.0.00	0.92	0.17
	II	0.49	0.33	0.25	.0.00	0.25	0.17
	III	0.42	0.17	0.25	.0.00	0.75	0.17
Co	I	0.25	0.17	0.17	0.00	0.17	0.00
	II	0.25	0.25	0.08	0.17	0.17	0.00
	III	0.33	0.08	0.12	0.00	0.17	0.00
Cr	I	0.00	0.00	0.06	0.06	0.06	0.06
	II	0.00	0.00	0.06	0.06	0.06	0.06
	III	0.00	0.00	0.06	0.06	0.06	0.06
Cd	I	0.05	0.01	0.00	0.00	0.00	0.00
	II	0.03	0.00	0.00	0.00	0.00	0.00
	III	0.00	0.00	0.00	0.00	0.00	0.00

Table 2.4 (Contd)

Trace Element	Determinations	Digestion methods (Concentration in ppm)					
		A	B	C	D	E	F
Cu	I	0.28	0.13	0.08	0.12	0.15	0.20
	II	0.17	0.10	0.08	0.12	0.17	0.23
	III	0.17	0.13	0.08	0.12	0.17	0.12
Zn	I	1.47	0.27	1.45	0.70	1.39	1.44
	II	1.55	0.64	0.34	0.89	1.50	1.67
	III	2.69	0.29	0.99	0.79	1.45	1.45

Table 2.5

Digestion method	A	B	C	D	E	F
Score	6	2	3	2	7	3

On the basis of the scores, method E was considered most suitable for spinach. The method is fast and gives little precipitate after warming. The method was considered to be the most suitable for studying trace metals in other leafy vegetables.

Method A was also quite good and would make a useful alternative. The blank values for A and E were also lowest. This meant that the reagents used here were the purest.

Table 2.6 shows the results for Hibiscus esculentus L.(okra). This is sample nine in the analysis.

Using the same criteria as for spinach, the score chart was prepared and is shown as Table 2.7.

Table 2.7

Digestion method	A	B	C	D	E	F
Score	4	6	5	6	4	4

From the score chart, methods B and D are considered most suitable for the digestion of okra and possibly all fruity vegetables for the purpose of trace element determination. Method B was however used because of the fear of precipitation of lead in the present of sulphuric acid and because it is faster than D.

Table 2.6

Digestion Methods(Concentration in ppm)

Trace Element	Determi-nations	A	B	C	D	E	F
Mn	I	0.14	0.18	0.15	0.17	0.14	0.14
	II	0.15	0.15	0.17	0.12	0.17	0.15
	III	0.17	0.16	0.15	0.15	0.18	0.15
Fe	I	0.57	2.50	0.93	0.14	1.07	2.29
	II	0.50	1.50	0.71	0.00	0.79	2.57
	III	0.50	2.29	0.71	0.00	1.21	2.34
Pb	I	0.17	0.17	0.17	0.42	0.08	0.08
	II	0.17	0.25	0.25	0.42	0.00	0.00
	III	0.25	0.17	0.17	0.42	0.08	0.00
Co	I	0.00	0.00	0.00	0.00	0.00	0.00
	II	0.00	0.00	0.00	0.00	0.00	0.00
	III	0.00	0.00	0.00	0.00	0.00	0.00
Cr	I	0.11	0.00	0.00	0.00	0.00	0.78
	II	0.22	0.00	0.00	0.00	0.00	0.00
	III	0.00	0.00	0.00	0.00	0.00	0.00
Cd	I	0.00	0.00	0.00	0.00	0.00	0.18
	II	0.01	0.00	0.00	0.00	0.00	0.00
	III	0.00	0.00	0.00	0.00	0.00	0.00

Table 2.6 (Contd)

Trace Element	Determi-nations	Digestion methods (Concentration in ppm)					
		A	B	C	D	E	F
Cu	I	0.00	0.00	0.07	0.12	0.03	0.00
	II	0.00	0.07	0.03	0.00	0.00	0.00
	III	0.00	0.00	0.00	0.00	0.00	0.00
Zn	I	0.00	0.75	0.60	0.84	0.00	0.97
	II	0.00	0.72	0.58	0.81	0.00	0.53
	III	0.00	0.72	0.65	0.82	0.00	0.70

2.5. Trace metal determination

For the preparation of sample solutions for elemental analysis, an oxidation process is necessary for the destruction of the organic matter. The elements desired must be obtained in a concentrated form and separated from as many sources of interferences as possible. For routine analysis, it is preferable to prepare the solution in such a way that several elements can be determined on it. The oxidation is usually by the action of acids if wet oxidation is used.

The next objective after the digestion of sample is the determination of the individual elements. In this study, the elements of interest are iron, manganese, copper, zinc, cadmium, lead, chromium and cobalt. These can be determined using various techniques including electrochemical techniques (polarography, coulometry, conductometry, potentiometry, amperometry), atomic spectroscopy (emission, absorption, flourescence), colorimetric/ spectrophotometric, chromatographic, complexometric, volumetric and gravimetric techniques. Of these, the atomic absorption spectrophotometry was used because it has a history of simple and reliable application to food analysis. It is also capable of determining minute quantities of element.

2.5.1. Atomic Absorption Spectrophotometer

This is an analytical method for the determination of elements

based on the absorption by free atoms. If a solution containing a metallic salt is aspirated into a flame, (e.g. air-acetylene flame) a vapour which contains atoms of the metal may be formed. If light of the resonance wavelength is passed through a ffame containing these atoms, part of the light would be absorbed and the extent of absorption will be proportional to the number of these atoms present in the flame. This is the underlying principle of atomic absorption spectrophotometry. The hollow cathode lamps emit rays of wavelength specific to the elements they are made of. The relationship between the absorbance A and the concentration of the element which produces this absorbance is known as Beer's law. It is represented by the formula.

$A = \log \frac{I_o}{I_t} = \epsilon c l$ where I_0 - intensity of incident light

C - Concentration; I_t - intensity of transmitted light.

l - Path length; ϵ - molar absorptivity

A - absorbance

2.5.2. Sample preparation

Two methods of wet digestion were used. Digestion method E described in section 2.4 was used for S_1 - S_8 while method B was used for S_9 - S_{26}.

2.5.2.1 Digestion method E

Materials

(i) Concentrated mitric acid (sp. gr. 1.42).

(ii) Perchloric acid 60-62% w/w.

(iii) Concentrated hydrochloric acid (sp. gr. 1.18)

(iv) Deionised water.

Method; as described in section 2.4.

2.5.2.2. Digestion method B

Materials

(i) Concentrated nitric acid (sp. gr. 1.42)

(ii) Perchloric acid 60 - 62 % w/w

(iii) Deionised water.

Method ; as described in section 2.4

2.5.3 Iron

Iron was determined with the Pye Unicam Atomic Absorption Spectrophotometer (Unicam SP 1900 with digital readout) at 248.3nm. The air-acetylene flame was used. No interferance by other metals was observed.

Materials

(i) Hydrochloric acid, 3M: prepared by diluting 93cm^3 of concentrated hydrochloric acid (sp. gr. 1.18) to 1dm^3 with deionised water.

(ii) Stock iron solution: This was prepared by dissolving

4.84g of ferric chloride hexahydrate ($FeCl_3 . 6H_2O$) analytical grade in 3M hydrochloric acid and diluting to $1dm^3$ with deionised water. This solution contained 1000mg dm^{-3} of iron.

(iii) Working standard solution: $10cm^3$ of the stock solution was diluted to $100cm^3$ with deionised water to give a solution containing 100mg dm^{-3} of iron ions. 0,1.0, 2.0, 3.0, 4.0, $5.0cm^3$ of this solution were pipetted into separate $100cm^3$ volumetric flasks and the volumes made up with deionised water. These gave solutions containing 0, 1.0,2.0,3.0,4.0,5.0mg dm^{-3} (ppm) of iron respectively.

Method

A calibration curve was constructed from the absorbances of the working standard solution. The absorbances of the sample solutions were also measured. In some cases, the sample solutions were diluted so that their absorbance readings could fall within the limit of detection of iron at 248.3nm by the instrument. The concentrations of these solutions were read off from the calibration curve.

