SOME CHEMICAL STUDIES ON TAMARINDUS INPICA

BY

MUINAT MODUPEOLA ISHDLA

A Thesis submitted to the Postgraduate School, Ahmadu Bello University, in partial fulfillment of the requirements for the degree of Master of Science in Chemistry

Department of Chemistry
Faculty of Science
Ahmadu Bello University
Zaria - Nigeria.

November, 1988

DECLARATION

This is to certify that the work reported in this thesis is original and was carried out by myself. It has not been accepted in any previous application for a higher degree, and all information cited from published and unpublished sources are specifically acknowledged in the text by means of references.



M. ISHOLA.

This thesis entitled "Some Chemical Studies on Tamarindus indica" by Muinat M. Ishola meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

Supervisor	16/11/89 Date
Supervisor	16/1/89 Date
Head of Department	Date
Dean, Postgraduate School	9-3-89 Date

DEDICATION.

To my Parents, Brothers and Sisters for their

LOVE,

PATIENCE,

SUPPORT

$ GUIDANCE

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to Dr. E.B. Agbaji and Dr. A.S. Agbaji who supervised this work for their commitment, keen interest, invaluable suggestions, encouragement, and above all for their easy accessibility through out the work. I would like to thank Prof. J.Y. Olayemi and Dr. G.H. Ogbadu, heads of the departments of Chemistry and Biochemistry for making available the research facilities, within the departments, for this work. I also wish to express my appreciation to Dr. J.A. Elegbede and Dr. O. Olakanmi for their useful contributions. I must also express my sincere gratitude to the entire technical staff of chemistry and biochemistry departments for their continued co-operation throughout the work. I am grateful to all members of the teaching staff of chemistry department who have, in various capacities, contributed to the success of this work. I should also thank my colleagues for easing the tension common to research laboratories. My special thanks to A.I. Raji for his efforts and contributions at every stage of this work. I am equally grateful to Dr. and Mrs K. A. Bello.

ABSTRACT

Both the pulp and the seed of the Tamarind fruit were analysed for proximate composition, mineral elements and the antinutritional substances - phytate and trypsin inhibitor.

The protein content of the seed (26.93%), is higher than that of the pulp (8.79%). The seed could be considered as a good source of protein when compared with groundnut (22-27%, Oyenuga, 1968), which is considered a good source of protein. The true protein was determined by both the Lowry and Biuret methods and the values obtained are comparable (33.12% and 35.40% - for seed by Lowry and Biuret methods respectively; and 9.71% and 11.26% - for pulp by Lowry and Biuret methods respectively). The estimated molecular weight of the seed proteins range from 14.8-50.7 x 10^3. The effect of metal ions on the extraction of these proteins was also studied. The pulp was found to be quite acidic with a pH value as low as 2.7 and this is responsible for its soury taste.

The petroleum ether extract (lipid) from the seed and pulp samples were studied in detail. Some of their physico-chemical properties are reported. No detectable amount of phytate was obtained from the pulp though it contains trypsin inhibitor (68% and 82% for water and 0.5M NaCl extracts respectively). The seed samples contain significant amount of phytic acid (47mg/100g) but lower amount of trypsin inhibitor (18% and 28% for water and 0.5M NaCl extracts respectively) than the pulp. The two antinutritional factors were found to be heat labile, and are therefore not likely to constitute a health hazard after adequate heat treatment. The storage ability of the pulp samples were also investigated and discussed.

TABLE OF CONTENTS

	Page
Title Page.	i
Declaration.	ii
Certification.	iii
Dedication.	iv
Acknowledgement.	v
Abstract.	vi
Table of contents.	vii
List of Tables.	xii
List of Figures.	xiii
CHAPTER 1	
INTRODUCTION.	1
1.1 Fruits and Vegetables.	2
1.2 Nutritive value of fruits.	2
1.3 Legumes in nutrition.	4
1.4 The Tamarind fruit tree.	6
1.5 Uses of Tamarind.	8
1.5.1 Nutritional uses.	8
1.5.2 Tamarind in folk medicine.	9
1.5.3 Other uses.	10
1.6 Justification of research.	11

CHAPTER 2		Page
MATERIALS AND METHODS.		13
2.1	Source of samples.	14
2.1.1	Sample preparation.	14
2.2	Moisture and dry matter determination.	14
2.3	Ash determination.	15
2.4	Determination of mineral elements.	16
2.4.1	Sample preparation.	17
2.4.1.1	Dry ashing method.	17
2.4.1.2	Mixed acid digestion method.	18
2.4.2	Preparation of standard solutions.	19
2.4.3	Determination of the elements in sample solution.	21
2.4.4	Phosphorus determination.	21
2.5	Determination of protein content in samples.	23
2.5.1	Determination of nitrogen and crude protein.	23
2.5.2	True protein determination.	24
2.5.2.1	Lowry method.	24
2.5.2.2	The biuret reaction method.	27
2.5.2.3	Lowry method using SDS-urea solvent mixture.	30
2.6	Gel Filtration separation of proteins in Tamarind seed.	32
2.7	Determination of the acidity of the pulp and seed samples.	34
2.7.1	Preparation of sample solutions.	34
2.7.2	Determination of the pH of the sample solutions.	34
2.7.3	Determination of titratable acidity.	35
2.7.4	Determination of volatile acidity.	35

		Page
2.8	Carbohydrate determination.	36
2.8.1	Determination of total carbohydrate.	36
2.8.2	Determination of reducing sugars.	39
2.9	Alcohol-insoluble solids determination.	41
2.10	Determination of water-insoluble solids.	42
2.11	Crude fibre determination.	42
2.12	Determination of crude lipid.	44
2.13	Determination of some properties of the extracted crude lipid.	44
2.13.1	Saponification value.	44
2.13.2	Iodine value.	45
2.13.3	Unsaponifiable matter.	47
2.13.4	Acid value and the free fatty acid.	47
2.13.5	Peroxide value.	48
2.14	Thin layer chromatography (TLC) of the extracted lipids.	49
2.15	Determination of antinutritional factors.	50
2.15.1	Determination of phytic acid level in unprocessed pulp and seed samples.	50
2.15.1.1	Processing.	52
2.15.2	Determination of trypsin inhibitor activity.	52
2.15.2.1	Processing.	55
2.16	Sorption isotherm of Tamarind fruit.	55

CHAPTER 3.		Page
RESULTS AND DISCUSSION		57
3.1	Moisture content.	58
3.2	Ash content and mineral composition.	60
3.3	Protein.	65
3.3.1	Crude protein.	65
3.3.2	True protein.	66
3.3.2.1	Effect of metal ions on the extraction of true protein.	66
3.3.3	Molecular weight determination of the proteins from Tamarind seed.	71
3.4	Acidity of Tamarind fruit.	73
3.5.1	Carbohydrates.	76
3..5.2	Alcohol- and water -insoluble fractions. ..	79
3.5.3	Crude fibre.	80
3.6	Lipids.	81
3.6.1	Some physico-chemical properties of the extracted lipids.	81
3.6.2	Thin layer chromatographic (TLC) separation of the extracted lipids.	86
3.7	Antinutritional factors in Tamarind fruit.	88
3.7.1	Phytic acid.	88
3.7.1.1	Effect of processing on phytic acid level in Tamarind seed.	90
3.7.2	Trypsin inhibitor.	92
3.7.2.1	Effect of heat on antitryptic activities.	94
3.8	Sorption isotherm.	94

Page

3.9 Conclusion and suggestion for further work. 100

References. 102

Appendices. 113

List of Tables

Table		Page
3.1	Proximate composition of the pulp and seed of Tamarind as percent of dry matter.	59
3.2	Mineral content of pulp and seed of Tamarind in mg/100g.	61
3.3	Comparison of assay methods for the estimation of true protein from Tamarind fruit.	67
3.4	Effect of sodium (Na^+) and magnesium (Mg^{2+}) ions on the extraction of protein from Tamarind fruit.	70
3.5	Molecular weight of proteins in Tamarind seed separated by Gel filtration.	74
3.6	Acidity of the pulp and seed of Tamarind fruit.	77
3.7	*Some physico-chemical properties of the lipid extracted* from the pulp and seed of Tamarind fruit.	83
3.8	R_f values of the lipids extracted from both the pulp and seed of Tamarind fruit.	87

List of Figures

Figure		Page
2.1	True protein estimation by Lowry method. Calibration curve of BSA (1mg/cm^3) in water.	26
2.2	True protein estimation by Biuret reaction . Calibration Curve of BSA (10mg/cm^3) in SDS-urea solvent.	29
2.3	True protein estimation by Lowry method. Calibration Curve of BSA (1mg/cm^3) in SDS-urea solvent.	31
2.4	Standard trypsin curve using 2% casein as substrate	54
3.1	Protein extraction from Tamarind seed using sodium chloride and magnesium chloride at different concentrations.	68
3.2	Protein extraction from Tamarind pulp using sodium chloride at different concentrations.	69
3.3	Gel permeation profile of some standard proteins and proteins from Tamarind seeds on sephadex G-75.	72
3.4	Elution volume Vs Log. molecular weight of protein standards separated on sephadex G-75.	75
3.5	Gas chromatogram of methyl esters of the Tamarind seed lipids.	89
3.6	The effect of cooking and soaking on phytate-P. level in Tamarind seed.	91
3.7	Antitryptic activities of Tamarind pulp and seed in both sodium chloride (0.5M) and water extracts.	93

Page

3.8 The effect of heat (waterbath) on the trypsin inhibitor activity in 0.50cm³ of sample extract. 95

3.9 Effect of autoclaving on the trypsin inhibitor activity in 0.50cm³ of sample extract. 96

3.10 Sorption isotherm for Tamarind pulp at 27°C. 98

CHAPTER ONE

INTRODUCTION

KASHIM IBRAHIM LIBRARY
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA.

1.1 Fruits and Vegetables

The word "fruit" botanically means the mature seed-bearing structures of the flowering plants. This definition would however include, the fruits of the grass family which are harvested dry and generally milled into a meal or flour and are commonly known as cereals; Other edible fruits which naturally mature in the dry state and can be stored in this condition for a relatively long time, are the pulses, the nuts and the spice fruits; and lastly, the edible fleshy fruits which inspite of the variety they exhibit, form a useful and well-defined class on their own (Duckworth, 1966). The term is more commonly restricted in its use to those botanical fruits which have fragnant aromatic flavours and are either, naturally sweet or are sweetened with sugar before eating and hence are essentially dessert items in those countries with sophisticated eating habits.

These edible fruits also have much in common, from the culinary point of view, with the soft edible structures developed mainly from other parts of the plant and are popularly referred to as vegetables. Fruits and vegetables have the characteristic feature of being soft edible plant products which, because of their relatively high moisture content, are perishable in the freshly harvested state (Duckworth, 1966).

1.2 Nutritive Value Of Fruits

The nutitive value of plants in general, is a measure of how nearly it can supply the nutrient requirements of the animal. It is therefore necessary to specify the amounts of energy, minerals, vitamins, protein quantity and quality as well as their availability to the animals. Fruits,

vegetables and their products play a very important role in human diet, they supply in the cheapest form, some of the essential nutrients in which other food materials are deficient (Davadas et al 1964, Oyenuga et al, 1975). They constitute an important source of soluble vitamins and mineral elements in addition to being a source of essential amino acids and fatty acids.

The fibrous parts of fruits, consisting largerly of cellulose and hemicellulose, which though cannot be digested in the human body, are still beneficial. They usually serve as roughages which help in accelerating the passage of digested food by stimulating intestinal and bowel movements, thus reducing the cases of intestinal disorders (Wilson et al, 1979).

Most fruits are however poor sources of energy and protein (Chrispeels et al, 1977), the few exceptions include bananas, dates which contain a significant amount of starch and sugar respectively (Brouk, 1975), and avocados pear with calorific value three times that of banana and half that of beefsteak (Wardlaw, 1937). Fruits are generally acidic and play a role in neutralizing excess basic substances produced during digestion of some akaline materials.

Most fleshy fruits are eaten raw and are valued by people because they also provide such a rich variety of flavour, colour and texture in the diet. Hence, they help to raise the process of eating from the level of a mere satisfaction of hunger and of nutritional requirements to that of a pleasurable occupation, delight of which can be enhanced by the application of a well-directed culinary art (Duckworth, 1966).

By virtue of all the aforementioned, fruits and vegetables are the cheapest sources of essential nutrients in most developing countries. Adequate use of these cheap sources cannot however be made if our knowledge of the nutritional values of the fruit-trees, that are largely available in Nigeria (either native or cultivated) is not broadened, since energy and nutritional values of plants as food depend on their chemical make up. These plant sources can also be studied with the view of compounding animal feeds on purely local materials. The presence of toxic substances can however limit the nutritive value of plants and these should also be accorded intensive study when assessing plant materials for food and feed.

1.3 Legumes in Nutrition.

The family leguminosae is one of the largest and most important family in the entire plant kingdom (de Wit, 1966). Leguminosae is further divided into three subfamilies viz: Mimosaceae,

Caesalpiniaceae and

Papilionaceae.

The subfamily papilionaceae is made up of herbs and shrubs with characteristic pea-like flowers which give rise to pods with one or more seeds. Members of this subfamily have often been loosely referred to as legumes mainly because their seeds are used for food which include all true beans. The other two subfamilies are mainly trees and shrubs which contain many plants of great economic importance, supplying for example gums, tannins and some functioning as timber trees (de Wit, 1966).

The legume family include among others, those protein-rich seeds which are also called pulses or food legumes. They can be eaten after minimal preparation such as soaking or boiling (Duffus et al, 1980) and these include peas, kidney beans, lima beans, lentils, chick peas, cow peas, soybeans, peanuts, broadbeans etc. Legumes in 'poor countries' are the most important high-protein food and play the role of meat or other animal proteins common in 'rich countries'. Generally , legumes contain 15-40% protein, 20-50% starch and variable amounts of fat which ranges from 2-3% in beans and peas to 50% in peanuts (Chrispeels et al, 1977).

Dry legumes contribute little vitamin A, riboflavin and ascorbic acid, but their content of thiamine and nicotinic acid is fairly high (Stanton,1966). They contain reasonable amounts of nutritionally important minerals such as calcium and iron. On the whole, legumes contain much more iron, riboflavin, thiamine than in the same amounts of cereals. As a result, legumes have been referred to as "poor man's meat" and on the average, they form a considerable portion of diets in Africa (Stanton, 1966). In underdeveloped countries of Latin America, Africa and Asia, which are also characterized by high rates of population growth, legumes also play an important part in the prevention of ill-health and diseases associated with protein calorie deficiency.

Some plants which are used to make hay or silage for animals, for example clover, alfalfa as well as a large number of trees are also legumes (Chrispeels et al, 1977).

Though legumes are rich and less expensive sources of dietary proteins and contribute substantially to the protein content of the diets of a large

part of the world's population, presence of antinutritional factors is one of the main drawbacks limiting the nutritional qualities of these legumes (Liener, 1976). Some of these antinutritional factors include oxalic acid, phytic acid, saponins and trypsin inhibitors.

They may also, at low concentrations, adversely affect feed intake, palatability and feed conversion while at higher concentrations can result in the death of the animals (Cookerell et al, 1975). Oxalic and phytic acids for example form insoluble salts with mineral elements thus lowering their bioavailability (Erdman, 1979; Eka, 1980). Phytate is also known to inhibit several proteolytic enzymes and amylases (Singh and Krikorian, 1982; Deshpande and Cheryan, 1984). Saponin (triterpene glycosides) impart bitter taste to these plant foods and causes physiological disturbances and toxicity to human system (George, 1965, Oakenfull, 1981). Trypsin inhibitors act by impairing protein digestion as a result of formation of stable complex with digestive enzymes trypsin and chymotrypsin (Baough and Travis, 1976; Duffus et al, 1980).

It is however important to mention that some of these anti-nutritional factors are largely destroyed during the cooking process (Ward et al, 1977; Eka, 1980; Khokhar and Chauhan, 1986).

1.4 The Tamarind Fruit Tree

Tamarindus indica despite its name comes from tropical Africa and not from India (de Wit, 1966). It is known as Tamarind in English; Tamarinier in French; Voamadilo in Madagascar, and Tamrhindi in Arabic. It is also known in Senegal as Dakar which is the origin of the name "Dakar" the

capital city of Senegal (Jardin, 1967). Tamarind belongs to the leguminosae family and to the caesalpiniaceae subfamily (de Wit, 1966).

The caesalpiniaceae subfamily have zygomorphic (bilaterally symmetrically) flowers. All leguminosae have in common, the production of gumes that is, fruits formed from a single carpel, splitting along the rsal and ventral sutures, which usually contain a row of seeds borne on e innerside of the ventral suture as in a pea pod (de Wit, 1966). The esalpiniaceae include mostly woody genera and among them are large trees nstituting an important part of the tropical forest. Many of them are ants of great economic importance, supplying gums, resins and tannins and nctioning as timber trees. They also play a role in soil fertility intenance and anti-erosion measures, and the seeds of a few of them are athered and eaten by human beings and others are of medicinal value anton, 1966).

Tamarindus indica is popularly known as "Tsamiya" by the natives of northern parts of Nigeria amongst whom the fruit is very popular. Though tamarind is a native of Tropical Africa and southern Asia, it is widely cultivated in other regions as an ornamental tree and for its edible fruit (Tirah, 1981). It is also cultivated as a shade tree and for its attractive evergreen appearance and its acidic pulpy fruits.

The tree grows to about 24metres in height; the trunk of a mature tamarind has a diameter of 1.8-2.4metres and has alternate, pinnately compound leaves; with about 20-40cm long. The yellow flowers, which are about 2.5cm across, are borne in small clusters. The fruit is a plump pod of irregular sausage shape of 7.5-24cm long that does not split open and

it contains up to 12 large flat, brown, shiny seeds embedded in a soft, brownish pulp (Tirah, 1981). Flowering occurs two times in a year November-December and May-August, while fruiting takes place around October to December.

1.5 Uses of Tamarind

1.5.1 Nutritional uses

The acidic pulpy fruits of tamarind are widely used for foods, beverages and medicines. Refreshing drinks can be prepared by cold infusion of the pulp with sugar or honey and water, which is then left for some days to mature, this is usually taken as an appetizer. "Fresco de Tamarindo" is a favourite beverage in many Latin America countries and it is bottled commercially (Duke et al, 1981). The pulp is also made into cakes and sold as a delicacy in India and Arabia, while in Nigeria, the fruits are [illegible] are dissolved in water to make a porridge, with or without some cereal, known as "Kunun tsamiya".

In Latin America countries and the Orient, the pulp is used to flavour confections, curries, sweet and sour sauces and in chutneys. It can also be made into brine for pickling fish while in some parts of Africa, they are used to detoxify poisonous Dioscorea dishes and to deodorise fish dishes.

In Nigeria, the testa of the seeds of tamarind fruits is removed by roasting and the seed kernels left are made into "daddawa" - a soup seasoner. Otherwise, they are boiled or fried and then eaten. In India,

once the seeds are boiled and peeled they are eaten as starch foodstuff and also as a source of carbohydrate for sizing cloth, paper and jute products. In some places, the flowers, seedlings and even the leaves are eaten as vegetables in curries. The flowers are also made into a sort of salad called "fate fate" in Nigeria.

1.5.2 Tamarind in folk Medicine.

Uses of tamarind in folk medicine is well recognised in many parts of the world. Laxative properties of the fruit pulp have been recognised and are said to be due to tartaric acid (about 9%, Dalziel, 1948), salts of this acid namely potassium hydrogen tartrate or "cream of tartar" and sodium potasium tartrate (Hamman, 1976). The sour infusion of the pods is used to cook cereal foods and as a drink for women after childbirth. The evaporated pulp extract which is partly soluble when mixed with water is widely used all over the Sudan specifically for fever, but it is also

pulp is used in Eritrea, to cure dysentry and malaria; in Indonesia for worms and stomach disorders; in Mauritius as a liniment for rheumatism; in Kenya for snakebite; in Srilanka for jaundice, eye diseases and ulcers; in Cambodia for conjuctivitis; and in Brazil as a diaphoretic, emollent or purgative medicine and in treatment of haemorrhoids. The astringent seed is used to treat dysentry.

A concoction of the bark is used as astringent for diarrhoea, as a tonic and febrifuge . The cooked bark can also be used as an anti-asthmatic, antiseptic, for apoplexy, hangover, lenitive and leprosy (Duke

et al, 1981). The root is used to cure chest complaints.
A mixture of the leaves of tamarind with those of Baobab Adansonia digitata is used as a prescription in cases of male and female infertility (Owen, 1970).

1.5.3 Other uses of Tamarind

The bark of tamarind is used as a masticatory. Its wood is very hard, durable and resistant to termite attack and hence its use in making tool handles, rice-pounders, oil and sugar mills, furniture and turnery. It is also used to produce the finest grade of gun powder charcoal (Duke, et al, 1981). Tamarind is frequently grown as a dooryard, roadside or windbreak tree. The bark ash and fruit coat (epicarp) are used in tanning goatskin and in a mixture with other preparations, for hides (Odilison, 1985). In damp weather, the leaves of tamarind tree are said to have a corrosive effect on fabrics on which they fall, and this is probably due to the high content of tannin (about 7%), which also affords it, its economic importance in tanning, dyeing and dehairing (Oke, 1966). The twig makes a very good chewing stick and is used in test trial of manhood, "Sharo", a flogging culture among Fulani youths.
The seeds contain some rarely eaten edible oil which is suitable for use in varnishes (Dalziel, 1948). Tamarind is a host to a silkworm called 'Tsamiyan tsamiya' formed in hollows of the tree and the silk can be obtained by boiling the cocoon (Odilison, 1985).

1.6 Justification of Research

Local food sources could go a long way in solving the malnutrition problems of most developing countries, but only if, they are put into effective use. In Nigeria, fruits and vegetables constitute the next major food items consumed after cereals and tubers (Oke, 1972). There are some fruit plants that can thrive in savanna regions which grow wildly and are being used for food in one form or the other. These local sources can be adequately relied upon to provide balanced diets only if, the essential nutrients they contain as well as the presence and level of toxic substances in them are known. A knowledge of the chemical composition of these foods would therefore permit their use with appropriate supplementation where deficiences are found.

Tamarind grows wildly in savanna regions and is most commonly used by the natives of northern Nigeria as vegetable and for its flavour, while some variety of *Tamarindus indica* fruits have been done elsewhere, there has been very little report on the chemical analysis of the whole fruit in Nigeria. This is significant because apart from differences in species, the chemical composition of the same fruits can differ significantly from place to place depending on a number of factors such as climatic condition and soil type. Thus, the aim of this work is to provide some basic chemical data about the tamarind fruit grown in Nigeria.

In this study, the moisture sorption characteristics of tamarind pulp

KASHIM IBRAHIM LIBRARY
BELLO UNIVERSITY

was also investigated with a view of having an insight into its storage ability and probably devising an effective storage condition for this seasonal fruit.

CHAPTER TWO

Materials and Methods

Chemical:

All chemicals were of analytical grade unless otherwise stated.

2.1 Source of samples

Tamarind pods were obtained from different geographical locations in Kano and Zaria towns.

2.2.1 Sample Preparation

The epicarp was removed from each of the pod, and the pulp was carefully separated from the seeds by means of scapels. The pulp and the seed were treated as two diferent samples. All the pulp obtained from pods from the various locations were then properly mixed up for homogeneity and were stored in polythene bags to protect them from dust particles. The seeds were also mixed, cracked and grated into smaller fine particle sizes in a Pifco blender, and this was also stored in polythene bags. The pulp and seed samples were later stored in large glass sample bottles after drying.

2.2 Moisture and Dry Matter Determination

(AOAC, 1980)

Procedure:

Ten grammes each of the pulp and seed sample was accurately weighed into clean, dry and pre-weighed crucibles. The samples were then dried in an air oven set at 70°C, until constant weights were obtained, usually after 48 and 12 hours for the pulp and seed samples respectively. Triplicate determinations were carried out on each sample and the moisture content was represented as the loss in weight of the samples upon drying.

$$\text{Moisture content (\%)} = \frac{100(W_2 - W_3)}{W_2 - W_1}$$

Where,

W_1 = weight of crucible.

W_2 = weight of crucible plus sample before drying.

W_3 = weight of crucible plus sample after drying.

2.3 Determination of Ash.
(AOAC, 1980)

Procedure:

Five grammes of the dried ground sample was accurately weighed into a clean, dry, pre-weighed porcelain crucible which was then heated on a bunsen burner until the smoke ceased. This was then transferred to a muffle furnace set at 525°C, and the sample was left in the furnace for

sample but the pulp sample was not completely ashed. The crucible was then allowed to cool in a desiccator and the ash moistened with distilled water was dried first on steambath and then on a hot plate before returning it to the furnace for re-ashing. This was done twice before the pulp became completely ashed. When ashing was completed, the crucible was cooled in a desiccator and weighed. Triplicate determinations were carried out for each sample.

$$\text{Ash (\%)} = \frac{100(W_3 - W_1)}{(W_2 - W_1)}$$

Where,

W1 = weight of crucible.

W_2 = weight of crucible plus sample.

W_3 = weight of crucible plus ash.

2.4 Determination of Mineral Elements.

The analytical methods for the determination of these elements are based on flame photometry, atomic absorption spectrophotometry and colorimetry. Potassium and sodium were determined by flame photometry, calcium, magnesium, copper, iron, zinc and nickel were determined by atomic absorption spectrophotometry while phosphorous was by colorimetry.

In flame photometry, the atoms of the element are excited by flame and on returning to the ground state, they emit radiations characteristic of the element concerned. The intensity of the radiation emitted is directly proportional to the concentration of the element present. A calibration curve is normally plotted and the concentration of the element in the sample, corresponding to its intensity is read from the curve.

In atomic absorption spectroscopy, a solution of the element of interest is passed into a cool flame where the bulk of the atoms remain in the ground state. Radiation of a characteristic wavelength from a hollow cathode lamp is passed through the flame. The atoms absorb some of this

radiation and become excited. The decrease in intensity of the radiation is then measured using a monochromator and a detector system. The extent of absorption is dependent on the number of atoms in the ground state, and the decrease in intensity is therefore related to the concentration of the element in solution.

2.4.1 Sample Preparation

The mineral elements of the pulp and seed samples were taken into solution by one of the two methods described below.

2.4.1.1 Dry Ashing Method.
(Allen, 1974)

Reagents:

Hydrochloric acid 1:1

Hydrochloric acid concentrated

Nitric acid concentrated.

Procedure:

The ash obtained in section 2.3 was treated with 5cm^3 of concentrated hydrochloric acid and 1cm^3 of concentrated nitric acid to dissolve it. The solution was evaporated to dryness on a waterbath and heating was continued for one hour to dehydrate silica. The residue was then dissolved in 1cm^3 of 1:1 hydrochloric acid and diluted to 10cm^3 with distilled water, warming to complete dissolution. The solution obtained was filtered through a No 44 filter paper into a 50cm^3 volumetric flask and made up to the mark with distilled water. Blank determinations were

carried out in the same way and the sample solutions were prepared in triplicate. Appropriate dilutions of this solution were made for the determination of potassium, sodium, calcium, magnesium, copper, iron, zinc and nickel.

2.4.1.2 Mixed Acid Digestion Method.
(Allen, 1974).

Reagents:

Perchloric acid 60%

Nitric acid concentrated

Sulphuric acid concentrated.

Procedure:

Accurately weighed 0.50g of the dried sample was placed in a 100 cm^3 Kjeldahl flask and to it were added 1cm^3 60% perchloric acid, 5cm^3 nitric acid and 0.5 cm^3 sulphuric acid. The mixture was digested slowly, initially at moderate heat, and later more vigorously for 15 minutes after the appearance of white fumes. The cooled digest was diluted with distilled water before filtering into a 50 cm^3 volumetric flask and made up to the mark with distilled water. Blank determinations were taken through the same process and triplicate digestions were carried out on each sample. This solution was used for the determination of phosphorous.

2.4.2 Preparation of the standard solutions
(Whiteside, 1975)

(i) Sodium Stock Solution (100ppm)

Prepared by dissolving 0.2542g of dry sodium chloride in 1000cm³ distilled water. Working standards contained 0 to 5ppm of sodium.

(ii) Potassium Stock solution (1000ppm)

This was prepareed by dissolving 1.9068g of potassium chloride in distilled water and making up the volume to 1000cm³ with water. Working standards range between 0 to 10ppm.

(iii) Calcium stock solution (1000ppm)

Prepared by dissolving 2.49973g of dry anhydrous calcium carbonate in about 200cm³ of distilled water containing 5cm³ concentrated hydrochloric acid. The mixture was warmed to drive off carbon dioxide gas, cooled and made up to 1000cm³ with distilled water. A working range of standard solutions of concentrations between 0 to 10ppm calcium were prepared. However sufficient stock Lanthanum chloride was added to these working standards to bring the concentration of Lanthanum to 400ppm in each of them. Lanthanum helps to remove interferences from other elements such as aluminium, phosphorous, silicon and sulphur. Sufficient quantities of the stock Lanthanum, were also added to the sample and blank solutions and appropriate quantity of 10% sulphuric acid were then added to produce a final acid concentration of 1% (v/v).

The Lanthanum stock solution (5000ppm) was prepared by dissolving 6.6837g of Lanthanum chloride ($LaCl_3 .7H_2O$) in distilled water containing 1cm³ of 2M hydrochloric acid and the solution was made up to 500cm³ with

distilled water.

(iv) Magnesium stock solution (1000ppm)

This was prepared by dissolving 3.9106g magnesium chloride in 200cm^3 distilled water and diluting the solution to 1000cm^3. Working standards contained 0 to 3ppm magnesium. Sufficient stock Lanthanum (5000ppm) were added to the standard, sample and blank solutions to obtain 400ppm in each. Appropriate quantity of 10% sulphuric acid were also added to the sample and blank solutions to obtain an acid concentration of 1% (v/v).

(v) Copper stock solution (1000ppm)

Prepared by dissolving 0.3930g copper sulphate in distilled water and making up the solution to 1000cm^3. Working concentrations were between 0 to 10ppm.

(vi) Zinc stock solution (1000ppm).

Prepared by dissolving 1.0g of zinc metal in 30cm^3 5M hydrochloric acid and making up to 1000cm^3 with distilled water. Working standards ranged between 0 to 1ppm.

(vii) Iron stock solution (1000ppm).

This was prepared by disolving 7.0213g of iron (II) ammonium sulphate in 200cm^3 distilled water and making it up to 1000cm^3. Working standards of concentration range between 0 to 20ppm were prepared.

(viii) Nickel stock solution (1000ppm).

This was prepared by dissolving 4.0503g of nickel chloride in 100cm^3 of distilled water and making up to 1000cm^3. Working standard concentrations range between 0 to 10ppm.

2.4.4 Determination of the Elements in sample solutions

Procedure:

The various instrumental conditions necessary for maximum out-put of the instrument used were adjusted as recommended for each element (Whiteside, 1975). The absorbance readings of the sample solutions were subsequently taken under the same conditions as the standards. The amount of the element present in the sample solutions (Cppm) were read off the calibration curves (appendix A-H). The amount of each element present in the sample is calculated from the expression;

$$\text{Element (mg/100g)} = \frac{C(ppm) \times \text{sample volume } (cm^3)}{10 \times \text{weight of sample (g)}}$$

2.4.5 Phosphorous Determination
(Allen, 1974)

Reagents:

(i) Phosphorus standards.

A 100ppm stock solution ($1cm^3 \equiv 0.1$ mg P) was prepared by dissolving 0.4393g dry potassium di-hydrogen orthophosphate in distilled water and diluting to $1000cm^3$. An intermediate working standard ($1cm^3 \equiv$ 0.002mgP) was freshly prepared when required, from the stock solution.

(ii) Ammonium molybdate - suphuric acid reagent.

Ammonium molybdate (25.0g) was dissolved in about $200cm^3$ water in a beaker and warmed slightly, to dissolve. $280cm^3$ of concentrated sulphuric acid was added to about $400cm^3$ distilled water and the molybdate solution was then filtered into this acid solution, with thorough mixing. The solution was made up to $1000cm^3$ after cooling and stored in the dark.

(iii) Stannous Chloride reagent.

Stannous chloride (0.50g) was dissolved in 250cm³ 2% (v/v) hydrochloric acid. This reagent was always prepared fresh before use.

Procedure:

Working standards containing 0 to 0.03mg phosphorous were prepared in 50cm³ volumetric flask. Suitable aliquots of the sample solution were also pipetted into 50cm³ volumetric flasks and these flasks were filled to two-third capacity with water, 2cm³ ammonium molybdate reagent was added to each and mixed followed by 2cm³ stannous Chloride reagent. The flask contents were mixed, made up to volume and the absorbance readings were taken after 30 minutes at 700nm using water as reference. A calibration curve was plotted using the standard solutions (appendix 1). The amount of phosphorus in the sample was read off from the graph and the mg/100g calculated as below:

$$\text{Phosphorus (mg/100g)} = \frac{C(mg) \times \text{solution volume } (cm^3) \times 100}{\text{Aliquot } (cm^3) \times \text{sample weight (g)}}$$

Where,

C(mg) = amount of phosphorus in milligram obtained from the calibration curve. Factors for dilution were applied where necessary.

2.5 Determination of protein content in samples

2.5.1 Determination of Nitrogen and crude protein
(Pearson, 1976)

Reagents:

(i) Mixed catalyst.

This contained anhydrous sodium sulphate, copper sulphate and selenium dioxide in the ratio 96.0:3.5:0.5 (w/w).

(ii) Screened Methyl red indicator.