2.5.4 Manganese

Atomic absorption spectrophotometry is recommended and was used because of its sensitivity and convenience. Manganese is relatively free from interference. It is essential that the standard and sample solution contain manganese in the same valency state. This can be achieved by the mixed acid digestion method.

Materials

(i) Hydrochloric acid; prepared as in section 2.5.3.

(ii) Stock manganese solution: This was prepared by dissolving 4.06g of manganous sulphate $MnSO_4.4H_2O$ (analytical grade) in hydrochloric acid and diluting to $1dm^3$ with deionised water.

(iii) Working standard solution: $10cm^3$ of the stock solution was diluted to $100cm^3$ with deionised water. 0, 0.5, 1.0, 1.5, 2.0, 2.5, $3.0cm^3$ of this solution were pipetted into separate $100cm^3$ volumetric flasks and the volume made up with deionised water.

Method

Manganese was determined at 279.5nm. A calibration curve was constructed and the concentration of the sample read off.

2.5.5. Lead

Materials

(i) Hydrochloric acid: as in section 2.5.3.

(ii) Stock lead solution: This was prepared by dissolving 1.598g of lead nitrate $Pb(NO_3)_2$ (analytical grade) in 3M hydrochloric acid and diluting to $1dm^3$ with deionised water.

(iii) Working standard solution: $10cm^3$ of the stock solution was diluted to $100cm^3$ with deionised water. 0, 1.0, 2.0, 4.0, 6.0, 8.0, $10.0cm^3$ of the solution was pipetted into separate $100cm^3$ volumetric flasks and the volume made

up with deionised water.

Method

Lead was determined at 213.9nm. The concentrations of the sample were read off from the calibration curves. It was not certain if the presence of iron actually increased the sensitivity of determining lead as was claimed[26].

2.5.6 Cobalt

Materials

(i) Hydrochloric acid: prepared as in section 2.5.3.

(ii) Stock cobalt solution: This was prepared by dissolving 4.9379g of cobaltous nitrate $Co(NO_3)_2 . 6H_2O$ (analytical grade) in 3M hydrochloric acid and diluting to $1dm^3$ with deionised water.

(iii) Working standard solution: $10cm^3$ of the stock solution was diluted to $100cm^3$ with deionised water to give a solution containing 100mg dm^{-3} of cobaltous ions. 0, 0.5, 1.0, 2.0, 3.0 4.0, $5.0cm^3$ of this solution was pipetted into separate $100cm^3$ volumetric flask and made up to the mark.

Method

Cobalt was determined at 241.0nm. The concentrations of the sample solutions were read from the calibration curve.

2.5.7 Chromium

A literature on the analysis of chromium by atomic absorption spectrophotometry reported that the factors controlling the formation of chromium atoms in flames are complex. It has been claimed that in air-acetylene flames, sensitivity for chromium is dependent on the stoichiometry of the flame[27]. The sensitivity is expected to be highest in rich flames (i.e. almost luminous), thus rich flames are recommended for the chromium analysis despite the knowledge that the extent of interference is also higher in these flames. Interference, as reported, could be eliminated by the addition of ammonium chloride or lanthanum chloride to both the sample and reference solutions.

Materials

(i) Hydrochloric acid: as in section 2.5.3.

(ii) Stock chromium solution: This was prepared by dissolving 3.735g of potassium chromate K_2CrO_4 (analytical grade) in hydrochloric acid and diluting to $1dm^3$ with deionised water.

(iii) Working standard solution: $10cm^3$ of the stock solution was diluted to $100cm^3$. 0, 0.5, 1.0, 2.0, 3.0, 4.0, $5.0cm^3$ of this solution was pipetted into separate $100cm^3$ volumetric flasks and made up to mark with deionised water.

Method

Chromium was determined at 358.0nm. The flame was adjusted so that it was near luminous (yellow flame). Lanthanum chloride

(5%) was added to all experimental solutions to eliminate interference by iron. Calibration graphs were constructed under the same modified conditions, and the concentration of the sample solution was read off the curves.

2.5.8. Cadmium

Materials

(i) Hydrochloric acid: prepared as in section 2.5.3.

(ii) Stock cadmium solution: This was prepared by dissolving 1.1423g of cadmium oxide CdO (analytical grade) in hydrochloric acid and made up to $1dm^3$ with deionised water,

(iii) Working standard solution: $10cm^3$ of the stock solution was diluted to $100cm^3$. 0, 0.2, 0.4, 0.5, 1.0, 1.5, $2.0cm^3$ of this solution was pippetted into separate $100cm^3$ volumetric flasks and made up to mark with deionised water.

Method

Cadmium was determined at 229.0nm.

2.5.9 Copper

Materials

(i) Hydrochloric acid: as in section 2.5.3.

(ii) Stock copper solution: This was prepared by dissolving 3.798g of cupric nitrate $Cu(NO_3)_2 . 3H_2O$ (analytical grade in hydrochloric acid and made up to $1dm^3$ with

deionised water.

(iii) Working standard solution: $10cm^3$ of the stock solution was diluted to $100cm^3$. 0, 0.5, 1.0, 2.0, 3.0, 4.0, $5.0cm^3$ of this solution was pipetted into separate $100cm^3$ volumetric flasks and made up to mark with deionised water.

Method

Copper was determined at 324.8 nm. The concentrations of the sample solutions were read from the calibration curves.

2.5.10. Zinc

Atomic absorption procedure is suitable for zinc and is preferred to other techniques. It was used here. Sensitivity is good and the method is virtually free from interference .

Materials

(i) Hydrochloric acid: as in section 2.5.3.

(ii) Stock zinc solution: This was prepared by dissolving accurately weighed 1g of zinc powder in hydrochloric acid and making it up to $1dm^3$ with deionised water.

(iii) Working standard solution: $10cm^3$ of the stock solution diluted to $100cm^3$. 0, 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, $1.0cm^3$ of this solution was pipetted into separate $100cm^3$ volumetric flasks and made up to mark with deionised water.

Method

Zinc was determined at 213.9nm. The concentration of the sample solutions were read from the calibration curves.

For all the metals determined, working standard solutions were prepared fresh, and the absorbances read together with that of the samples. This corrected for variations in pressure of gas, flame conditions and air pressure.

CHAPTER III

RESULTS AND DISCUSSION

For the purpose of discussion, the samples have been divided into five groups. GI, samples S_1 - S_8, for leafy vegetables; GII, samples S_9 - S_{11}, the okra group; GIII, samples S_{12} - S_{15}, the pepper group; GIV, samples S_{16} - S_{20}, the garden egg (yalo) group while GV, samples S_{21} - S_{26} represents the onion group.

3.1. Moisture Content

The results of the moisture content determination of the samples are shown in table 3.1. The table reflects each of the groups. The values of the moisture content of the leafy vegetables range between 74.81 percent to 90.38 percent with a mean value of 87.02 percent. The range in GII is between 89.88 percent and 90.50 percent, with a mean of 90.11 percent. Moisture content values in GIII ranges between 55.26 percent and 87.03 percent, with a mean of 77.48 percent. In GIV, the values range between 78.43 percent and 90.63 percent, with a mean value of 86.90 percent, while that in GV ranges between 85.37 percent and 92.36 percent,with a mean of 89.12 percent. Considerable variations are noticed within members of each group. Samples S_1 and S_2 though are in the same family but different species, were grown under the same climate and soil conditions, They have different moisture contents. This might be due to the differences in their ability to absorb water from the soil. With the exception of S_6, all the

samples in GpI were collected from the same source. It is difficult to explain the unusually low value of the moisture content of S_5 here. There is no appreciable difference in the values of the water content of Gp II members. Also, all the samples in Gp III were grown under the same soil and climatic conditions. With the exception of S_{15}, there is no appreciable difference in their moisture content. S_{15} is of a different species from the rest of the group. The remaining three, though have different shapes and sizes, are all called sweet pepper and are the same species. S_{15} is called 'hot pepper'. In Gp IV, water content for S_{20} is comparatively different from the rest of the group. In this group, the first three vegetable samples are all the same species while the last two are of the same species. Gp V is the onion group. S_{21} has the lowest moisture value. S_{21} is an imported species, while the others are the same local species grown at different locations. This might account for the differences in the values of moisture content. Of all the samples studied, S_{15} has the lowest moisture content while S_{24} has the highest. It has been reported[30] that many factors affect the moisture content of materials; these include climate and soil. In the light of this known variability, it is not possible to predict the exact value of the moisture content of any of the vegetables. The individual values and ranges of values could be accepted only as a general guide. The overall range in moisture content is