This was prepared by dissolving 0.016g of methyl red and 0.083g of bromocresol green in 100cm³ of ethanol

(iii) Boric acid indicator (2%)

Twenty grammes of very pure boric acid was weighed and dissolved in 200cm³ ethanol by vigorous shaking. 10cm³ of the screened methyl red indicator was then added to this alcohol solution and the volume made up to [illegible]

(iv) Sodium hydroxide solution, 50%

(v) Hydrochloric acid solution, 0.1M

(vi) Concentrated Sulphuric acid.

Procedure:

Accurately weighed quantity (1.50g) of the dried sample was placed in a Kjeldahl flask, and to it were added 8.0g of the mixed catalyst, 20cm³ of concentrated sulphuric acid and some anti-bumping granules. The mixture was then gently heated until the initial frothing had ceased, after which

it was heated strongly until the digest became colourless. The digest was then quantitatively transferred into a 500cm³ volumetric flask, made up to the mark and mixed. This solution was made alkaline with at least 75cm³ 50% sodium hydroxide solution and distilled into 50cm³ of boric acid indicator solution which trapped the liberated ammonia. The ammonia was then titrated with 0.1M hydrochloric acid to a reddish brown end point. Triplicate determinations were caried out on each sample, blank determinations were also carried out using all reagents in the same quantities but without the sample. Percent total nitrogen of sample was calculated as:

$$\frac{A \times 0.0014 \times V_T \times 100}{\text{Weight of sample (g)} \times V_A}$$

Where,

A = volume of 0.1M hydrochloric acid used for titration.

V_T = volume of digested sample.

V_A = volume of distillate titrated.

Percent crude protein was then obtained by multiplyiing the percentage nitrogen by a conversion factor of 6.25.

2.5.2 True Protein Determination

2.5.2.1 Lowry Method (1951)

Reagents:

(i) Solution A: 2% sodium carbonate in 0.10M sodium hydroxide

solution.

(ii) Solution B: 0.5% copper sulphate in 1% sodium-potassium tartrate solution.

(iii) Solution C: This solution was prepared just before use by mixing 50cm³ of solution A with 1cm³ of solution B.

(iv) Folin-ciocalteu reagent:

The commercial reagent was diluted in the ratio 1:2 with distilled water just before use.

(v) Standard stock solution of Bovine serum albumin (BSA) of concentration 1mg/cm³. This solution was also prepared immediately before use.

(vi) Sodium chloride solutions of concentration range of 1% to 20%.

(vii) Magnesium chloride solutions also of concentration range of 1% to 20%.

Procedure:

Calibration curve:

A stock solution containing 1mg/cm³ bovine serum albumin (BSA) was prepared in distilled water and from this were prepared 1cm³ portions of solutions containing 0 to 1.0mg BSA. 5cm³ of solution C was added to each of these solutions which were then left to stand for 15minutes at room temperature. After this time, 0.5cm³ of Folin - ciocalteu reagent was added while stirring and the absorbance readings of these solutions were taken after 30minutes at 700nm wavelength with water as the reference. A standard curve is shown in Figure 2.1.

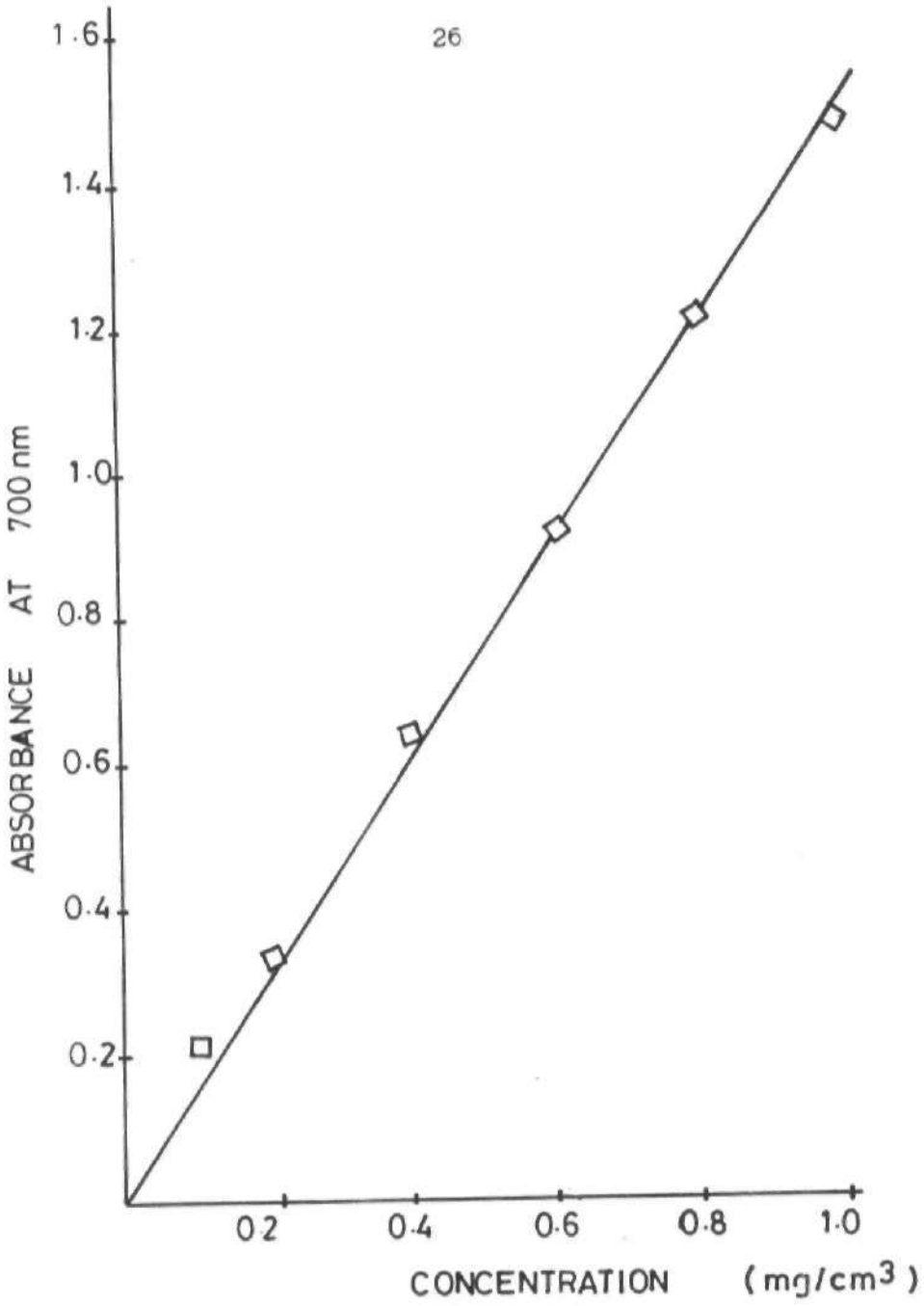


FIG. 2.1: True protein estimation by Lowry method. Calibration Curve of BSA (1mg/cm3) in wate

Sample Preparation.

To each of 0.50g of the dried pulp sample was added 10cm³ of distilled water, 10cm³ each of sodium chloride solutions and magnesium chloride solutions of concentrations 1, 5, 10, 15 and 20%. The mixtures were shaken for 12 hours after which they were centrifuged at 4,000rpm and the volumes of the supernatant were noted. 0.1cm³ portions of each were diluted to 1.0cm³ with distilled water, and were then treated as described in the preparation of the curve. Triplicate determinations and their respective blanks were carried out for the pulp and seed samples. The corresponding amount of protein in milligram were read off from the calibration curve.

$$\text{Protein (\%)} = \frac{C(mg) \times \text{volume of supernatant}(cm^3) \times 100}{\text{Weight of sample}(g) \times \text{Aliquot}(cm^3)}$$

Where,

C(mg) = amount of protein in milligram obtained from the calibration graph.

2.5.2.2 The Biuret Reaction Method
(Oyeleke, 1984)

Reagents

(i) Biuret Reagent

Copper sulphate (1.50g) and 6.0g of sodium potassium tartrate were dissolved in 500cm³ distilled water and to this solution was added 300cm³ of 10% carbonate-free sodium hydroxide with proper shaking and 2.0g of potassium iodide was also added to prevent auto reduction of copper(11)

oxide. The solution was made up to 1,000cm³ and stored in a polythene bottle.

(ii) Solvent Mixture

Accurately weighed 14.42g of sodium dodecyl sulphate (SDS) was carefully dissolved in about 100cm³ distilled water with stirring and 24.24g of urea was also dissolved in about 300cm³ distilled water. These two solutions were then mixed and made up to 500cm³ with distilled water. The mixture obtained was filtered through an ashless filter paper.

(iii) Bovine serum albumin (BSA) 10mg/cm³ in the solvent mixture.

Procedure:

Calibration curve.

A 10mg/cm³ BSA stock solution was prepared in SDS - urea solvent and from this, 1.0cm³ portions of solutions containing 1 to 10mg/cm³ BSA were prepared. 4.0cm³ of Biuret reagent was added to each of these solutions which were then left to develop for 30minutes before taking their absorbance at 540nm wavelength. From these readings, a calibration curve of absorbance against concentration was plotted and is shown in Figure 2.2.

Sample Preparation.

A 10cm³ portion of SDS-urea solvent was added to 500mg of the dried sample, centrifuged at 4,000rpm for 30minutes and the volume of the supernantant was noted. A 1cm³ portion of it was then treated as in the calibration curve above before taking the absorbance at 540nm wavelength.

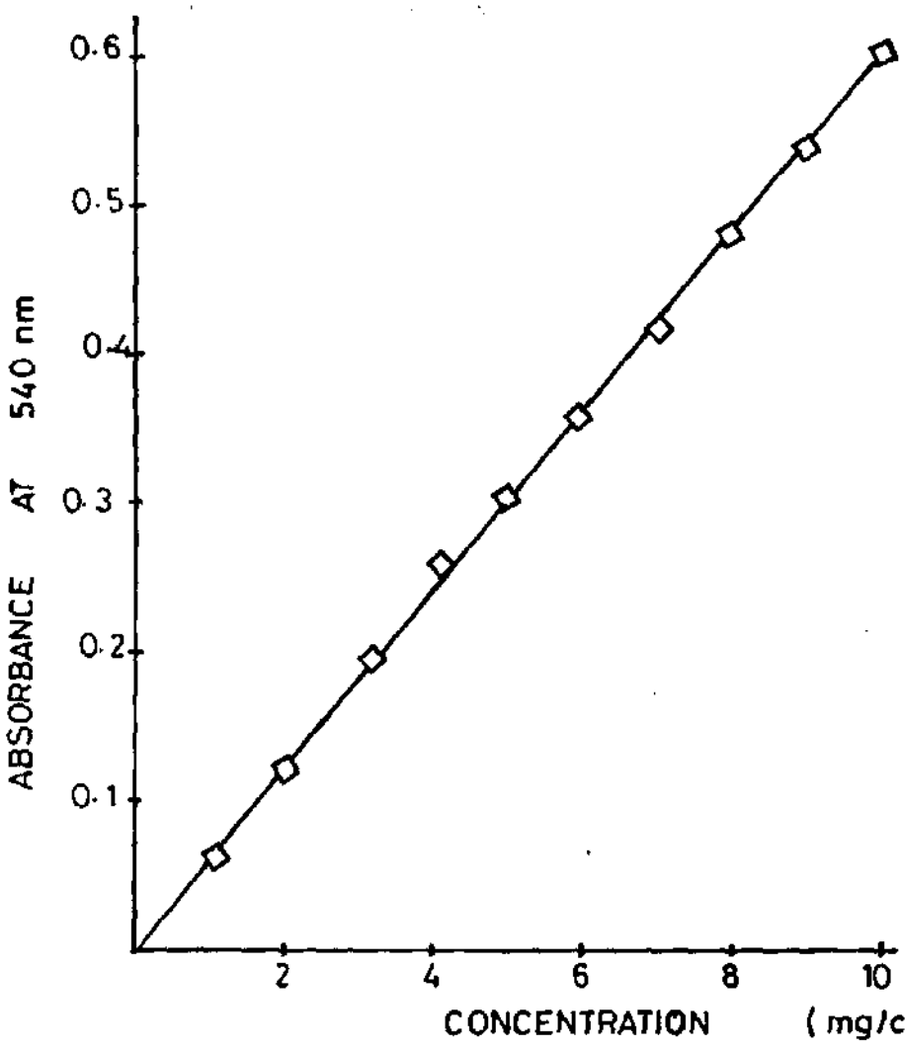


FIG 2·2: True protein estimation by Biuret reaction. Calibration Curve of BSA (10 mg/cm^3) in SDS - urea Solvent

Blank determinations were carried out on each sample. The corresponding amount of the protein was read off the caliberation curve.

$$\text{Protein (\%)} = \frac{C(mg) \times \text{Volume of supernatant}(cm^3) \times 100}{\text{weight of sample (mg)}}$$

Where,

C(mg) = amount of protein in milligram obtained from the calibration graph.

2.5.2.3 Lowry Method Using SDS - Urea Solvent Mixture.

Reagents:

Solutions A, B, C and Folin - ciocalteu reagent were prepared as described in 2.5.2.1.

SDS - urea solvent mixture was prepared as in 2.5.2.2.

Standard stock solution of BSA (1mg/cm^3) was prepared in SDS - urea solvent immediately before use.

Procedure:

Calibration Curve

The calibration curve (Figure 2.3) was prepared as in section 2.5.2.1 but using SDS - urea as solvent and reference in place of distilled water.

Sample Preparation.

A 10cm^3 aliquot of SDS - urea solvent was added to 0.50g of sample, centrifuged at 4,000rpm for 30 minutes and the volume of supernatant noted. 0.1cm^3 portion of the supernatant was diluted to 1.0cm^3 with SDS - urea

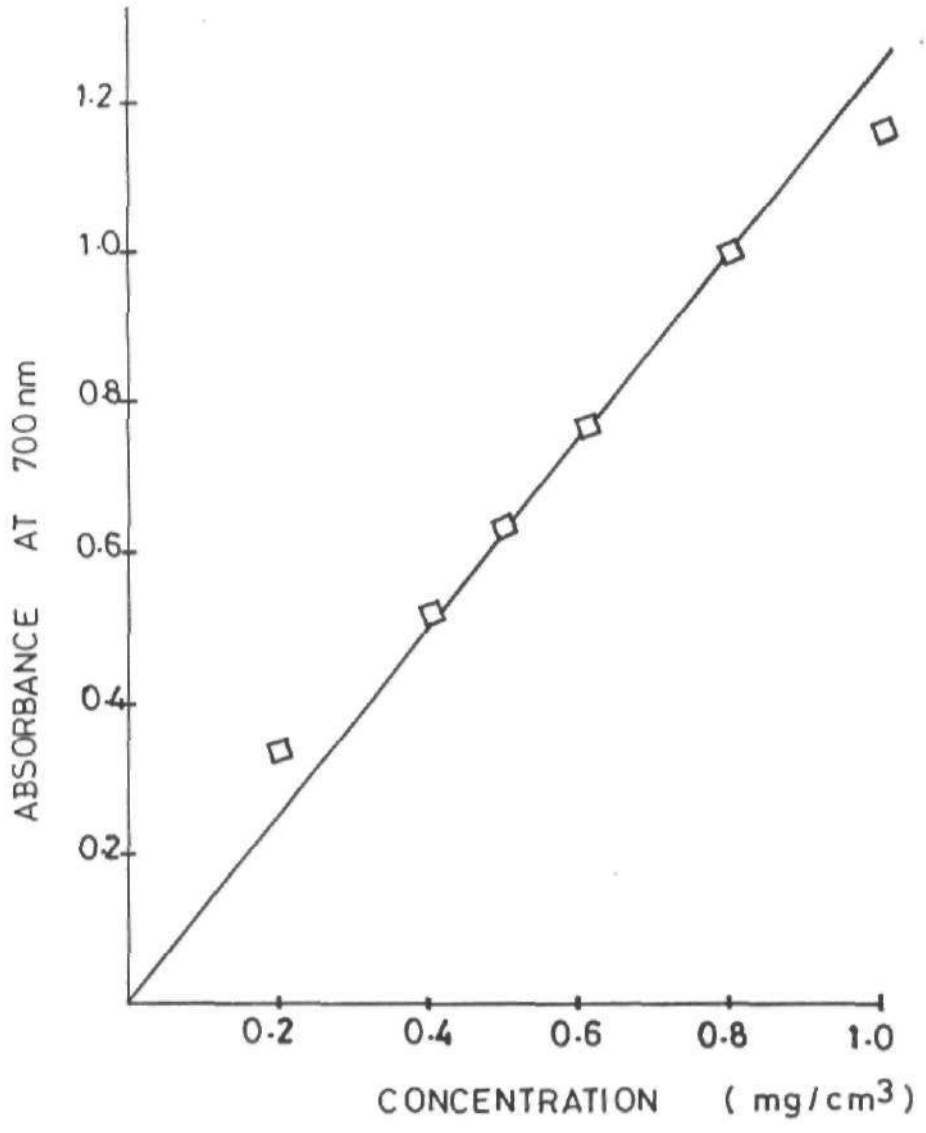


FIG. 2.3: True Protein estimation by Lowry Method. Calibration Curve of BSA (1 mg/cm^3) in SDS-urea solvent.

solvent and this was treated as in section 2.5.2.1. Triplicate determination with their respective blanks were carried out for the samples and the corresponding amount of protein in milligram were read off from the calibration curve (Figure 2.3).

$$\text{Protein (\%)} = \frac{(\text{Cmg}) \times \text{volume of supernatant}(\text{cm}^3) \times 100}{\text{Weight of sample}(\text{g}) \times \text{Aliquot}(\text{cm}^3)}$$

Where,

C(mg) = amount of protein in milligram obtained from the calibration graph.

2.6 Gel filtration separation of Proteins in Tamarind seed.

Gel filration in principle separates proteins according to their molecular size.

Materials and reagents

(i) Sephadex G-75, fractionation range of which is 50,000 to 1,000.

(ii) Protein standards

Blue Dextran	2×10^6.
Casein	1.92×10^5
Haemoglobin	6.80×10^4
Pepsin	3.55×10^4
Egg Albumin	3.38×10^4
Trypsinogen	2.38×10^4

(iii) Tris (hydroxymethyl) methylamine-hydrochloric acid buffer solution (0.01M, pH 7.5).

About 2,500cm³ of this solution was prepared and the pH was adjusted to 7.5 with 0.1M hydrochloric acid.

(iv) Sodium dodecyl sulphate (SDS) urea solvent mixture was prepared as in section 2.5.2.2.

(v) Chromatography column: Length 30.70cm; radius 1.25cm and volume 150.62cm³.

Procedure:

Sephadex G-75 (fine) was treated according to standard procedures (Pharmacia Fine Chemicals, 1969). 50.0g of the gel was swollen in excess Tris-HCl buffer (0.1M, pH 7.5) for 24 hours with several changes of the eluting buffer and the fines decanted off. The column was then packed, equilibrated with the Tris-HCl buffer for 48 hours and a flow rate of 4cm³ min^{-1} was set.

The column was calibrated with some standard proteins and this was done by carefully applying, in drops, a mixture of the standards (4.0mg

top of the bed. Thirty 5cm³ portions of eluants were collected at the set flow rate and the absorbance of these eluted portions were taken at 254nm.

One gram (1.0g) of seed sample was dissolved in 20cm³ of SDS - urea solution, stirred and centrifuged at 4000rpm. The supernatant obtained was applied to the top of the bed as done with the standards, again collecting thirty 5cm³ portions of eluants whose absorbance readings were taken at 254nm. Considering that the fractionation range of the gel used (50,000 - 1,000) and that species are eluted from the column inorder of their decreasing molecular size (Moore, 1964 and pharmacia fine chemicals, 1969).

KASHIM IBRAHIM LIBRARY
BELLO UNIVERSITY

Over a considerable range, elution volume (Ve) is a linear function of the logarithm of molecular weight (Andrews, 1965 and Carnegie, 1965) and is given by the expression:

$$Ve = A - B \text{ Log Mol. Wt.}$$

where,

A and B are constants (Pecsok et al, 1976).

2.7 Determination of the Acidity of the Pulp and seed samples.

2.7.1 Preparation of samples solutions
(AOAC, 1980)

One percent solutions of the pulp and seed samples were prepared in distilled water.

Procedure

Two grammes of the dried sample and 50cm^3 of boiled distilled water were homogeneized in a blender for 20 minutes. More water was added, stirred and allowed to stand for 2 hours at room temperature.
The mixture was then filtered and the filtrate was made up to the mark with water in a 200cm^3 volumetric flask. This solution was always freshly prepared before use.

2.7.2 Determination of the pH of the sample solutions.

Procedure:

The pH meter was first calibrated with buffer solutions of pH 4, 7 and 9, after which the pH of the pulp and seed solutions were read off.

2.7.3 Determination of Titratable Acidity
(AOAC, 1980)

Reagents

Sodium hydroxide solution 0.1N

phenolphthalein indicator solution (%) (1%).

Procedure:

The sample solution, after appropriate dilution, was titrated with 0.1N sodium hydroxide solution using 0.3cm^3 phenolphthalein solution for each 100cm^3 solution titrated. Triplicate determinations were carried out on each sample. Titratable acidity was reported as cm^3 0.1N alkali per gram of sample.

2.7.4 Determination of volatile Acidity
(AOAC, 1980).

Reagents:

Sodium hydroxide solution o.1N

phenolphthalein indicator solution (1%).

Procedure:

The steam delivery tube of the steam can was allowed to dip well into a two-necked round-bottom flask containing 25 cm^3 of freshly prepared one percent solution. The water in the can was allowed to boil for about five minutes with the other side of the two-necked flask open. The set up was then closed and about 300cm^3 liquid was allowed to be distilled into an Erlenmeyer flask. 0.5cm^3. phenolphthalein was added to the collected distillate and the solution was rapidly titrated with 0.1N sodium hydroxide solution until a pink colour which persisted for 15 seconds was

obtained. Triplicate determinations were carried out on each sample. Total volatile acidity was expressed as:

gHOAc/100cm³ of 1% sample solution, that is, gHOAc/g sample, and is equal to cm³ 0.1N NaOH x 0.006 x 4 (1cm³ 0.1N NaOH ≡ 0.006g HOAc)

2.8 Carbohydrate Determination.

2.8.1 Determination of Total carbohydrate (Clegg, 1956).

Reagents

(i) Anthrone reagent

Accurately weighed 1.0g of anthrone was dissolved in 1000cm³ of 13.6M sulphuric acid solution. Fresh reagent was prepared daily.

(ii) Ethanol (80%)

(iii) Perchloric acid (52%)

This was prepared by adding 270cm³ of concentrated perchloric acid to 100cm³ of distilled water.

(iv) Standard glucose solution (1mg/cm³)

This solution was diluted daily to give a solution of 100µg glucose per cm³.

Procedure

Extraction of sugars from sample.

To 0.20g of the dried sample in a centrifuge tube was added two drops of 80% ethanol, to aid mixing, then 5cm³ of water and the whole mixture was thoroughly stirred. 25cm³ of hot 80% ethanol was then added, stirred thoroughly, set aside for 5 minutes and centrifuged. The alcoholic

solution was decanted and extraction procedure was repeated with 30cm³ hot 80% ethanol. The alcohol in the extracts left was then diluted with water to 50cm³. This aqueous solution of sugars was then ready for analysis with the reagent and was marked thus. Triplicate extractions were caried out on each sample.

Extraction of starch from sample

Distilled water (5cm³) followed by 6.5cm³ of 52% perchloric acid were added to the sample residue above after extraction with alcohol. After stirring for about 20 minutes, 20cm3 of water was added, the mixture was then centrifuged and the supernatant decanted into a 100cm³ volumetric flask. Another 5cm³ quantity of water was again added to the residue and extraction with perchloric acid was repeated with occasional stirring for the next 3 minutes. The supernatant was again decanted into the 100cm³ volumetric flask which was then made up to mark with distilled water. This was filtered with the first 5cm³ of the filtrate discarded. A 2.0cm³ aliquot of the filtrate was diluted to 20cm³ with water and this extract solution was used for analysis with the reagent. Triplicate extraction of the sample was carried out.

Analysis of the Extracts.

For each determination, the following were prepared in boiling tubes:-

(i) "the blank" - containing 2cm³ of distilled water. Prepared in duplicate.

(ii) "test extract alone" - containing 1cm³ of the diluted extract (sugar or starch extract) and 1cm³ of distilled water. Prepared in

triplicate.

(iii) "test extract plus glucose standard" -containing 1cm³ of the diluted extact (sugar or starch extract) and 1cm³ of glucose standard (100µg). Prepared in triplicate.

To each of these tubes was added 10cm³ of the anthrone reagent and the contents were properly mixed before covering them with wooden corks. The tubes were heated in a boiling waterbath for exactly 12 minutes, cooled to room temperature under a running tap and the developed colour intensities were read off at 630nm wavelength. The reagent blank was used as reference and the green colour developed was stable for at least 3 hours. The quantity of glucose in the test extract was calculated as follows:

(i) Milligram glucose in sample (Amg) = $\frac{X \times 0.1\text{mg} \times D.F \times \text{volume of supernatant (cm}^3\text{)}}{(y-X)}$

where,

X = absorbance due to test extract only minus the absorbance due to the blank.

y = absorbance due to test extract and glucose standard minus the absorbance due to the blank.

D.F = dilution factor of sample extract.

(ii) Percent sugar in the sample = $\frac{A(\text{mg}) \times 100}{\text{weight of sample (mg)}}$.

For the starch, the expresion (ii) was multiplied by a factor of 0.9 to give the starch in the original sample. The total carbohydrate was given as the sum of the percent sugars and percent starch in the sample.

2.8.2 Reducing sugars Determinaion
(Hodge and Davies, 1952)

This method is based on the reduction of alkaline ferricyanide to ferrocyanide in the presence of reducing sugar, the amount of reduction of which is a measure of sugar in a sample and is determined as the difference between the added ferricyanide and the quantity left after reduction.

Reagents:

(i) Glucose standard 1mg/cm^3

(ii) Sodium thiosulphate aproximately 0.01M

Sodium thiosulphate (2.50g) was dissolved in freshly boiled distilled water and made up to 1000cm^3. 0.01g of sodium carbonate was added to stabilize the solution.

(iii) Potassium ferricyanide - sodium carbonate reagent.

Accurately weighed 4.125g of potassium ferricyanide and 5.30g of sodium carbonate were dissolved in distilled water and made up to 500cm^3. The solution was stored in the dark.

(iv) Potassium iodide - zinc sulphate - sodium chloride reagent.

Accurately weighed 6.25g of potassium iodide, 12.50g zinc sulphate and 62.5g sodium chloride were dissolved in distilled water and made up to 250cm^3. Solution was stored in the dark.

(v) Acetic acid

(vi) Starch indicator.

Extraction of soluble carbohydrate

Accurately weighed 0.20g of the dried sample with 30cm^3 of distilled water were allowed to simmer gently on a hot plate for 2 hours and cooled.

This was then filtered noting the volume of the filtrate, and made up to the mark in a 50cm³ volumetric flask. This extract solution was prepared shortly before colour development. The water blank was also taken through the same procedure and triplicate extractions were carried out on each sample.

Procedure:

A 5cm³ aliquot of the extract solution was pipetted into a boiling tube, 5cm³ each of glucose standard and distilled water were also pipetted into separate boiling tubes and from this stage, all the tubes were treated in the same way.

Portions of the potassium ferricyanide - sodium carbonate reagent (5cm³ each) were added and the tubes were covered with wooden corks and allowed to stand in boiling water bath for 15 minutes.

These were then cooled for 3 minutes under running water, before adding 5cm³ of the potassium iodide-zinc sulphate-sodium chloride reagent which results in the formation of precipitate and liberation of iodine vapour. 3cm³ of 5% acetic acid was then added and the mixture was titrated with approximately 0.01M thiosulphate using starch indicator. Blank determinations were also treated in the same way. The percent reducing sugars as glucose was calculated as shown below;

$$\frac{A - C\ (cm^3) \times \text{Extract volume } (cm^3)}{A - B\ (cm^3) \times \text{sample weight (g)} \times 2}$$

where,

A(cm^3) = the volume of 0.01M thiosulphate required for the ferricyanide reagent only.

$B(cm^3)$ = the volume of 0.01M sodium thiosulphate required for the glucose reagent.

$C(cm^3)$ = the volume of 0.01M thiosulphate required for the sample extract.

Factors for dilution were applied where necessary.

2.9 Alcohol - insoluble solids Determination (AOAC, 1980)

Procedure

Ten grammes of the dried sample (W_1) was boiled with $300cm^3$ of 80% ethanol, then allowed to simmer slowly for 30 minutes. The mixture was filtered through a buchner funnel fitted with a prepared filter paper of appropriate size. The filter paper having been prepared by drying in an uncovered flat - bottom dish for 2 hours at 100°C, the dish was then covered with a tight cover, cooled in a desiccator and weighed (W_2) at once. The filter paper and the residue were washed with 80% ethanol until washings were clear and colourless, and they were then transfered to the dish used in preparing the filter paper, dried uncovered for 2 hours at 100°C. The cover was again replaced and the dish was cooled in a desiccator and then weighed (W_3). Triplicate determinatios were carried out on each sample.

$$\text{Alcohol - insoluble solids (\%)} = \frac{100(W_3 - W_2}{W_1}$$

2.10 Determination of water - insoluble solids

(AOAC, 1980)

Procedure

A filter paper (Whatman No4) was washed with hot water and dried overnight at 100°C, then cooled in a desiccator and weighed (W_1). Accurately weighed 25.0g of the dried sample (W_2) was boiled with 200cm³ water for 20 minutes with occasional replacement of water lost by evaporation. The mixture was then filtered by gravity through the prepared filter paper in a buchner funnel and washed with about 80cm³ of hot water. Suction was applied to the buchner funnel to remove excess water. The filter paper was then dried overnight at 100°C, cooled in a desiccator and then weighed (W_3). Triplicate determinations were carried out for each sample and the percent water insoluble solids was calculated as follows:

$$\text{Water - insoluble solids (\%)} = \frac{100(W_3 - W_1)}{W_2}$$

2.11 Crude Fibre Determination

(Ranjhan and Krishna, 1980)

Reagents

Sulphuric acid 10%

Sodium hydroxide 10%

Ethanol 95%

Apparatus

A stick filter was made in the glass blowing section of the department and this was a 20cm long heavy wall glass tubing with an inner diameter of

0.2 - 0.3cm. One end of this was blown to give an outer diameter of 0.9cm and this opening was fitted with glasswool to give the appearance of a small broom, with a 2cm thick brush.

Procedure

Five grammes of the defatted sample (W_1) was extracted with some boiling water and 25 cm^3 of 10% sulphuric acid by boiling for 30 minutes. The acid solution was then removed by suction through the stick filter. The residue was washed at least three times with boiling water, after which the procedure was repeated with 25cm^3 of 10% sodium hydroxide in place of the acid. The alkaline solution was also removed using the stick filter and the residue obtained here was quantitatively transferred to a large porcelain crucible. The residue was further extracted with small portions of ethanol after which the glasswool was removed from the stick and left in the crucible along with the residue. The porcelain crucible with its contents were dried at 100°C to constant weight, cooled and weighed (W_2). The contents of the crucible were then incinerated at 600°C (about 2 hours) in a muffle furnace until the carbonaceous matter were burnt. The crucible now containing the ash and the unburnt glasswool were then cooled in a desiccator and weighed (W_3). Triplicate determinations were carried out on each sample.

$$\text{percent crude fibre} = \frac{100(W_2 - W_3)}{W_1}$$

2.12 Determination of crude lipid
Soxhlet Extraction Method
(A.C.O.C.S oil and soap, 1940)

Reagent

Petroleum Ether 40 - 60°C

Procedure

The dried sample (20.0g) was accurately weighed into a pre-weighed fat-free thimble. 350cm³ of petroleum ether was poured into a previously weighed 500cm³ round bottom flask containing anti-bumping granules. The soxhlet extractor containing the thimble with its content was then fitted into the round bottom flask and the extraction continued for 12 hours. The petroleum ether in the flask was distilled off using a vacuum rotary evaporator. The last traces of the solvent were removed by drying the flask in an oven to constant weight. The flask was cooled, weighed and the amount of lipid extracted was obtained from the difference between the weight of the flask before and after extraction.

$$\text{Crude lipid (\%)} = \frac{\text{Weight of lipid (g)} \times 100}{\text{weight of dry sample (g)}}$$

2.13 Determination of some Properties of the Extracted crude lipid

2.13.1 Saponification value (Pearson, 1976)

Reagents:

(i) Alcoholic solution of potassium hydroxide was prepared by dissolving 35.0g of potassium hydroxide in 20cm³ of distilled water and making up to 1000cm³ with 95% ethanol. The solution was left to stand

overnight before carefully decanting off the clear aqueous liquid.

(ii) Phenolphthalein (1%)

(iii) Hydrochloric acid 0.5M

Procedure:

Accurately weighed quantity (2.0g) of the extracted lipid was placed in a conical flask and exactly 25cm³ of the alcoholic potassium hydroxide solution was added to it. The flask was heated under reflux in boiling water for one hour and 1cm³ of phenolphthalein indicator was added to the hot solution. The excess alkali was then titrated with 0.5M hydrochloric acid (titre = acm³). Blank determination was also carried out at the same time (titre = bcm³). Triplicate determinations were carried out on each sample and saponification value of the lipid in milligram was calculated from the expression:

$$\frac{(b - a)\ cm^3 \times 28.05}{\text{weight of sample (g)}}$$

2.13.2 Iodine value (Dam's method)

Reagents

(i) Chloroform

(ii) Sodium thiosulphate solution 0.025N

(iii) Potassium iodide solution 10%

(iv) Dam's reagent

This was prepared by mixing 41cm³ pyridine and 30cm³ concentrated sulphuric acid with a solution of 13cm³ concentrated bromine solution in 100cm³ of concentrated acetic acid. The solution was made up to 2,500cm³ in acetic acid and was stored in a brown bottle.