Table 3.1 Moisture content and protein content of the vegetable samples

Sample	(%) Moisture and S.D	(%) Protein of dry weight and S.D.
GI		
S_1	87.78 ± 0.21	32.60 ± 0.26
S_2	90.38 ± 0.40	31.31 ± 0.04
S_3	85.67 ± 0.30	31.75 ± 0.13
S_4	90.26 ± 0.15	24.77 ± 0.54
S_5	74.81 ± 0.11	29.42 ± 0.64
S_6	89.05 ± 0.27	34.58 ± 0.62
S_7	88.48 ± 0.04	26.49 ± 0.19
S_8	89.74 ± 0.14	23.84 ± 0.46
GII		
S_9	89.94 ± 0.13	17.15 ± 0.41
S_{10}	89.88 ± 0.02	16.51 ± 0.31
S_{11}	90.50 ± 0.28	18.51 ± 0.08
GIII		
S_{12}	83.40 ± 0.43	16.02 ± 0.25
S_{13}	87.03 ± 0.27	17.11 ± 0.14
S_{14}	84.23 ± 0.14	19.06 ± 0.20
S_{15}	55.26 ± 0.34	13.81 ± 0.40

GIV		
S_{16}	88.99 ± 0.09	18.68 ± 0.30
S_{17}	89.38 ± 0.30	18.45 ± 0.11
S_{18}	87.08 ± 0.51	20.23 ± 0.37
S_{19}	90.63 ± 0.14	21.28 ± 0.07
S_{20}	78.43 ± 0.23	16.20 ± 0.07
GV		
S_{21}	85.37 ± 0.06	11.38 ± 0.07
S_{22}	87.92 ± 0.45	11.52 ± 0.30
S_{23}	89.76 ± 0.29	11.99 ± 0.06
S_{24}	92.36 ± 0.27	9.54 ± 0.12
S_{25}	90.67 ± 0.27	10.18 ± 0.65
S_{26}	88.64 ± 0.42	11.54 ± 0.14

N = 3 for moisture content determination

N = 5 for protein content determination

Some literature values for the moisture of vegetables from GI to GV.

Onion 81 -93% ; Pepper[30] 70 - 93%

Garden egg (yalo) 82.1% ; Okra[33] 89.2%

Spinach[33] 86.6% ; Spinach[30] 91 - 93%

Spinach[32] 84.8% ; Bitterleaf 83.6%

Some literature values for the percentage protein of the dry vegetables in GI - GV.

		Multiplying factors
Green leafy vegetables[31]	19 - 31	6.25
Spinach[32]	34.32	6.60
Bitterleaf[32]	34.98	6.60
Onion[30]	5.63 - 13.75	6.25
Pepper[30]	7.50 - 12.50	6.25

between 55.26 percent and 92.36 percent. It has been reported from literature that most vegetables contain a lot of water. Some literature values of moisture contents of some vegetables are given below the tables. It would be seen that most of the values agree with literature, though literature values may themselves have a wide range.

The moisture content is frequently regarded as an index of stability and quality of the foodstuff. It is also a measure of the yield of food solids. It is known that materials with a high moisture content has low keeping quality. The analysed vegetables have a high moisture content and so would only keep well in a refrigerator.

3.2 Protein content

The values for the protein content of the samples analysed are given in table 3.1. The range for Gp I is between 24.77 percent to 34.58 percent, with a mean of 29.35 percent. For Gp II, the range is between 16.51 percent and 18.51 percent, with a mean of 17.39 percent. The values for Gp III range between 13.81 percent and 19.06 percent, with a mean of 16.50 percent. For Gp IV members, the value ranges between 16.20 percent and 21.28 percent, with a mean of 18.97 percent, while for Gp V, it ranges between 9.54 percent and 11.99 percent, with a mean of 11.03 percent. In Gp I, bitterleaf and the two types of spinach have

the highest protein value, while sample S_4 and S_8 have the lowest values. It would be recalled that the samples in this group, with the exception of S_1 and S_2 which have about the same protein content are all of different species, so the variations in their protein values are expected. There are no drastic differences in the protein values of members of GII. However, it is noteworthy that S_9 and S_{10} are the same species, with fertilizer applied to S_{10}, while none was applied to S_9, grown around the same location. The differences in the protein and other factors studied might be traceable to the differences in the conditions of management. The higher value of S_{11} might be due to species differences. There are slight differences in the protein content of members of Gp III, with the exception of S_{15} which is the only different species in this group. Species differences are expected to account for the different value for S_{15} from those of other members of GIII. The values for GIV exhibit some variations. Onion group has quite low protein values compared to values for members of GI - GIV. Protein values compare very well for members of GV. It has been reported[30] that the protein content of a plant is dependent on the fertiliser applied. The literature values are shown below the table. If the literature value of green leafy vegetable is multiplied by 6.6 (this is the value used in this analysis) the range would be 20.06 - 32.74 percent. Most of the values with the exception of S_6 fall within this range.

Another literature value for S_6, however, agrees with the value obtained. The values obtained for GV also fall within that from literature. Higher values than that in literature were obtained for GIII. This might be due to differences in climatic conditions under which the plants were managed. Of all groups, the leafy vegetables, GI, have the highest crude protein content. This is followed by the garden egg, GIV. Okra and pepper groups, GII and GIII respectively are comparable, while the onions have the lowest protein contents.

The importance of protein is very well known. The role of easily available leaf protein has for long been overlooked. From this study, it can be realised that the dry matter of leaves contain reasonable amounts of crude protein. The amount of digestible proteins in leafy plants would be of interest especially as compared to the proteins in legumes.

3.3. Trace Element

The provision of minerals is one of the most important contributions which vegetables make to human growth. It has been reported[30] that there are considerable differences in the mineral content between species and varieties of plants and also between different batches of the same variety grown under different environmental conditions. Many factors, including climate, soil and fertiliser also contribute to the differences expected and sometimes observed in the contents of particular minerals in

plant products.

3.3.1 Iron content

The iron content of the samples analysed are given in table 3.2. The range of values in GI is between (11.00 and 44.00)mg/100g, with a mean of 23.67mg/100g. For GII, the range is (5.90 to 12.13)mg/100g, with a mean of 9.46 mg/100g and for GIII, it is (6.39 to 1888.88)mg/100g with a mean of 480.07 mg/100g.
For members of GIV, iron content ranges between (3.90 and 8.60) mg/100g with a mean of 5.8mg/100g while for GV it is (2.90 to 5.00) mg/100g with a mean of 3.51mg/100g. There is an appreciable difference in the values for S_1 and S_2, though they are of the same species but different varieties. The reason for this difference could be in the physiological differences in these two varieties. Sample five, S_7 and S_8 are not too different but S_5 has only about half the value of either of the latter two samples. This, again, could be due to differences in variety and soil. It is generally believed that spinach contains the highest amount of iron in the vegetable group. From the result, S_3 contains more iron than either of the two types of spinach.
It is interesting to note that in GII, S_{10} to which fertiliser was applied has a lower iron value than S_9, the same variety, to which no fertiliser was applied. The reason for

this is not known, but might not be unrelated to the composition of the fertiliser. In GIII, the values for the various varieties of sweet pepper S_{12}, S_{13} and S_{14} are low, with an average of 10.47 mg/100g but that for S_{15} (chill pepper) is unusually very high. The reason for this can be attributed mainly to the physiology of the plant. The variations in the iron values for members of GIV could be attributed to the differences in the species and varieties. With the exception of S_{24}, there appears to be no significant difference in iron values obtained for members of GV. From the table, it is obvious that the value for S_{15} is the highest. This, of course, is a new discovery as no previous report has indicated this iron level in pepper grown in Nigeria. Compared with literature values, the leafy vegetables were found to have lower values of iron than those recorded in literature[29] while the onion and pepper have much higher values[30]. The reason could be because of the differences in the soils and climates.