Procedure

Portions of chloroform and Dam's reagent, 5cm³ each, were measured into a dry conical flask and allowed to stand for 10minutes in the dark. Then 5cm³ of 10% potassium iodide and 20cm³ of water were added and mixed. The solution was then quickly titrated with 0.025N sodium thiosulphate shaking vigorously to ensure proper mixing of the organic and aqueous layers and the titration was continued dropwise, when the mixture had turned pale yellow, until both phases just turned colourless. This was a blank titration to determine the iodine equivalent of bromine in the reagent.

The measurement of the iodine equivalent of the bromine not absorbed by the lipid was then carried out as follows:-

To 5cm³ portion of 1% lipid solution in chloroform was added 5cm³ of Dam's reagent and the mixture was left to stand in the dark for 10minutes. 5cm³ of 10% potassium iodide and 20cm³ of water were then added, swirled and titrated with 0.025N sodium thiosulphate solution just as done with the blank. Triplicate determinations were carried out for the blank and the samples.

Calculation:

1cm³ 0.025N Sodium thiosulphate ≡ 0.00317g of iodine.

therefore,

Iodine equivalent of the reagent ≡ Volume (cm³) required for the titration x 0.00317.

The iodine equivalent of the bromine absorbed by the lipid sample was calculated and the iodine value of the lipid sample was the difference between these values.

2.13.3 Unsaponifiable Matter
(Pearson, 1976)

Reagents

Potassium hydroxide 3M and 0.5M

Diethyl ether.

Procedure:

The neutralized liquid obtained after titration for saponification value was again made alkaline with 1cm³ of 3M aqueous potassium hydroxide. The solution was then transferred to a separator, washed with water and extracted thrice, while still just warm, with 50cm³ portions of diethyl ether. The ether extracts were poured into another seperator and washed with three 20cm³ portions of water. The extract was then further washed with two 20cm³ portions of water until the wash water was no longer alkaline to phenolphthalein. The extract was then transfered into a pre-weighed flask (W_1) and the solvent was evaporated off using a rotary evaporator. The flask and the residue were then dried at 78°C (temperature should not exceed 80°C) until constant weight (W_2) was attained. Triplicate determinations were carried on each sample.

$$\text{Unsaponifiable Matter (\%)} = \frac{100(W_2 - W_1)}{\text{Weight of sample (g)}}$$

2.13.4 Acid Value and Free Fatty Acid Value (Pearson, 1976)

Reagents

Diethyl ether

Ethanol 95%

Sodium hydroxide 0.1M

Phenolphthalein 1%

Procedure

The solvent mixture consisting of 25cm³ each of diethy ether, ethanol and 1cm³ of phenolphalein solution, was carefully neutralized with 0.1M sodium hydroxide. The extracted lipid (1.0g) was then dissolved in the neutral solvent mixture and titrated with aqueous 0.1M sodium hydroxide with constant shaking until a pink colour which persisted for 15seconds was obtained.

$$\text{Acid Value (\%)} = \frac{\text{Titre value (cm}^3\text{)} \times 5.61}{\text{Weight of Sample (g)}}$$

Free fatty acid (FFA) value was calculated as Oleic acid and;

1cm³ 0.1M sodium hydroxide = 0.0282g Oleic acid in which case, the acid value equals twice the free fatty acid.

2.13.5 Peroxide Value (Pearson, 1976)

Reagents

Potassium iodide (powdered and 5% solution)

Glacial acetic acid

Chloroform

Sodium thiosulphate 0.002M

Starch indicator

Solvent mixture containing glacial acetic acid and chloroform in the ratio 2:1 (v/v).

Procedure

This determination was carried out in subdued daylight. Accurately weighed 0.50g of the extracted lipid was mixed with 1.0g of powdered potassium iodide and 20cm³ of the solvent mixture and boiled for 20-30seconds. The mixture was then quickly transferred to a conical flask containing 20cm³ of 5% potassium iodide solution. The mixture was titrated with 0.002M sodium thiosulphate solution using starch as indicator. A blank determination was carried out at the same time and triplicate determinations were carried out on the samples. The peroxide value was reported as milliequivalents of peroxide oxygen per Kg of sample (cm³ per Kg) was calculated as:

2 x the volume (cm³) of 0.002M sodium thiosulphate per gram of sample.

2.14 Thin Layer Chromatography (TLC) of the Extracted Lipids

Reagents:

This consisted of petroleum ether (b.p 40°-60°C), diethyl ether and acetic acid in the ratio 80:20:1 (v/v/v)

(ii) Silica Gel (Kieselgel G.)

(iii) Iodine Crystals.

Procedure

(a) One percent sample solutions of the crude lipid of pulp and seed samples were prepared in chloroform. These lipid solutions were then spotted on a TLC plate 20 x 20cm coated with silica gel (0.5mm thickness), and the relative movements of these spots, in the solvent mixture were

determined with iodine staining. The R_f of the components of each spot were calculated from the expression:

$$R_f = \frac{\text{Distance moved by spot (cm)}}{\text{Distance moved by solvent front (cm)}}$$

(b) The plate was left to stand out on the bench for the iodine stains to vapourize off, and was then turned at an angle of 90° in the solvent to observe any further seperation of the spots obtained in (a).

2.15 Determination of Anti - nutritional Factors.

2.15.1 Determination of Phytic acid level in unprocessed Pulp and seed samples. (Allen, 1974)

Reagents

(i) Hydrochloric acid 0.5M and 0.17M

(ii) Sodium hydroxide 0.5M

(iii) Ferric chloride 0.25% (w/v)

(iv) Digestion reagents as in section 2.4.5

(v) Colorimetric reagents and standards same as in phosphorus determination in section 2.4.5

Procedure

The dried pulp and seed samples (1.50g) were shaken in 100cm³ each of 0.5M hydrochloric acid for 2hours and filtered. An aliquot of the filtrate was neutralized with 0.5M sodium hydroxide solution and again made slightly acidic with 0.17M hydrochloric acid before making it up to 50cm³ with

distilled water. An aliquot of this solution was taken in a centrifuge tube, to it was added 4cm^3 of freshly prepared ferric chloride solution, which was then heated for 15minutes at 100°C, cooled and centrifuged. The supernatant was discarded and the residue washed with 0.5M and then 0.17M hydrochloric acid, discarding again the supernatant. Distilled water (2cm^3) was added to the residue, heated for a few minutes at 100°C. Sodium hydroxide (0.5M) was then added and heated for another 15minutes, then the contents were filtered into a Kjeldahl flask, washed with hot water while retaining washings in the flask. Concentrated sulphuric acid (0.5cm^3) was added and the mixture was boiled before digesting with nitric and perchloric acid mixture in the ratio 5:1 (v/v) (section 2.4.5). Triplicate determinations were carried out on each sample. The amount of phosphorus determined here was that present as phytate. The amount of phytate-phosphorus was caculated from the expression below:

$$\text{Phytate - P(mg/100g)} = \frac{A(mg) \times \text{digest solution(cm}^3) \times \text{neutral solution(cm}^3) \times \text{acid extract(cm}^3) \times 100}{B(cm^3) \times C(cm^3) \times D(cm^3) \times \text{Weight of sample(g)}}$$

where,

A(mg) = amount of phosphorus in the sample determined from the calibration curve.

B(cm^3) = volume of aliquot for phosphorus determination.

C(cm^3) = volume of aliiquot for digestion.

D(cm3) = volume of aliquot for neutralization.

2.15.1.1 Processing

Accurately weighed 1.50g of the dried pulp and seed samples were either cooked or soaked for different lengths of time in 10cm³ of distilled water. At the end of the time lapse, the samples were centrifuged and the supernatant discarded, leaving behind the processed samples which were then treated as in 2.15.1. Triplicate determinations were carried out on each sample at each time period.

2.15.2 Determination of Trypsin Inhibitor Activity.
(Roy and Rao, 1971)

Reagents:

(i) Phosphate buffer (0.1M, pH 7.5)

This was prepared by adding 16cm³ 0.2M solution of sodium di-hydrogen orthophosphate to 84cm³ of di-sodium hydrogen phosphate (0.2M) and making the mixture up to 200cm³ with distilled water. The pH of this solution was then adjusted to 7.5 with 0.1M hydrochloric acid.

(ii) Sodium chloride solution 0.5M

(iii) Casein solution 2% in phosphate buffer (0.1M, pH 7.5)

(iv) Trypsin solution 5mg/100cm³ in 0.001M hydrochloric acid.

(v) Trichloroacetic acid (TCA) 5% (w/v)

(vi) Hydrochloric acid (0.001M)

Trypsin and casein solutions were stored in a refrigerator.

Preparation of sample

The dried pulp and seed samples, 5.0g each, were shaken in 100cm^3 0.5M sodium chloride solution and in 100cm^3 of distilled water for 3 hours each at room temperature.

Procedure:

Trypsin standard curve.

Aliquots of the stock trypsin (0.2 to 1.0 cm^3) were pipetted into a triplicate set of test tubes, one set for each level of trypsin, and the final volume was adjusted to 1.5cm^3 with the phosphate buffer. To one of the set of tubes was added 6cm^3 5% (w/v) trichloroacetic acid which served as the blank, 2cm^3 of casein solution and 0.5cm^3 of 0.001M hydrochloric acid were added to each tube which were then incubated at 37°C for exactly 20 minutes. The reaction was stopped by adding 6cm^3 of 5% TCA to the experimental tubes and allowing them to stand for one hour at room temperature. The suspension was filtered and the absorbance of the filtrates measured at 280nm (Figure 2.4).

Trypsin Inhibitor Activity of the samples

Aliquots of the sample extract (0.2 - 1.0cm^3) were pipetted into a triplicate set of test tubes (one set for each level of extract) and the volume was brought to 1.0cm^3 with the phosphate bufer. Trypsin solution (0.5cm^3) and 2cm^3 casein solution were added to each tube and the mixture was made up to 4cm^3 with 0.5cm^3 0.001M hydrochloric acid. The mixture was then incubated for exactly 20 minutes at 37°C, and the reaction was stopped

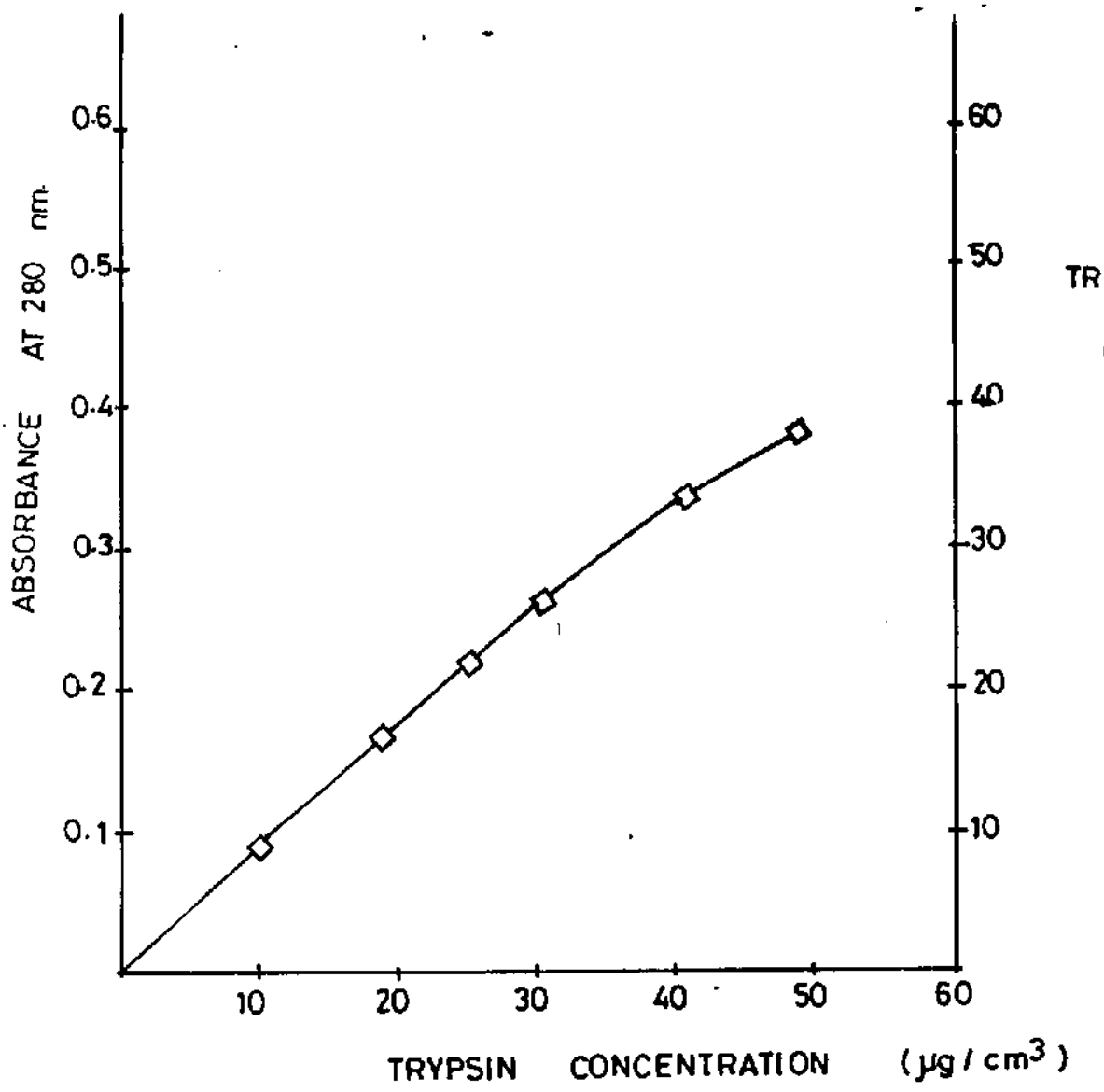


FIG. 2·4. Standard Trysin Curve using 2% Casein as Substra

by the addition of 6cm³ 5% TCA to each of the tubes, which were allowed to stand for one hour and then filtered. The absorbance of the filtrate was measured at 280nm against the blank.

Activity was expressed as trypsin unit (TU) Where one TU is arbitrarily defined as an increase of 0.01 absorbance units at 280nm in 20 minutes per 10cm³ of the reaction mixture. Trypsin activity is given as the number of trypsin units inhibited (TUI).

2.15.2.1 Processing

Some portions from the sodium chloride extracts and water extracts of the pulp and seed samples were heated in a boiling waterbath for 10, 20, 30, 60 and 120 minutes and some autoclaved at 15lb presure for 15 and 30minutes. The samples were cooled down to room temperature before pipetting out 0.4cm³ aliquots of the processed samples. The incubation mixture consisted of 0.4cm³ sample extract, 0.5cm³ trypsin solution, 2cm³ casein solution, 0.6cm³ phosphate buffer and 0.5cm³ of 0.001M hydrochloric acid. Incubation was carried out at 37°C and the procedure continued as with the unprocessed samples.

2.16 Sorption Isotherm of Tamarind Fruit
(Screedharan, 1985).

KASHIM IBRAHIM LIBRARY
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA.

Reagent

Concentrated sulphuric acid
(S.G 1.84)

Procedure:

Equilibrium moisture content of the oven dried tamarind pulp was

determined at room temperature by keeping triplicate samples in petri dishes, placed on metal gauze in medium sized desiccators maintained at different relative humudities (r.h.). Constant r.h. was maintained in each desiccator using sulphuric acid (S.G 1.84) and water mixtures prepared as shown in the appendix J (Raganna, 1977). The samples were weighed on a mettler balance at intervals of 24 hours until they attained constant weight. The percent equilibrium moisture content (e.m.c) was calculated as follows:

$$\% \text{ e.m.c} = \frac{W_2 - W_1 \times 100}{W_1}$$

where,

W_1 = Initial weight of sample

W_2 = Final weight of sample.

The time of the appearance of mould on the samples was also noted.

CHAPTER THREE

Results and Discussion

3.1 Moisture Content.

The moisture content of both the pulp and the seed are given in Table 3.1. The moisture content of the pulp (18.93%) is higher than that of the seed (10.10%). The value obtained for the pulp is lower than those reported in previous works. While Mohammed (1987) reported a value of 27.10%, Duke et al (1981) gave a value of 38.70%. All these values however fall within a range of 18-35% reported by Platt (1962). This wide range is probably due to differences in species and moreover, the moisture content of a fruit could vary depending on the stage of ripeness and the length of time for which the fruit had been stored (Addy, 1978). Tamarind fruit is generally considered ripe when the fruit is dry. In Zaria, the ripe fruits are obtainable in October - December, during the harmattan and it is probable that the samples used in this study were drier than the ones used by the first two workers. The moisture content of the seed, however agrees with that reported by Mohammed (1987); 10.10% and by Ega (1986); 10.98%.

The appearance, keeping quality and dry matter content of foods are closely related to the moisture content (Whyte and Selvey, 1969), with the amount of dry matter having an inverse relationship with moisture content. Foodstuffs are dried inorder to lower their moisture content, thereby preventing the growth of spoilage micro-organisms and also reducing the chemical and biochemical reactions to the minimum during storage. The moisture content of a food can therefore be used as an index of stability and susceptibility to fungal infections (Scott, 1956).

Table 3.1

Proximate Composition of the Pulp and Seed of Tamarind as Percent of Dry Matter.

	Pulp	Seed.
Moisture	18.93 ± 0.27	10.10 ± 0.25
Ash	2.88 ± 0.23	2.01 ± 0.01
Crude Protein	8.79 ± 0.44	26.93 ± 1.20
Carbohydrate (by difference)	66.87 ± 0.20	50.05 ± 0.23
Total Sugars (a)	41.20 ± 0.57	11.30 ± 0.45
Reducing Sugars (b)	24.68 ± 0.13	7.43 ± 0.47
Non-Reducing Sugars (a-b)	16.52	3.87
Starch (c)	0.57 ± 0.03	3.31 ± 0.13
Total Available Carbohydrate (a+c)	41.77 ± 0.30	14.61 ± 0.29
Crude Lipid	2.53 ± 0.43	10.91 ± 0.05
Crude Fibre	2.20 ± 0.47	7.40 ± 0.36
Alcohol - Insoluble Solids	22.70 ± 0.31	74.20 ± 1.01
Water - Insoluble Solids	20.50 ± 0.24	90.70 ± 0.21
Calories/100g	325.41	406.11

3.2 Ash Content and Mineral Composition

The ash content of the pulp (2.88%) was higher than that of the seeds (2.01%) (Table 3.1). These values do not vary much from those reported in literature. Purseglove (1968) reported an ash content of 2.10% for the pulp while Duke (1981) and Obodo (1982) reported 2.40% and 2.45% respectively. The ash content of tamarind pulp compares with those of brown beans and yam with values of 3.20% and 2.75% respectively while that of the seed compares fairly with millet, groundnut and coconut pulp with values of 2.13%, 2.22% and 2.34% respectively (Oyenuga, 1968 and Abede, 1981).

3.2.1 Mineral Composition

The mineral constituent of the samples are shown in Table 3.2

3.2.1.1 Calcium

The calcium content of the pulp and the seed were found to be 465.70mg and 786.86mg per 100g sample respectively. These values were much higher than values obtained for most fruits but they compare well with values obtained for leafy vegetables such as spinach (400mg/100g), baobab leaves (562mg/100g) (Abede,1981) and cocoyam (409.10mg/100g) (Oyenuga, 1968). The high calcium -containing legumes include soyabean (220mg/100g) and locust bean seed (310mg/100g) (Oyenuga, 1968).

It has been estimated that pregnant and lactating women usually require large amounts of calcium and this ranges from 800mg to 2000mg daily (FAO/WHO, 1962). It would therefore appear that tamarind fruit contribute a significant amount of calcium to diets, especially for those it forms a major part of their diets. The seeds which are most often thrown away, in

Table 3.2

Mineral Content of pulp and seed of Tamarind in mg/100g

	pulp	seed
Calcium	465.75 ± 29.10	786.86 ± 76.21
Phosphorus	91.00 ± 0.02	165.00 ± 0.10
Magnesium	72.03 ± 3.54	118.33 ± 0.29
Potassium	62.00 ± 0.03	610.00 ± 0.02
Sodium	76.66 ± 4.53	19.17 ± 0.68
Copper	21.83 ± 1.73	18.97 ± 0.20
Iron	8.49 ± 0.24	Not Detected
Zinc	1.06 ± 0.03	3.00 ± 0.14
Nickel	0.52 ± 0.03	Not Detected

the northern parts of Nigeria, could be a good source of calcium for both man and poults, though their bioavailability have to be considered.

Dietary Calcium could be made unavailable to the body by precipitation under alkaline conditions in the intestine, as calcium oxalate. The effect of oxalate in a food item can only be determined when compared with the amount of calcium intake, and it has been reported that toxic symptoms will not occur, if the calcium to oxalate ratio is 1:1 (Oke, 1969). Chakraborty (1976) reported a total oxalate content in tamarind fruit as (0.39mg/100g), of which only 28.21% is soluble and this would be of negligible effect on the available calcium of the fruit. Large amounts of calcium and phosphorus are required for bone formation and are incorporated into the bones by vitamin D. Inadequate supply of calcium in young animals usualy result in a bone deformation disease.

3.2.1.2 Phosphorus.

The phosphorus content of tamarind pulp and seed were 91.00mg and 165.00mg per 100g sample respectively. Duke et al (1981) recorded 86mg per 100g of the pulp which agrees with the values obtained in this work. Tamarind seed may be considered as a good source of phosphorus since cereals containing 140-330mg per 100g are reported to be the major source of phosphorus in African diets (Oyenuga, 1968). Tamarind pulp contains more phosphorus than most fruits (Oyenuga, 1968). It was found in this work that 28.5% of the phosphorus in the seed exists are phytic acid (section 3.7.1) while the amount of the pulp phosphorus present as phytic acid was not detectable. Calcium and phosphorus as constituents of the bones and

teeth give rigidity to these skeletal structures.

3.2.1.3 Magnesium

The magnesium content of tamarind pulp (72.03mg/100g) and seed (118.30mg/100g) were higher than those of most other fruits which ranged between 10.8mg/100g for mango to 42.2mg/100g for grape (Abede, 1981). Magnesium content of the pulp however compares with those of leafy vegetables such as sorrel (green) (69.0mg/100g) and Cochorus olitorius ('ayoyo') (70.0mg/100g), while the seed compares with okra (106.30mg/100g) and water leaf (103.0mg/100g) (Abede,1981). Daily requirement of magnesium is between 200mg - 700mg (WHO, 1973).

3.2.1.4 Sodium and Potassium

Tamarind pulp is a good source of sodium (76.66mg/100g) and compares fairly with groundnuts (67.60mg/100g), baobab leaves (88.0mg/100g) and locust bean pulp (99.0mg/100g) (Abede, 1981). Tamarind pulp is however fairly low in potassium (62.0mg/100g) but compares well with pineapple (66.0mg/100g). A balance in the potassium and sodium intake of diets containing high - protein foods such as beans with high ash content (3.0-3.2%) and very high potassium content (1240-1350mg/100g) (Abede, 1981) may be ensured if taken with porridge or juice made from tamarind pulp containing high sodium and fairly low potassium. This might be particularly useful in low fat diets of obese patients.

Tamarind seed has low sodium content (19.17mg/100g) (Table 3.2), similar to the value reported for banana (18.20mg/100g) (Abede, 1981). It

however has a high potassium content (610mg/100g) which compares favourably with those of melon (612.0mg/100g) and Irvingia gabonensis ('Ogbono') (543mg/100g) (Abede, 1981). From the above, it is clear that a balance in potassium and sodium will be ensured if both pulp and seed are included in the diet.

3.2.1.5 Copper

Tamarind fruit has a fairly high copper content with the pulp and seed containing 21.83mg and 18.97mg copper per 100g sample respectively. The body however requires copper in minute quantity (2.15mg daily for an average man of about 75kg weight) (Ekpete, 1985). Thus, copper requirement may be met when tamarind fruit is taken. Copper is essential for the utilization of iron in the haemoglobin synthesis.

3.2.1.6 Iron

Tamarind pulp contains an appreciable amount of iron (8.49mg/100g) which is much higher than values obtained for most fruits and vegetables. The value is however similar to that reported for the pulp of locust bean (8.2mg/100g) (Abede, 1981). Surprisingly, tamarind seed did not show any detectable amount of iron. Iron being a constituent of the respiratory pigment haemoglobin is vital for the functioning of all tissues and organs of the body.

3.2.1.7 Zinc

Zinc content of tamarind fruit was found to be low. The pulp had

a zinc content of about three times less than that of the seed (Table 3.2). This is not surprising as fruits generally have low zinc content. The pulp value however compares favourably with those of green vegetables such as okra (1.2mg/100g), Cochorus olitorius ('ayoyo') (0.7mg/100g), while on the other hand, the amount in tamarind seed compares with beans (3.5mg/100g) (Abede, 1981). Dietary requirement of zinc ranges from 1.25mg for infants to 5.45mg for lactating women daily (FAO/WHO, 1973).

3.2.1.8 Nickel

Nickel is present only in trace quantity in tamarind pulp (0.52mg/100g) and it was not detectable in the seed. Nickel is not of any known function in plants and animals and may have been present as a result of pollution from the soil.

3.3 Protein

3.3.1 Crude Protein

The crude protein contents of tamarind seed and pulp obtained in this study were 26.93% and 8.79% respectively (Table 3.1). These values are higher than those reported by Duke et al (1981) (15.4-22.7% and 2.3%) and Mohammed (1987) (20.1% and 7.0%) for seed and pulp respectively. Ega (1986) reported a very low value of 10.98% for the seed. Although the pulp is relatively poor in protein, the seed is a good source of protein and compares well with groundnut with a crude protein value of the range 22 - 27% (Oyenuga, 1968).

3.3.2 True Protein

An attempt was made in this work to estimate the amount of the true protein present in the samples using Lowry and Biuret methods. The results are shown in Table 3.3. There appears to be no major difference in values of protein using either of the methods. Lowry method is based on the reaction of the folin-ciocalteu reagent with the tyrosine residues in the protein and thus, the values obtained will depend on the number of tyrosine residues in the protein. Biuret method, on the otherhand, is based on the reaction of the biuret reagent with the peptide likages in protein. It is therefore expected that the latter method should give a more accurate value of protein than the former. However, in this study, either of the methods can be used.

3.3.2.1 Effect of Metal ions on the Extracttion of the protein.

The influence of inorganic cations on the amount of proteins extracted from both the seed and the pulp was studied. Water as extractant in the absence of salts was used as the control. The effects of varying salt concentration on the percent of protein extracted for both the seed and pulp are shown in Figures 3.1 and 3.2 respectively. For the seed sample, increase in concentration of Mg^{2+} and Na^{+} (except for the initial decrease with Na+), resulted in a corresponding increase in percent protein extracted upto a point (Figure 3.1). The optimum Na^{+} concentration is 10% with a corresponding protein content of 7.13% for the seed (Table 3.4) after which increase in salt concentration resulted in decrease in the amount of protein extracted. The optimum Mg^{2+} concentration in this study

Table 3.3

Comparison of Assay Methods for the Estimation of True Protein from Tamarind Fruit.

Method	Extractant	Pulp	Seed
Lowry	SDS/urea	9.71 ± 0.71	33.12 ± 4.50
Biuret	SDS/urea	11.26 ± 0.15	35.40 ± 3.74

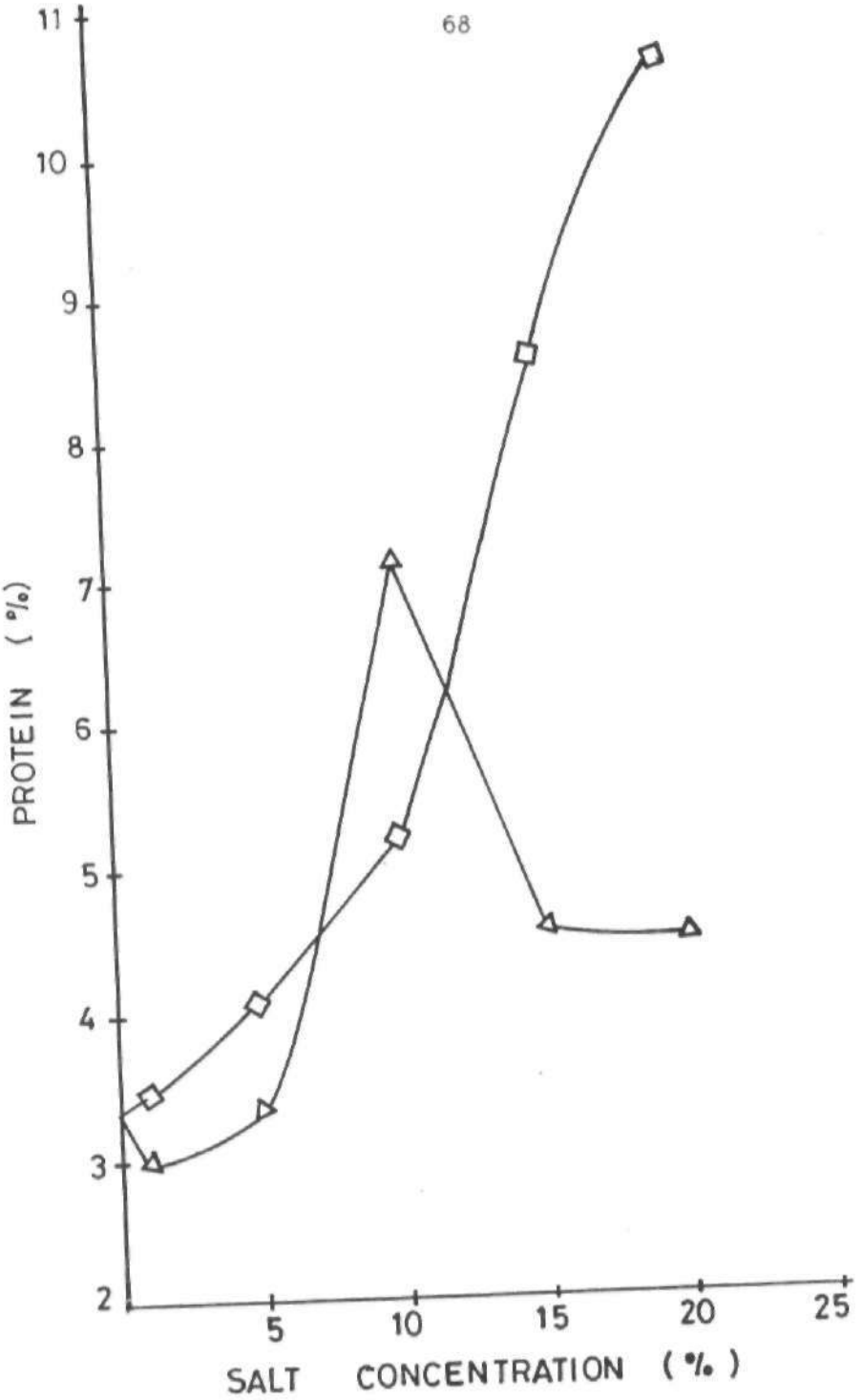


FIG. 3.1: Protein extraction from Tamarind seed using Sodium Chloride (Δ) and Magnesium Chloride (□) at different concentrations.

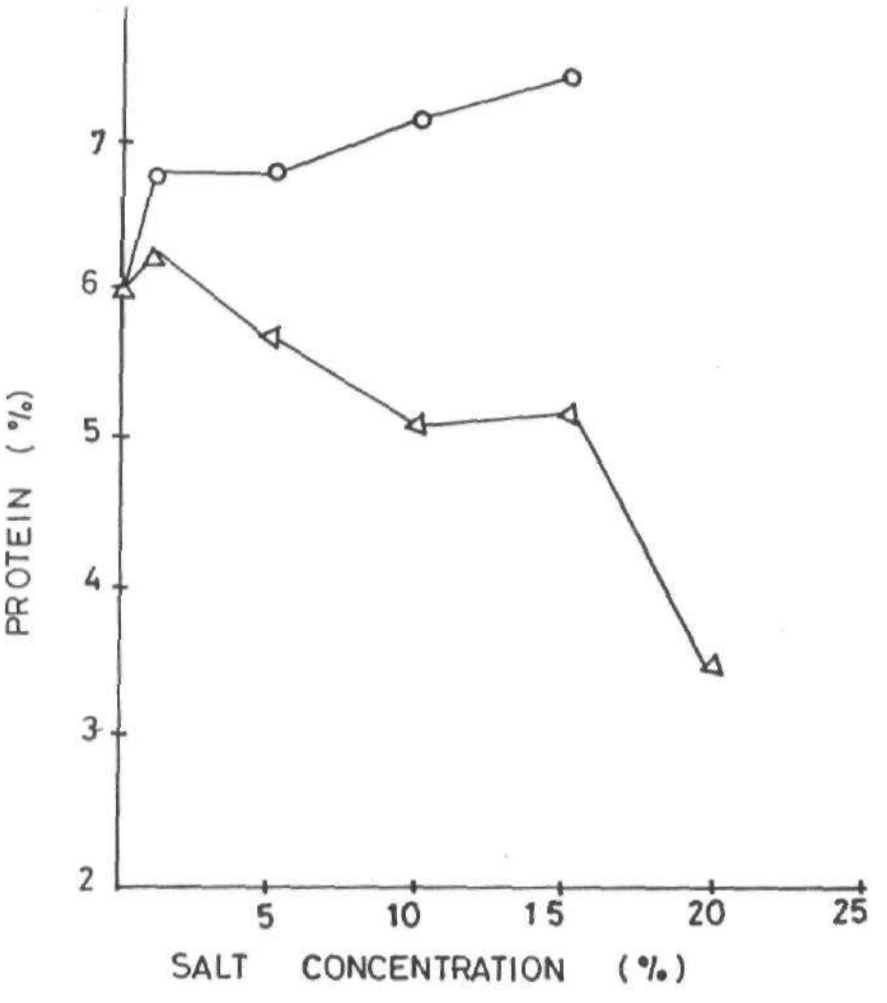


FIG. 3.2: Protein extraction from Tamarind pulp using sodium chloride (Δ) and Magnesium Chloride (O at different Concentrations

Table 3.4

Effect of sodium (Na^+) and Magnesium (Mg^{2+}) ions on the Extraction of Protein From Tamarind Fruit.