3.3.2. Manganese content

The values for the manganese content of the samples analysed are given in table 3.2. In GI, the range of values is (2.78 to 5.88)mg/100g, with a mean of 4.26mg/100g. For members of GII, the range is between (0.73 and 3.20)mg/100g, with a mean of 1.57 mg/100g. Values for members of GIII range between (1.75 and 5.40)

Table 3.2. Iron, manganese and lead contents of the vegetables samples.

Sample	Iron (mg/100g)of dry weight and S. D.	Manganese (mg/100g) of dry weight and S. D.	Lead(mg/100h) of dry weight and S.D.
GI			
S_1	20.33 ± 0.90	2.83 ± 0.04	1.80 ± 0.00
S_2	41.43 ± 0.52	3.73 ± 0.02	4.33 ± 0.00
S_3	44.00 ± 1.37	4.61 ± 0.16	1.50 ± 0.00
S_4	11.83 ± 0.47	2.78 ± 0.10	1.05 ± 0.00
S_5	11.00 ± 0.28	5.88 ± 0.31	0.75 ± 0.00
S_6	15.50 ± 0.28	5.64 ± 0.12	1.05 ± 0.00
S_7	21.25 ± 1.75	4.53 ± 0.29	0.75 ± 0.05
S_8	23.98 ± 0.44	4.05 ± 0.21	0.75 ± 0.05
GII			
S_9	12.13 ± 0.57	0.79 ± 0.07	0.85 ± 0.00
S_{10}	5.90 ± 0.46	0.73 ± 0.36	1.05 ± 0.05
S_{11}	10.35 ± 0.50	3.20 ± 0.13	0.90 ± 0.15
GIII			
S_{12}	6.39 ± 0.06	2.05 ± 0.05	0.39 ± 0.05
S_{13}	14.56 ± 0.19	1.75 ± 0.01	0.85 ± 0.05

GIII			
S_{14}	10.45 ± 0.52	3.12 ± 0.13	2.37 ± 0.20
S_{15}	1888.88 ± 43,90	5.40 ± 0.04	0.39 ± 0.00
GIV			
S_{16}	8.60 ± 0.28	2.80 ± 0.04	0.75 ± 0.20
S_{17}	3.90 ± 0.03	1.73 ± 0.03	0.00 ± 0.09
S_{18}	4.25 ± 0.10	1.85 ± 0.10	0.50 ± 0.09
S_{19}	8.00 ± 0.31	2.50 ± 0.09	0.00 ± 0.00
S_{20}	4.25 ± 0.14	2.33 ± 0.07	0.39 ± 0.00
GV			
S_{21}	3.23 ± 0.40	0.35 ± 0.10	0.85 ± 0.00
S_{22}	2.90 ± 0.00	1.00 ± 0.00	1.25 ± 0.30
S_{23}	3.25 ± 0.20	1.00 ± 0.00	0.85 ± 0.00
S_{24}	5.00 ± 0.22	1.00 ± 0.01	0.85 ± 0.00
S_{25}	3.30 ± 0.34	1.10 ± 0.13	2.00 ± 0.20
S_{26}	3.35 ± 0.09	1.50 ± 0.01	1.65 ± 0.05

N = 5 for the determinations.

Some literature values for the iron content of vegetables from GI - GV.

Bitterleaf[29] 48.0mg/100g ; Bitterleaf[32] 5.0mg/100g

Corchorus species[29] 74.0mg/100g ; Spinach[32] 4.1mg/100g

Spinach[29] 48.0mg/100g

Onion [30] 0.2-0.5mg/100g

Pepper[30] 0.7 - 1.5mg/100g

Spinach 0.8 - 48.0mg/100g [29,30,32] combined.

Some literature values for the manganese content of vegetables in GI.

Bitterleaf[29] 14,72mg/100g

Spinach[29] 5.25mg/100g

Corchorus species 6.75mg/100g.

No literature values for lead were available.

mg/100g, with a mean of 3.03mg/100g. Members of GIV have manganese values between (1.73 and 2.80)mg/100g, with a mean of 2.42mg/100g, while those of GV have values ranging from (0.35 to 1.50)mg/100g, with a mean of 0.99mg/100g. In GI, the corchorus species and bitterleaf have the highest manganese level, while the Hibiscus articulatus has the lowest value. The differences in values could be due to differences in species. Though S_1 and S_2 are of the same species, they are of different varieties and this could account for the difference in manganese content. The same could apply to S_5, and S_8 which are also of the same species but different varieties. S_5 has the highest value in this group. All the samples, except S_5, were grown in the same area. The differences could be due to the physiology of the plants. The values obtained are all lower than literature values[29]. This could be due to climatic and soil differences. The values obtained for S_9 and S_{10} are close while S_{11} value is comparatively different. These three samples are all one species but S_{11} is a different variety and was grown in a different area. Both of these factors could account for the higher manganese value of S_{11}. Literature values for the manganese content of this group (okra) were not found.

3.3.3. Lead content

The values for the lead content of the vegetable samples are

given in table 3.2. The range of values in GI is (0.75 to 4.33)mg /100g, with a mean of 1.50mg/100g. For GII, the range is (0.85 to 1.05)mg/100g, with a mean of 0.93mg/100g, while for GIII, the range is (0.39 to 2.37)mg/100g with a mean of 1.00mg/100g. Values of lead in members of GIV range from (0.00 to 0.75)mg/100g, with a mean of 0.33mg/100g, while for members of GV, the values range from (0.85 to 2.00)mg/100g, with a mean of 1.24mg/100g. The values for members of GI are varied with S_2 having exceptionally high value, followed by S_1, both of which belong to the same species. Members of GII have values of lead content which are sufficiently close to one another. Sample fourteen in GIII is abnormally high in lead content. The reason for this is not known. There is no appreciable difference in GIV values, while in GV, the only exceptional case is the S_{25} value. The reason for this could be due to the soil. Generally the values for lead on the table are low. This is consistent as lead is not a desirable element. No values of lead were recorded in all the literature consulted.

3.3.4. Cobalt Content

Cobalt content of the samples studied are given in table 3.3. The values for members of GI range from (0.00 to 1.25)mg/100g, with a mean of 0.62mg/100g. For members of GII, the range is (0.00 to 1.25)mg/100g, with a mean of 0.67mg/100g, while for GIII, the range is from (0.00 to 1.44)mg/100g, with a mean of 0.36mg/100g. Cobalt contents of members of GIV have a range between (0.00 and 0.70)mg/100g, with a mean of 0.31mg/100g.

Table 3.3 Cobalt, chromium and cadmium content of the vegetable samples.

Sample	Cobalt (mg/100g) of dry weight and S. D.	Chromium (mg/100g of dry weight and S.D.	Cadmium (mg/100g of dry weight and S.D.
GI			
S_1	1.25 ± 0.00	0.00 ± 0.00	0.20 ± 0.00
S_2	1.25 ± 0.00	0.30 ± 0.00	0.00 ± 0.00
S_3	1.25 ± 0.00	0.00 ± 0.00	0.50 ± 0.00
S_4	0.40 ± 0.00	0.00 ± 0.00	0.20 ± 0.00
S_5	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.00
S_6	0.40 ± 0.05	0.00 ± 0.00	0.50 ± 0.12
S_7	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.16
S_8	0.40 ± 0.05	0.00 ± 0.00	0.50 ± 0.15
GII			
S_9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S_{10}	0.75 ± 0.10	0.00 ± 0.00	0.22 ± 0.03
S_{11}	1.25 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
GIII			
S_{12}	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.05
S_{13}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S_{14}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S_{15}	1.44 ± 0.01	0.87 ± 0.00	0.75 ± 0.06

GIV			
S_{16}	0.70 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
S_{17}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S_{18}	0.25 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
S_{19}	0.60 ± 0.17	0.00 ± 0.00	0.00 ± 0.00
S_{20}	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.00
GV			
S_{21}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S_{22}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S_{23}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S_{24}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S_{25}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S_{26}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

N = 5 for the determinations.