Extractant	Pulp		Seed	
	Protein (%)	Optimum salt concentration(%)	Protein (%)	Optimum salt concentration(%)
Water (control)	5.91 ± 0.28	-	3.34 ± 0.18	-
Sodium ion (Na^+)	6.22 ± 0.12	1	7.13 ± 0.14	10
Magnesium ion (Mg^{2+})	7.45 ± 0.10	15	10.60 ± 0.12	20

is about 20% with a corresponding protein content of about 10.6%.

For the pulp sample, there was again an initial increase in the percent protein extracted with increase in Na^+ concentration up to 1% with a corresponding percent protein of 6.22%. After which, an increase in the concentration resulted in a decrease in percent protein extracted. Mg^{2+} on the otherhand, after the initial sharp increase, showed a steady inrease with ion concentration (Figure 3.2). The optimum Mg^{2+} concentration is about 15% with a correponding protein content of about 7.45%. Generally in both samples, the efffect of monovalent Na^+ was less than that of the divalent Mg2+. This may likely be due to different ionic strength effect.

It is interesting to note that using SDS - urea as the extractant, the seed was found to contain significantly higher value of protein than the pulp (Table 3.3), but using water (Table 3.4) and low ion concentrations of both salts (Figures 3.1 and 3.2) as extractants, higher percentage of protein was extracted from the pulp than the seed. This observation suggests that most of the seed proteins are membrane bound and can only be solubilized with either a strong salt solution or a detergent such as SDS.

3.3.3 Molecular Weight Determination of the Protein from Tamarind Seed.

The seed powder was extracted with SDS - urea solution and the insoluble material was removed by centrifugation at 4,000rpm for 30minutes. The clear supernatant was applied to the column (Sephadex G-75) and eluted as described in section 2.3.3. The elution profile is given in Figure 3.3 along with that of standard proteins for easy comparison. The seed proteins were separated into four components numbered peaks 1, 11, 111

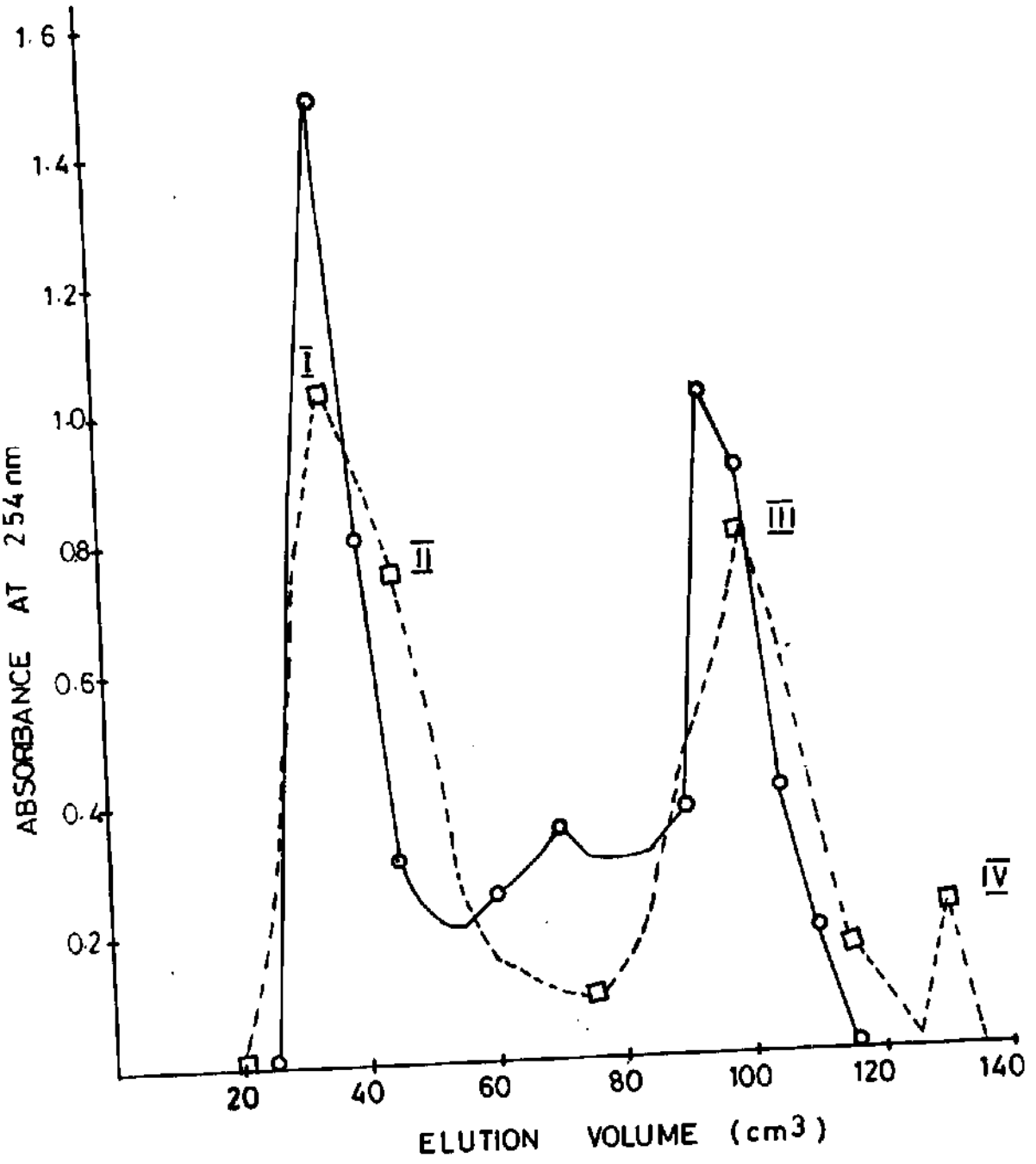


FIG 3.3: Gel Permeation Profile of some standard Proteins (
and Proteins from Tamarind seeds (□) on sephad
G - 75. Elution was with Tris - HCL buffer (0.1M,
PH 7.5) at 4 cm^3/min.

and IV. Peaks I and II were however not clearly separated. Their corresponding molecular weight were determined using the calibration curve shown in Figure 3.4 and are given in Table 3.5.

3.4 Acidity of Tamarind Fruit.

Many fruits are rich in organic acids which are an important source of respiratory energy in the plant cell. They are usually present either in the free or combined form as salts, esters and glycosides. Acids in foods impart their own characteristic flavour and degree of tartness. The acidic properties of organic acids are due to the presence in the molecule, of the carboxylic group (-COOH) in the free state. Other fruit constituents may have acidic properties without possessing the group in the free state for example, phenols and ascorbic acid.

The pH measures all free weak acids, and these are usually associated with their potassium salts to constitute buffer systems in the cell. The pH of fruit juice is generally low between 2 and 4 (Hulme, 1970). The pH of tamarind pulp obtained in this study is 2.7 (Table 3.6). Duke et al (1981) however recorded a pH value of 3.15. The value obtained in this study compares well with those of lemon (2.2 - 2.4), grape fruit (3.0 - 3.3), lime (1.8 - 2.0) and vinegar (2.4 - 3.4) (Jacobs, ed. 1951). The seed however is not quite acidic with a pH value of 6.3 which compares favourably with cereals such as corn (6.0-6.5) and wheat flour (6.0-6.5) (Jacobs, ed. 1951).

Titratable acidity is a measure of all the organic acids present in the free state, that is, uncombined with cations. It does not measure the

Table 3.5

Observed Molecular Weight of Proteins in Tamarind Seed separated by Gel Filtration.

Peak	Molecular Weight
1	50.70 x 10^3
11	44.98 x 10^3
111	23.55 x 10^3
1V	14.79 x 10^3

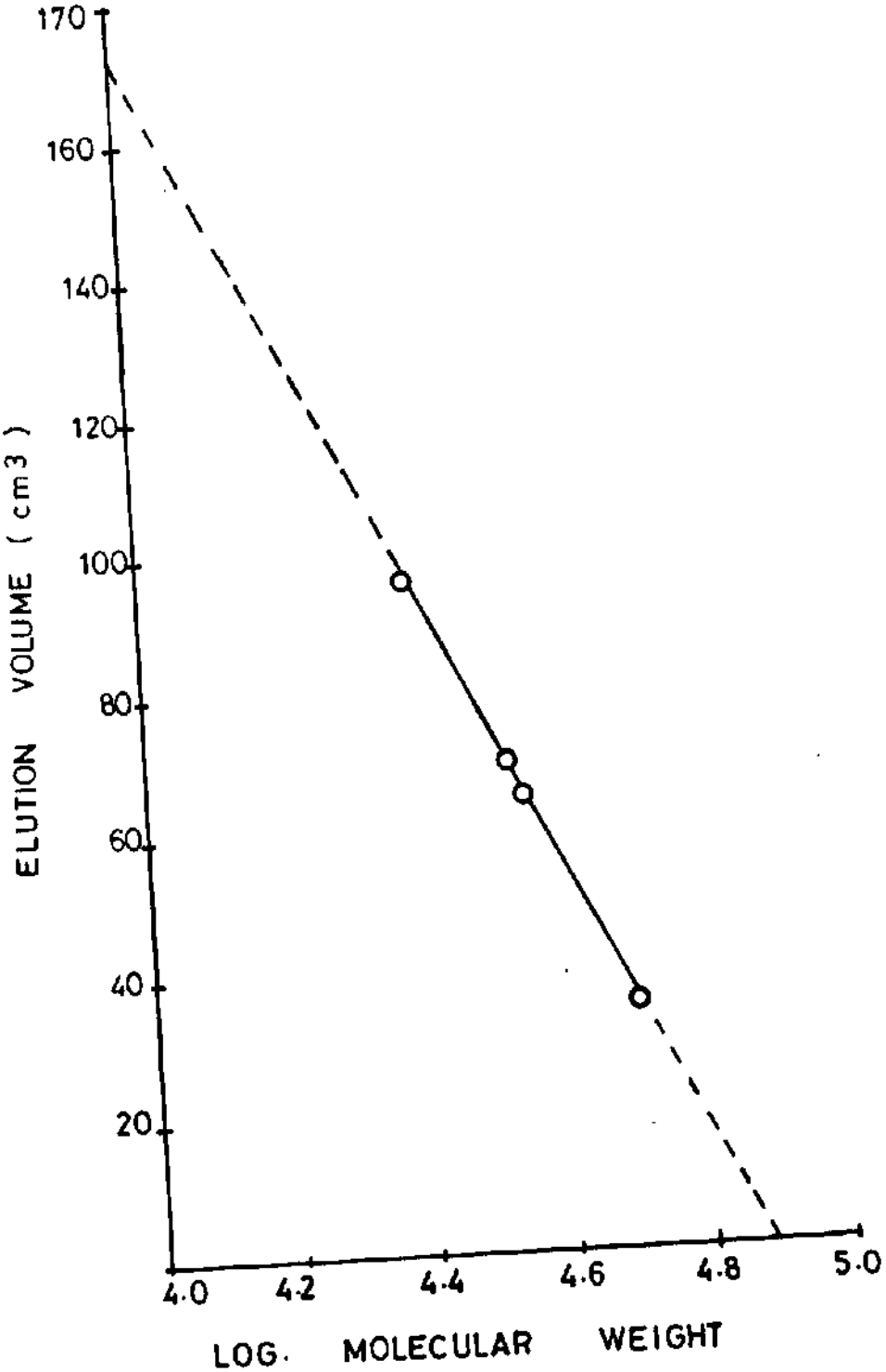


FIG 3·4: Elution Volume Vs log. Molecular weight of Prote standards separated on Sephadex G -75

total available acidity since many food products are highly buffered (Jacobs, ed. 1951). The pulp again has a higher titratable acidity value than the seed with values of 42.5cm³ and 3.3cm³ 0.1N NaOH per gram sample respectively (Table3.6). The pulp thus contains relatively greater amount of organic acids in uncombined state than the seed. The relatively high acidity of the pulp has been attributed to the presence of organic acids such as tartaric acid, citric acid and malic acid (Ulrich, 1970 and Duke et al, 1981); sodium and potassium salts of tartaric acid (Hamman, 1979) and also the presence of hydrocyanic acid (Chakraborty, 1976, and Duke et al, 1981).

Volatile acidity measures the percent of the total acidity, present free or combined that is volatile with steam. Such acids include water soluble butyric acid, caproic acid and the slightly water soluble caprylic acid and capric acid (Jacobs, 1951), which are mould inhibitors (Jacobs, ed. 1951). The volatile acid value of the pulp is close to about twice that of the seed (0.020 and 0.014g HOAc per gram of sample respectively (Table 3.6). Tamarind pulp owes its characteristic flavour to the balance between its sugar content (Table 3.1) and acid contents (Whiting, 1970).

3.5.1 Carbohydrates

Values of total carbohydrate (by difference) obtained for both pulp and seed are given in Table 3.1. Clearly, the pulp is richer in both total and available carbohydrates than the seeds. Available carbohydrate refers to that portion of the total carbohydrate that can be digested and absorbed in the alimentary tract of monogastric animals and utilized for the

Table 3.6

Acidity of the pulp and seed of Tamarind Fruit

	Pulp	Seed
pH	2.7	6.3
Titratable Acidity (cm^3 0.1N NaOH/g sample)	42.50 ± 0.37	3.30 ± 0.03
Volatile Acidity (g HOAc/g sample)	0.020 ± 0.05	0.014 ± 0.01

production of energy. This include starch and soluble sugars (Potter, 1968). It does not inlcude those fibrous materials such as cellulose, hemicellulose and lignin which constitute the structural components of the plant itself.

The carbohydrate (by difference) value for pulp obtained here (66.87%) compares with that reported by Duke et al (1981) (70.70%) and by Mohammed, (1987) (73.45%). The value obtained for the seed is however lower (50.05%) than those reported by both Duke et al (1981) and Mohammed (1987) (65.0% and 65.7% respectively). The calorie values (Table 3.1) calculated for the pulp and seed compare well with those reported by Mohammed (1987) (327.2 and 428.8cal. for the pulp and seed respectively). It is common practice in the northern parts of this country to add the pulp of the tamarind fruit to porridge to give a sour taste. This addition of tamarind pulp will certainly increase its calorific value.

While the total sugar content obtained for pulp and seed are 41.20% and 11.30% respectively, the starch content of these are 0.57% and 3.31% respectively (Table 3.1). These values indicate the high sugar content of tamarind pulp which makes up to about 61.60% of the total carbohydrate and 98.60% of the available carbohydrate. The sugars in the seed sample make up just about 22.60% of the total carbohydrate and 77.30% of the total carbohydrate.

About 59.90% of the simple sugars contained in the pulp is present as reducing sugars namely fructose and glucose while about 65.80% of the seed sugars is present as reducing sugars. This is not unexpected as it has been reported (Whiting, 1970) that the concentration of sucrose (non-

reducing) hardly exceeds that of the total reducing sugar in fruits. Part of this sugar is converted to the storage product-starch while the rest is used for the synthesis of pectic substances and cell wall materials. Fruits may however show considerable changes in sugar content between harvesting and eating ripeness (Whiting, 1970).

3.5.2 Alcohol-and Water-Insoluble Fractions.

Alcohol-soluble fraction of a fruit contains mainly sugars and organic acids (Jacobs,1951 and Ulrich, 1970). Alcohol-insoluble solids is used as a criterion of the quality of some vegetables. Tamarind pulp has a lower alcohol-insoluble solids value (22.70%) than the seed (74.20%) (Table 3.1). These values confirm that higher sugar and organic acid contents of the pulp over the seed.

Water-insoluble solids fraction is of value in determining the fruit content of some foods (Pomeranz and Meloan, 1978). The percent water - insoluble solids of the pulp and the seed samples are 20.50 and 90.70 respectively (Table 3.1). These are in agreement with the values of 29.90% and 89.90% reported by Mohammed (1987) for the pulp and seed respectively. The water-soluble fraction is usually turbid due to dissolved proteins and soluble starch. Tamarind pulp with a very low water-insoluble solid fraction indicates the solubility of a large percentage of the pulp in water. The insoluble solids of the pulp are mainly made up of fibrous materials.