No literature values for cobalt, chromium and cadmium were available.

Cobalt was found to be absent in members of GV.

The variations observed within the groups are expected due to differences in species. The level of cobalt is generally low in all the samples. The level of cobalt content of S_1, S_2, S_3, S_{11} and S_{15} are comparable and significantly greater than those of the other samples. These analyses suggest that cobalt level in vegetables might not be expected to be much higher than 2.0mg/100g dry sample. No values were recorded in the literature consulted.

3.3.5. Chromium Content

Chromium contents of the vegetables are given in table 3.3. With the exception of S_2 and S_{15}, the other vegetables have no chromium. Additionally, the values obtained for S_2 and S_{15} are low. It could be reasonably assumed that the level of chromium is low in most vegetables, and sometimes so low that it could not be reliably estimated by the atomic absorption spectrophotometry, and possibly other conventional analytical methods. No values were recorded in literature.

3.3.6. Cadmium content

Cadmium contents of the vegetable samples are given in table 3.3. Within GI, the range of values is (0.00 to 0.50)mg/100g, with a mean of 0.325mg/100g. For members of GII, the range is (0.00 to 0.22)mg/100g, with a mean of 0.07mg/100g, while for GIII, the

range is (0.00 to 0.75)mg/100g, with a mean of 0.22mg/100g. Cadmium content of members of GIV ranges between (0.00 and 0.08) mg/100g, with a mean of 0.016mg/100g. GV members all have no cadmium. On the whole, the highest value is from S_{15}, and is only 0.75 ± 0.06mg/100g dry weight. The cadmium values are low and this is expected because cadmium in large excess is toxic to the body and might be so to plants also. No literature value for cadmium in vegetables were found.

3.3.7. Copper Content

Table 3.4 gives the copper content of the vegetables analysed. For GI, the range is from (0.65 to 1.35)mg/100g, with a mean of 0.92mg/100g, GII has a range between (0.00 and 1.20)mg/100g, with a mean of 0.60mg/100g. GIII has a range from (0.98 to 2.50)mg/100g, with a mean of 1.46mg/100g. For GIV, the range is (0.72 to 1.35) mg/100g, with a mean of 0.97mg/100g. Members of GV have values between (0.39 to 0.49)mg/100g, with a mean of 0.44mg/100g. With the exception of S_6, there is no appreciable difference in GI values. S_{10} in GII is the sample with fertiliser and its relatively high value of copper might be due to the applied fertiliser. In GII, S_{15} has the highest value which is infact, about twice any of the others. This could be because of the difference in species to the others. In GIV, S_{18} and S_{19} have higher values than the others, but the copper values are generally varied, possibly in a pattern which is dictated by the physiology

of the varieties as they were all from the same area. The values in GV are comparable. S_{15} has the highest value for copper out of all the vegetables analysed. The values of copper obtained from literature are only slightly higher than those from the analysis, except those for onion, which are significantly higher than the literature value.

3.3.8. Zinc content

Zinc contents of the samples analysed are given in table 3.4 In GI, the range of values is between (2.10 to 7.35)mg/100g, with a mean of 3.70mg/100g. For members of GII, zinc content ranges from (3.60 to 7.45)mg/100g, with a mean of 5.43mg/100g, while for GIII, the range is (1.50 to 4.25)mg/100g, with a mean of 2.70mg/100g. The values for members of GIV range between (1.20 and 3.70)mg/100g with a mean of 2.71mg/100g, and for GV, the range of values is from (0.38 to 3.40)mg/100g, with a mean of 1.74mg/100g. In GI, S_1 and S_2 have values much higher than the mean for the group. These two are of the same species. The values of S_4 - S_7 compare with themselves very well. Values of zinc for members of GII are so varied though S_9 and S_{10} are of the same variety. The use of fertilizer for growing S_{10} and S_{11} might be responsible for these glaring differences in zinc content. In GIII, S_{14} has the highest value. The variations in GIII might be due to differences in variety. The variations observed in

Table 3.4 Copper and zinc contents of the vegetable samples.

Sample	Copper (mg/100g)of dry weight a.d S.D.	Zinc (mg/100g) of dry weight and S.D.
GI		
S_1	0.80 ±0.00	5.33 ± 0.25
S_2	0.85 ± 0.00	7.35 ± 0.10
S_3	0.85 ± 0.04	3.25 ± 0.04
S_4	1.65 ± 0.08	2.10 ± 0.10
S_5	0.65 ± 0.08	2.30 ± 0.03
S_6	1.35 ± 0.04	2.15 ± 0.07
S_7	0.90 ± 0.00	2.30 ± 0.16
S_8	0.90 ± 0.05	4.85 ± 0.70
GII		
S_9	0.00 ± 0.00	3.60 ± 0.07
S_{10}	1.20 ± 0.05	5.25 ± 0.14
S_{11}	0.60 ± 0.08	7.45 ± 0.69
GIII		
S_{12}	1.09 ± 0.03	2.27 ± 0.11
S_{13}	1.28 ± 0.04	2.76 ± 0.05
S_{14}	0.98 ± 0.00	4.25 ± 0.13

GIII				
S_{15}	2.50	0.20	1.50	0.20
GIV				
S_{16}	0.85	0.06	3.70	0.10
S_{17}	0.72	0.03	1.20	0.16
S_{18}	1.10	0.18	3.40	0.50
S_{19}	1.35	0.05	3.60	0.20
S_{20}	0.84	0.03	1.67	0.20
GV				
S_{21}	0.39	0.03	3.40	0.09
S_{22}	0.39	0.00	2.80	0.04
S_{23}	0.49	0.00	0.38	0.02
S_{24}	0.49	0.00	0.66	0.10
S_{25}	0.49	0.04	1.50	0.13
S_{26}	0.39	0.00	1.67	0.05

N = 5 for all the determinations

Some literature values for the copper content of vegetables from G_1 and GV

Corchorus species[29]	1.0mg/100g
Bitterleaf[29]	2.75mg/100g

Spinach[29]	1.00mg/100g
Onion[33]	0.08mg/100g

Some literature values for the zinc content of the vegetables from GI

Bitterleaf[29]	9.50mg/100g
Spinach[29]	7.50mg/100g
Corchorus species[29]	7.85mg/100g

GIV could also be due to species and variety differences. In GV, S_{21} gave the highest value. With the exception of S_{21}, all the other samples in this group are of the same species. So the much higher value of S_{21} might not be unexpected. The variation in zinc values from S_{22} - S_{26} might be due to soil and climatic differences, since they were planted on different plots. Sample two and S_{11} have the highest values for zinc. Literature values for spinach and corchorus species are higher than those obtained for these samples. This is also true for bitterleaf. The value for S_2 compares with that reported in literature.

3.3.9. Essential trace elements

The total values of the essential trace elements (Fe, Mn, Co, Cr, Cu, and Zn), analysed were calculated and the results are shown in table 3.5. In GI, the range is from (18.16 to 54.91)mg/100g, with a mean of 33.20mg/100g. For members of GII, the values range from (13.83 to 22.85)mg/100g with a mean of 17.73mg/100g, for GIII, the range is from (11.80 to 1900.59)mg/100g, with a mean of487.89mg/100g. Excluding the value from S_{15}, which has an abnormally high value for Fe, the range would be (11.80 to 20.35)mg/100g, with a mean of 16.98mg/100g. The values for the members of GIV range from (7.55 to 16.65)mg/100g, with a mean of 12.04mg/100g. In GV, the values range from

(5.12 to 7.37)mg/100g, with a mean of 6.67mg/100g. In GI, S_2 and S_3 have the highest values. It would be seen that S_2 has a higher value for the total amount of trace elements than S_1 though they are the same species but different varieties and were grown under the same soil and climatic conditions. This result evidently shows that the physiology of the plant affects the amount of trace elements in the plant. Sample five, S_7 and S_8, it would be recalled, are all one species but different varieties. The values for S_7 and S_8 are comparable, but S_5 is much lower, and in fact, it is about half of S_8. The reason could be attributed to the soil conditions as S_5 was grown in a separate area from the other two. Group I has a wide range for the total essential trace elements analysed. In GII, it would be recalled that only S_{11} was grown in a separate area from the rest. It also has the highest value. It is suprising that S_{10} to which fertiliser was applied has the least amount of trace elements. The reason for this is not known and it is hoped, would be of interest to plant scientists. All the members of GIII, with the exception of S_{15}, are one species but different varieties. The amount of trace elements for S_{13} and S_{14} are comparable but S_{12} is about half of either values. This again could be due to its physiology. S_{15} has the highest amount of essential trace elements. It would be recalled that all the members of GIV are one species, but different varieties. The first three are all

Table 3.5 Zinc/cadmium ratio and other ratios in the vegetable samples.

Sample	(Mn, Fe, Co, Cr, Cu, Zn) mg/100g	[Mn, Fe, Co, Cu, Zn)/(Cd, Pb)]	Zn/Cd
GI			
S_1	30.54	15.27	26.65
S_2	54.91	12.68	> 73.65
S_3	53.96	26.98	6.50
S_4	18.16	14.53	10.50
S_5	19.33	20.87	11.50
S_6	26.04	16.15	4.30
S_7	28.98	23.18	4.60
S_8	34.18	27.34	9.70
GII			
S_9	16.52	19.44	>36.00
S_{10}	13.83	10.89	23.86
S_{11}	22.85	25.39	>74.45
GIII			
S_{12}	11.80	21.85	15.13
S_{13}	20.35	23.94	>27.60
S_{14}	18.80	7.93	>42.50
S_{15}	1900.59	1667.18	2.00

GIV			
S_{16}	16.65	22.20	>37.0
S_{17}	7.55	>75.50	>12.0
S_{18}	10.85	21.70	>34.0
S_{19}	16.05	> 160.5	36,0
S_{20}	9.09	19.34	20.88
GV			
S_{21}	7.37	8.67	34.00
S_{22}	7.09	5.67	28.00
S_{23}	5.12	6.02	> 3.80
S_{24}	7.15	8.41	> 6.60
S_{25}	6.39	3.19	>15.00
S_{26}	6.91	4.19	>16.70

N = 5 for all the determinations.

Some literature values for the $^{Z}n/_{cd}$ ratio in vegetables from GI - GV.

Roots 49

Leaves and fruits 13

one variety but are different in sizes, colours and shapes. The last two are also one variety but different in sizes and shapes. The values for total essential trace elements for GIV do not follow any trend consistent with their varieties. Values for S_{16} and S_{19} are comparable, while those for S_{18} and S_{20} are also close. With the exception of S_{23}, the total amounts of trace elements in GV are comparable.

From the table, it would be seen that S_{15} has the highest total amount of trace elements. The values in GI are also high and much higher than any of the other values with the exception of S_{15}. Generally, the onions have the lowest values of total essential trace elements determined as shown by table 3.5.

3.3.10. The ratio of the essential elements to the non-essential trace elements.

The ratios for the samples are given in table 3.5. The range of ratios in GI is from 12,68 to 27.34 ; in GII, the range is between 10,89 and 25,39. Values in GIII range from 7.93 to 1667.18. In GIV, the ratios are from 19.34 to >160, and in GV, from 3.19 to 8.67. The lowest ratios are from GV. In GI, apart from S_3, the corchorus species S_5, S_7, and S_8 have the highest values. The values of S_1 and S_2 are comparable. In GII, a wide range of values is obtained, with the highest value being from S_{11}, a sample grown in a separate place from the other two, and

also a different variety. In GIII, S_{14} has an exceptionally low value while S_{15} has the highest value. With the exception of S_{17} and S_{19}, values in GIV are comparable. All the values in GIV are low and below 10.

3.3.11. Zinc/Cadmium ratio

The zinc/cadmium ratio of foods is important as it enables one to know what food might be harmful to the body on the basis of the previous report[9]. The zinc/cadmium ratio of the vegetables analysed are given in table 3.5. The range of values in GI is from 4.30 to 73.50. For GII, the range is from 23.86 to 36.00. Group III has a range of 2.00 to 42.50, while GIV and GV have the range of 12.00 to 37.00 and 3.80 to 34.00 respectively. Onion (roots) studied here have values below that from literature. For the leaves and fruits, some values are below and others are above that from literature. Sample two and S_{11}, in particular, have very high zinc/cadmium ratio, while S_6, S_7, S_{23} have very low values. Sample fifteen has an unusually low value of zinc/cadmium ratio.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions.

The results of these analyses show that leafy vegetables contain a significant amount of protein and could be regarded as proteinous foods. It contains about 29.35 percent of protein, though it is not known what proportion of this is digestible. This is comparable to cowpea seed which is known to be high in protein and regarded as a protein food. Cowpea seeds contain about 24.67 percent of crude protein[23]. The major apparent difference in these two classes of foodstuff protein is that vegetables contain about 80 percent water while cowpea seeds contain only 8.7 percent water, so that a larger quantity of vegetables is required. Studies by Schmidt[31] have shown that about 75% of total nitrogen in vegetables is protein nitrogen. The rest is non-protein nitrogen e.g. nitrates and amides. Thus we can assume that the true protein of leafy vegetables is about 22.00 percent; the true protein value for cowpea is reported to be 22.75 percent. Vegetable proteins contain adequate amounts of most essential amino acids[30]. Lack of good quality protein i.e. protein containing adequate and balanced amounts of the various essential amino acids, is certainly a major factor in the incidence of malnutrition. Some vegetables contain only small quantities of protein, nevertheless, they make a useful contribution by providing

supplementary amounts of the commodities supplying the bulk of dietary protein. From the foregoing, it is held that vegetables could be a good substitute for animal proteins in places where such are not available.

As for the trace elements, they can be considered on the basis of their daily minimal requirements for man. The daily minimal requirement of manganese is 0.0243mg/kg body weight. In Nigeria, an average man eats about 10g (wet weight) of leafy vegetables, 10g of okra, 10g of pepper, 7.0g of onion and 20g of tomatoes at a meal of soup. Since these contain about 80 percent water, it is assumed that the dry matter is 11.40g. Calculating from the observed result, the dry matter contains about 0.5mg from the okra, 0.2mg from the pepper and 0.1mg from the onion. This gives a total of about 1.1mg manganese without adding the contributions from tomatoes and the garden egg eaten for refreshment. This therefore shows that vegetables contain enough manganese to meet the daily requirements in man. The daily minimal requirement per kilogramme of body weight (i) of iron in man is about 0.0286 mg, (ii) of chromium, 0.014mg, (iii) of cobalt, less than 1.43×10^{-5} mg, (iv) of copper, 0,0286 mg and, (v) of zinc, 0.0572mg. If similar calculations like that for manganese are carried out for the other e.ements in the meal of vegetable soup; for iron, the calculated result is about 2.90mg per meal without adding the contribution from tomatoes and garden egg; for cobalt

0.157mg per meal if chilli pepper is used and 0.107mg if any of the sweet pepper is used (this does not include cobalt from tomatoes or garden egg). For chromium, about 0.07 mg is taken per meal, using chilli pepper. For copper, about 1.1mg per meal, excluding that from tomatoes or garden egg, and for zinc, 1.4mg per meal is taken. The values given here are approximate and for a meal. In all cases, the contributions from tomatoes and garden egg were not added. This type of meal could be eaten twice a day by a Nigerian. From the foregoing, it is obvious that the vegetables contain enough trace elements to meet the daily requirements in man.

From the results of the zinc/cadmium ratio, it would be seen that most of the vegetables have enough zinc to cover the cadmium they contain. It is not known here what a safe ratio is, but if it is assumed that a safe ratio is 10, then most of the vegetables are above it. Sample fifteen has a conspicuously low ratio. The reason for this is not known. The toxic level of lead is not known but most of the vegetables analysed, with the exception of S_2 and S_{14}, have low lead values. The values for the total amounts of essential trace metals enable us to see which of the vegetables are rich in terms of trace metals. From the table, it would be seen that the leafy vegetables have the highest amount of trace elements with the exception of S_{15}. Group five members have the lowest values. From the whole table, S_{15} gives the highest

value of trace elements.

From these analyses for trace elements, we can conclude that leafy vegetables are major sources of trace elements especially iron. Usually spinach is said to be the richest source of iron, this has been disproved as chilli pepper has a much higher amount.

Two primary requirements for good human nutrition are (i) provision of an adequate total amount of food in terms of calories and energy (ii) the food intake must be balanced so that there is sufficient intake of protein necessary for the building and replacement of tissues and also minerals, without which the body cannot be maintained in a state of good health. Fruit and vegetables meet both these requirements. In the latest world survey prepared by the Food and Agricultural Organisation of the United Nations,[30] it is estimated that about half of the world population still suffers from malnutrition as a result of lack of sufficient supply of essential nutrients.

Recommendations

The value of vegetable and fruits is becoming increasingly appreciated in Nigeria due to scarcity and rising prices, particularly in urban areas. The establishment of a research institute for Horticulture (fruits and vegetables in particular) becomes relevant in this direction, and if well organised and made to perform its functions properly, it should stimulate and optimize the production of these essential food sources and

encourage their utilization, storage and proper distribution in the country.

REFERENCES

1. Nelson, A. Medical Botany. A handbook for medical men and all who are concerned in the use of plants: nutritionists, dieticians, pharmacists and veterinarians. E. & S. Livingstone, Ltd. Edinburgh (1951). pp 3, 31-33, 47-53, 62,63, 65-67.

2. Aykroyd, W.R. Food for man. Pergamon press, Oxford (1969) pp 36 - 37.

3. Vail, G.E.; Phillips, J.A; Rust, L.O.; Grisnold, R.M; Justin, M.M. Foods. Sixth edition (1973) pp. 100 - 118.

4. Herklots, G.A.C. Vegetables in South East Asia. George Allen & Unwin Ltd, London. (1972) pp. 108.

5. Underwood, E.J. Trace elements in Human and Animal nutrition. Fourth edition. Academic press, New York (1977) pp. 1 - 11.

6. Mertz, W and Cornatzer, W.F. Newer trace elements in nutrition Proceedings of the national symposium on the newer trace elements in nutrition held at Grand Forks, North Dakota. Marcel Dekker, INC. New York (1971) pp. 5 - 7.

7. Fleck, H. Introduction to nutrition. Third edition Macmillan New York (1976) pp. 220 - 237.

8. Jacobs, M.B. The chemistry and technology of food and food products. Second edition. Interscience publishers, New York.

(1951) pp. 205 - 209.

9. Schroeder, H.A. Trace elements and nutrition. Some positive and negative aspects. Faber and Faber Limited, London. (1976) pp. 1 - 107.

10. Mitchell, R.L. Trace elements in soils and factors that affect their availability. A paper presented at AAAS symposium in geochemical environment in relation to health and disease. Geological society of America bulletin. 33 (1972) 1069 - 1076.

11. Dalziel, J.M. The Useful plants of west tropical Africa. An appendix to the flora of west tropical Africa. Crown agents for the colonies (1937) pp. 224, 225, 289, 345-347.

12. Kolthoff, I.M; and Elving P.J. Treatise on analytical chemistry. Part I. Theory and Practice. Volume two. Interscience publishers, INC. (1961) pp. 1051 - 1065.

13. Stock, R; and Rice, C.B.F. Chromatographic methods. Third edition, Chapman and Hall Ltd, London. (1974) pp. 37.

14. Osborne, D.R, and Voogt, P. The analysis of nutrients in Food, Academic press, INC, (London) Ltd. (1978) pp. 168.

15. Middleton, G; and Stuckey, R.E. The Analyst 78 (1953) 532.

16. Heanes, D.L. The Analyst 106 (1981) 172 - 181.

17. Gorsuch T.T. The Analyst 84 (1959) 135 - 165.

18. Moss, M.L. in MG. Mellon, Analytical absorption spectroscopy Wiley, New York. 1950, Chapter I cited in Treatise in Analytical chemistry.

19. Sub committee to the Analytical Methods Committee. Metallic impurities in organic matter. The Analyst 85 (1960) 643.

20. Middleton, G; and Stuckey, R.E. The Analyst 79 (1954) 140.

21. Scott, K. The Analyst 105 (1980) 883.

22. A.O.A.C. 15th Edition. 1930 pp. 463.

23. Oyenuga, V.A. Nigeria's foods and feeding stuffs. Their chemistry and nutritive value. Third edition. Ibadan University press. (1968) pp. 4, 5, 8 - 19.

24. Pearson, D. The chemical analysis of Foods. Seventh Edition, Churchill Livingstone, Edinburgh (1976) pp. 6, 9-11.

25. Whiteside, P.J. Atomic absorption data book Pye Unicam Ltd First edition (1975).

26. Price, W.J. Analytical atomic absorption spectrometry. Heyden, London (1972) pp. 45, 46, 56, 58.

27. Aggett, J. ; and O'Brienn. G. The Analyst 106 (1981). 497.

28. Nwogu, V.I. Chemical analysis of some Millet samples. M.Sc. Thesis, Ahmadu Bello University, Zaria. (1981).

29. Oyenuga, V.A.; and Fetuga, B.L. Dietary importance of fruits and vegetables. (Paper submitted at the National seminar on fruits and vegetables held at Ibadan, 13th - 17th October, 1975.) Ibadan, 1976.

30. Duckworth, R. B. Fruits and vegetables. Pergamon press (1966) pp. 1 - 20.

31. Schmidt, D.R. Yield and chemical components of tropical leafy vegetable.species as influenced by environmental variables and plant nutrition. A thesis present to the Graduate School of Cornell University for the degree of Doctor of Philosophy. Anni Arbor, Michigan (1971).

32. Oomen, H. A. ; and Grubben, G.J. Tropical leaf vegetable in human nutrition. Koninklijk Institute voor de Tropen. (1977) pp. 29, 26 - 37.

33. Food and Agricultural Organisation. Food composition tables: Minerals and vitamins for international use. Rome (1954) pp. 10 - 15.

34. Oyejola, ; and Bassir, O. The physiological availability of the iron content of some Nigerian leafy vegetables. Plant foods for man 1 (1975) 177 - 183.

35. Stryer, L. Biochemistry. Second edition. W.H. Freeman and company, San Francisco. (1981) pp. 11, 37.

36. Allen, E.S. ; Grimshaw, H.M.; Parkinson, J.A. ; and Quarmby, C. Chemical analysis of ecological materials. Pergamon press. (1974) pp. 23, 24, 56 - 62.

37. Vogel, A.I, Textbook of quantitative inorganic chemistry. Third edition. Longman press. (1975) pp. 301 - 304.

38. Basset, J, ; Denney, R.C. ; Jefferey, G.H. ; Mendham, J. Vogel textbook of quantitative inorganic analysis. Fourth edition. Longmans and English language book Society. (1978).

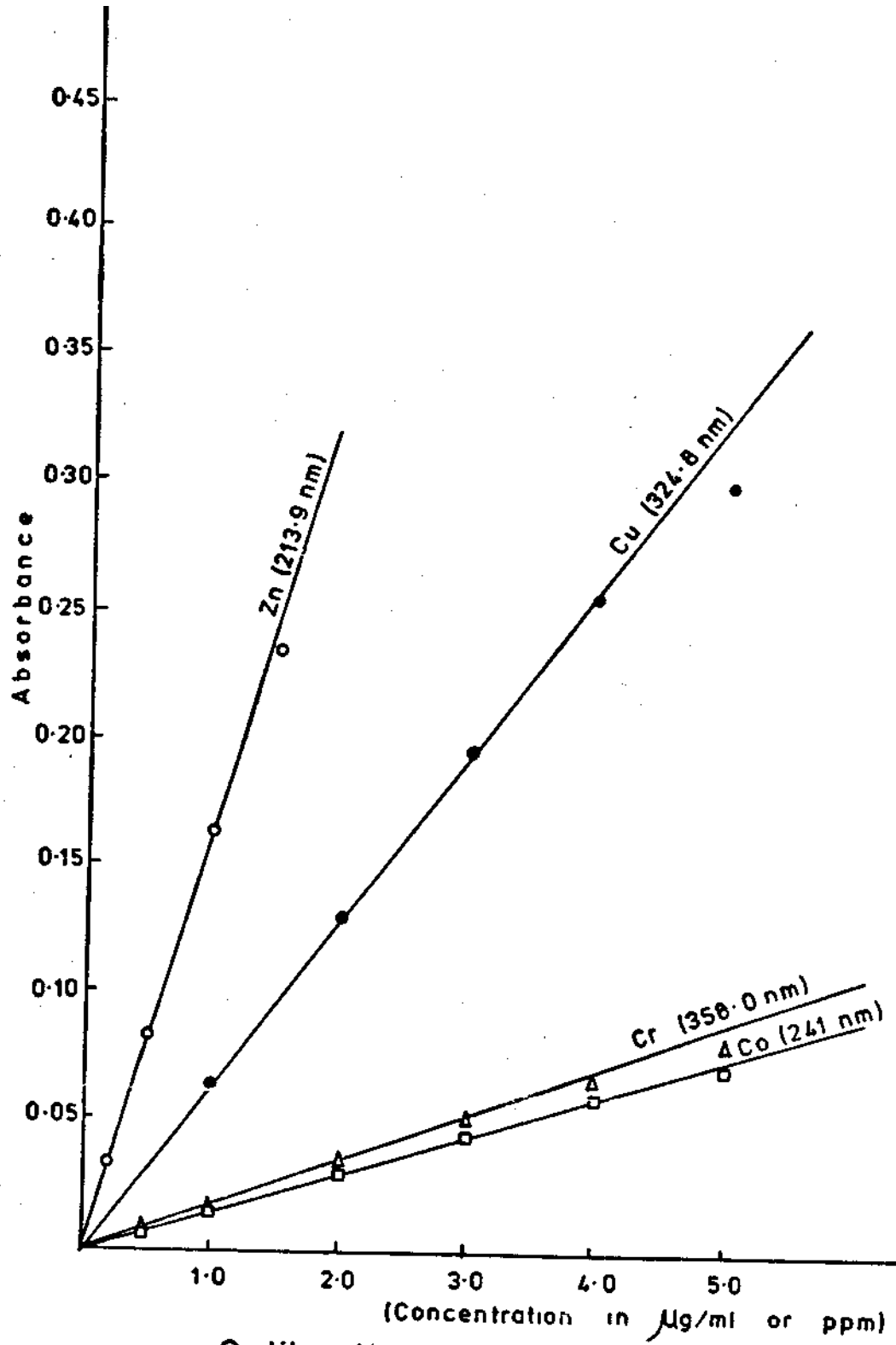
A P P E N D I X

The working range i.e. the range of concentration within which Beer's Law was obeyed using the Pye Unicam SP1900 Atomic Absorption Spectrophotometer (with digital readout) was determined for each of the eight elements analysed. The table shows the working range observed and in some cases that of the literature is also given.

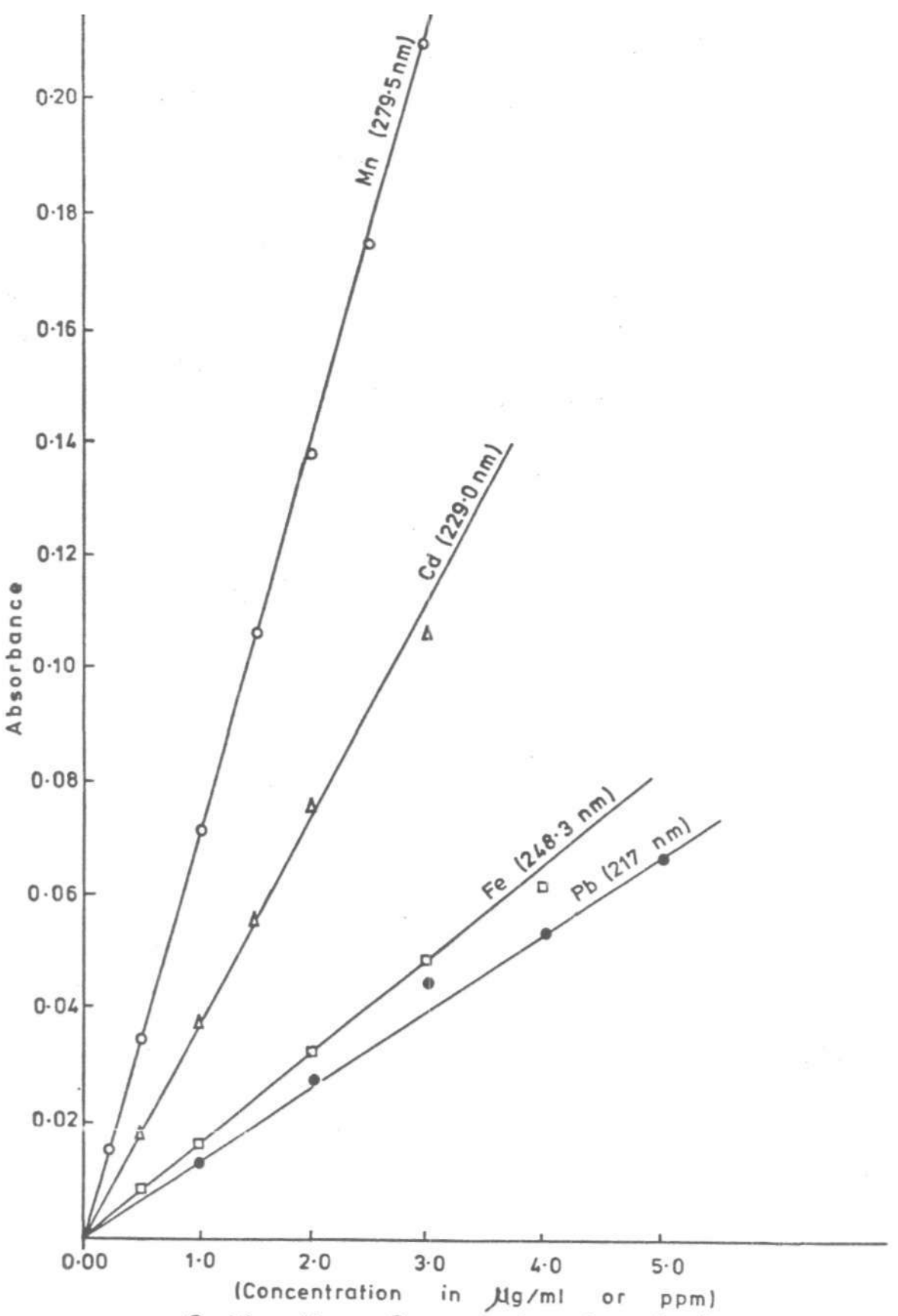
Table

Element	Working range (ug/ml or ppm)	Literature[14] working range (ug/ml or ppm)
Iron	0.00 - 4.00	0.05 - 5.00
Manganese	0.00 - 3.50	0.05 - 5.00 (3)
Lead	0.00 - 10.00	
Cobalt	0.00 - 5.00	
Cadmium	0.00 - 2.50	
Chromium	0.00 - 5.00	
Copper	0.00 - 4.50	0.05 - 5.00
Zinc	0.00 - 1.50	0.10 - 2.00 (1)

Values within the bracket shows the calibration curve is linear up to thøt value.



Calibration Curve for Zinc (Zn)
Copper (Cu) Chromium (Cr) Cobalt (Co)



Calibration Curves for iron(Fe) manganese (Mn) lead (Pb) and Cobalt (Co)