3.5.3 Crude Fibre.

Crude fibre refers to the unprocessed cellulosic component, with a little lignin, of a plant and its determination is an attempt to measure the non-digestible carbohydrate material, that is, unavailable (Ockerman, 1978), to human and animals except ruminants. The determination is usually affected by particle size, the finer the material is ground, the lower the determined fibre content. As the recovery never exceeds four fifth of the amount actually present, the crude fibre content of a food, therefore, does not represent a measure of the cellulosic component of the food and the values obtained tend to vary with the conditions employed (Pomeranz and Meloan, 1978).

The dry tamarind pulp has a lower fibre content than the seed (2.20% and 7.40% respectively) (Table 3.1). These values fall within the range reported by Duke et al (1981) 1.90% and 0.70-8.20% for pulp and seed respectively. Crude fibre is normally used as an index of the feeding value of poultry and stock feeds. Seeds high in crude fibre content are of low nutritional value (Pomeranz and Meloan, 1978). Crude fibre of tamarind seed compares well with those of groundnut seed (decorticated) (9.61%) and soyabean seed (5.71%) (Oyenuga, 1968), while grains of guinea corn, millet (bulrush) and yellow maize have much lower fibre values of 2.94%, 2.00% and 2.00% respectively (Platt, 1962 and Oyenuga, 1968).

Though high fibre diets could cause constipation (Crampton and Harris, 1969), low fibre diets could equally be harmful (Eastwood, 1974; Walker, 1976 and Mendeloff, 1978). A range of diseases of the bowel such as piles, appendicitis and cancer of the colon and rectum have been attributed

to low fibre diets (Walker, 1976). These diseases have however been found to be rare among Africans since fruits and vegetables constitute a large portion in African dietary pattern. Tamarind seeds with a crude fibre (7.4%) comparable with that of spinach (9.0%, Oyenuga, 1968) could be considered as a good source of dietary fibre.

3.6 Lipids

The seed sample contained much more crude lipid (10.91%) than the pulp sample (2.53%) (Table 3.1). Mohammed (1987) recorded 9.50% for the seed. Other workers recorded lower values for the pulp (about 0.60%) than that obtained here. Tamarind seed contains about twice the amount of lipid present in maize (whole grain) (4.50%, Platt, 1962). Soyabean seed however has a higher lipid content (19.10%, Oyenuga, 1968).

3.6.1 Some Physico-Chemical Properties of the Extracted Lipids.

Data on oil composition tend to be variable and they are affected by such factors as; the growth environment of the seeds, their condiiton on harvesting, method of drying and conditions of storage (Duffus and Slaughter, 1980). The pulp lipid was greenish-yellow in colour while the seed lipid was golden yellow and both are oils at room temperature.

3.6.1.1 Saponification Value

Saponification value is an indication of the average molecular weight of an oil. A high saponification value indicates a greater proportion of fatty acids of low molecular weight (Thorpe et al, 1970).

For example, the saponification value of butterfat (210-230mg/g) is higher than that of beef tallow (196-200mg/g) because the former contains the low weight fatty acid, butyric acid while beef tallow contains mainly the higher weight palmitic and stearic acids (Jacobs, 1951). Hence, the saponification values of the pulp oil (301.3mg/g sample) (Table 3.7) indicate that the oils are made up of a greater proportion of low molecular weight fatty acids, with the pulp oil having more of these fatty acids.

3.6.1.2 Iodine Number.

Iodine number measures the degree of unsaturation and therefore the reactivity of the oil. Tamarind pulp oil with iodine number of 120.60 seems to have a higher degree of unsaturation than the seed oil with iodine number of 78.10 (Table 3.7). The iodine number of tamarind pulp oil compares well with those of soyabean oil which has a range of 120-136 and with corn oil with a range of 111-128. Both corn oil and soyabean oil have been classified as semi-drying oils by virtue of their iodine numbers, it therefore implies that the pulp oil is also a semi-drying oil. A drying oil is one which has the property of drying, or of being oxidized thus becoming thick, viscous, and forming a membrane on exposure to air. The terms semi-drying and non-drying refer to slower drying oils and oils which lack the ability to dry respectively (Jacobs, 1951).

For the seed oil, iodine value is comparable with those of groundnut (80-110) and olive oil (78-90) (Pearson, 1976 and Ockerman, 1978), both of which are classified as non-drying oils, thus implying the non-drying nature of tamarind seed oil. Generally, non-drying oils have iodine

Table 3.7

Some Physico-Chemical Properties of the Lipid Extracted from the pulp and seed of Tamarind.

	Pulp	Seed
Saponification value (mgKOH/g)	301.30 ± 6.50	266.60 ± 3.55
Iodine value	120.60 ± 0.03	78.10 ± 0.01
Unsaponified Matter (%)	13.90 ± 1.13	3.13 ± 0.17
Acid value (%)	89.60 ± 1.20	29.26 ± 0.32
Free Fatty Acid (%)	44.80 ± 1.20	14.63 ± 0.32
Peroxide value (mEq/kg)	123.26 ± 0.25	98.86 ± 0.15

numbers below 100 while drying oils have iodine numbers between 130-200 (Pomeranz and Meloan, 1978). From the iodine values obtained in this study, it would appear that the pulp oil might be more suitable in the production of oils for vanishes and paints since it is more drying than the seed oil although the oil content of the pulp is much lower (2.53%) than that of the seed (10.91%) (Table 3.1). Dalziel (1948) had earlier reported the edible nature of the seed oil and indeed its suitability in the production of varnishes. However, the low iodine value of the seed oil indicate that it is more stable than the pulp oil.

3.6.1.3 Unsaponified Matter.

Unsaponified matter which is usually dissolved in fats and fatty acids include sterols, higher alcohols and some hydrocarbons but it consists principally of steroids and terpenes originally present in the lipid sample (Christie, 1973). Tamarind seed oil contains 3.13% of unsaponifiable matter while he pulp oil contains 13.9%. The terpenes contained in the unsaponifiable matter of the pulp oil are probably the active ingredient that is responsible for the medicinal properties of tamarind fruit especially as it has been shown that the pulp does not contain alkaloids (Hamman, 1976 and Olakanmi - personal communication). The vitamins A, D, E and K which are also non-saponifiable may form part of this unsaponified matter.

3.6.1.4 Acid value

The acid value of a lipid measures the extent to which the

constituent glycerides have been decomposed by lipase action, and this decomposition is usually accelerated by heat and light. Deterioration of grain and milled products have been reported to be accompanied by increased acidity and the acids formed include free fatty acids, acid phosphates and amino acids. The free fatty acids however are formed at a much faster rate during early stages of deterioration, than the other types of acidity combined (Pomeranz and Meloan, 1978). The pulp oil has a free fatty acid value and an acid value of 44.8% and 89.6% respectively (Table 3.7). These values are much higher than those of the seed oil which are 14.63% and 29.26% respectively (Jacobs, 1951). Generally, high free fatty acids of an oil indicates the deteriorating condition and lower edibility of the oil because of the development of objectionable flavours and odours and hence reducing the consumer acceptance of the oil.

3.6.1.5 Peroxide value.

On exposure to oxygen, lipids form peroxides which leads to the deterioration of tissues in vivo. This is responsible for the spoilage of foods (Gurr and James, 1971) and is caused by the free radicals produced during the peroxide formation. Oxidative rancidity involves the addition of atmospheric oxygen across the double bonds in unsaturated fatty acids in the presence of enzymes or certain chemical compounds. The odours and flavours associated with oxidative rancidity are mostly due to liberation of short chain carboxylic acids. The peroxide value of the pulp oil (123.26mEq/kg) is higher than that of the seed oil (98.86mEq/kg) (Table 3.7).

A comparison of the properties of the pulp and seed oils of tamarind (Table 3.7) reveals that the former has higher free fatty acid value, acid value and perioxide value. This is not unexpected in view of the higher saponification value and iodine number possessed by tamarind pulp oil. The high saponification value indicates the presence of gycerides of low molecular weight fatty acids, which will undergo hydrolytic rancidity with the formation of free fatty acids and glycerol. The high iodine number also indicates that the pulp oil will be more liable to undergo oxidative rancidity due to its higher degree of unsaturation, thus increasing the free fatty acids present.

3.6.2 Thin layer Chromatographic (TLC) Separation of the Extracted Lipids

Thin layer chromatographic separation of the lipids from both pulp and seed show that both the pattern of separation and chromatogram were dissimilar. However both of them were resolved into five components numbered consecutively (I-V) from the base. Their Rf values are shown in Table 3.8. Marked difference could be seen in the Rf values of both samples showing clearly, that the two lipids have different composition. Two dimensional TLC showed that component II of the pulp sample is a mixture of at least two components. On the otherhand, the two dimensional TLC of the seed oil showed that components II and IV were further resolved into two each and component V into four. These components were however not identified for lack of lipid standards.

Preliminary gas chromatographic run of the seed oil (courtesy of

Table 3.8

Rf values of the Lipids Extracted from both the pulp and the seed of Tamarind Fruit. Developing solvent mixture was petroleum Ether: Diethyl Ether: Acetic Acid (80:20:1 v/v/v).

Component	Rf Values	
	Pulp	Seed
I	0.23^{++}	0.36^{+}
II	0.35^{++}	0.44^{++}
III	0.47^{++}	0.58^{+++}
IV	0.87^{+}	0.73^{+++}
V	0.99^{+}	0.94^{++++}

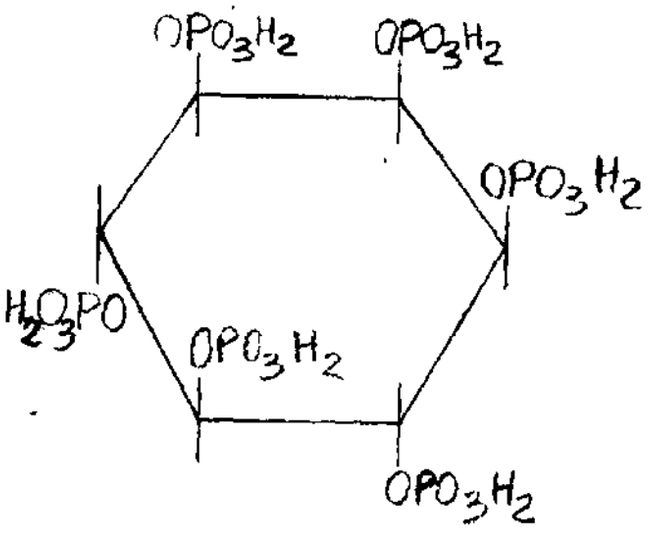
(+) gives the relative intensity of each spot where ++++ > +++ > ++ > +.

Dr. Thomas, Chemistry Department, Universita Degli Studi di Bologna, Italy) showed that there are at least ten fatty acid components (Figure 3.5). Identification of these fatty acids is being pursued. However, an earlier worker had reported the fatty acid components of Tamarindus indica from Sudan to contain 19.2% C_{16} and C_{18} saturated, 11.8% C_{20}, C_{22}, C_{24} saturated, 27.0% Oleic acid and 42.0% Linoleic acid (Hilditch and Williams, 1964).

3.7 Anti-nutritional Factors in Tamarind Fruit.

3.7.1 Phytic Acid

Phytic acid (inositol hexaphosphoric acid) with the following structure:



has 12 replaceable hydrogen atoms and it can form stable insoluble salts with metals like calcium, magnesium, iron and copper (Oberleas, 1973). This therefore makes the metals unavailable to the human body and to those of other animals such as rat and dog (Harris, 1955) except the ruminants

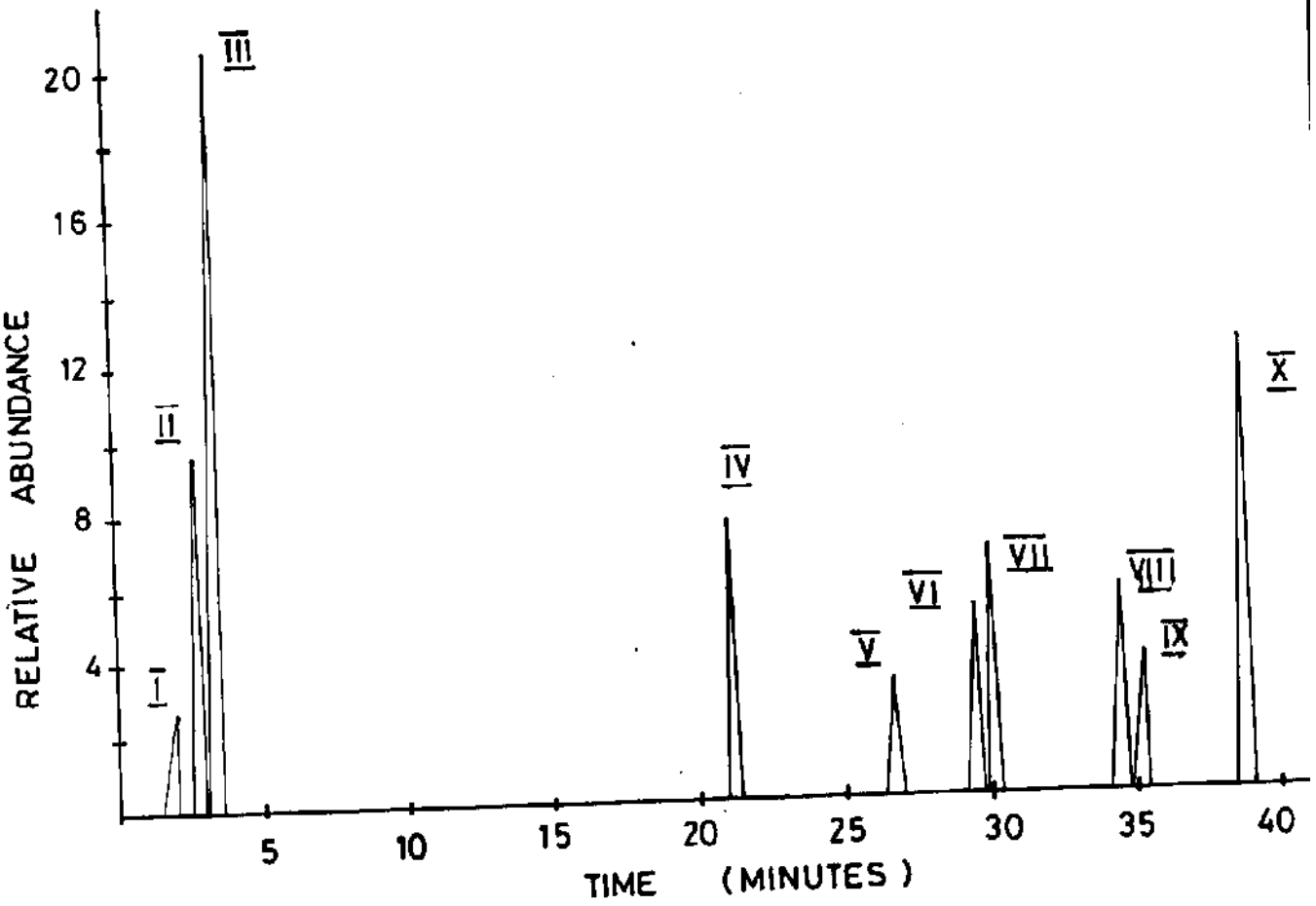


FIG. 3.5. Gas Chromatogram of Methyl Esters of the Tamarind seed Lipid.

(Tillman and Brethour, 1958). Availability of zinc can also be influenced by a high dietary content of phytate (Reinhold, 1971). While the phosphorus in phytic acid cannot be hydrolysed by intestinal enzymes of man and these other animals, the ruminants, on the otherhand, have the ability to utilize it efficiently and this is said to be due to the hydrolysis of phytic acid by the phytase produced by the rumen micro-organism (Raun et al, 1956). Influence of phytic acid on the nutritional value of specific diets varies depending on the mineral composition of that diet, and it appears that a relatively high level of dietary phytate would be necessary to cause an antinutritional effect under normal condition (Eggum and Campbell, 1979).

The tamarind pulp is found, in this study, not to contain any detectable quantity of phytate - phosphorus (phytate - P), that is, phytic acid. The seed however contains 47mg per 100g sample. It has been reported that the nutritive value of raw moth bean seed cultivar which contains phytate-P ranging between 852 - 899mg per 100g is impaired but to a lesser extent than soya beans (Khokhar and Chauhan, 1986). Comparing the values of the phytate-P obtained in this study with the above, it is expected that the effect of phytic acid in tamarind seed on its nutritional value would be minimal. Phytic acid is reported to lead to hard-cook in legume grains (Boulter, 1982).

3.7.1.1 Effect of Processing on Phytic acid Level in Tamarind seed.

Heat has a considerable effect (Figure 3.6A) on the level of phytic acid in seed. Ordinary cooking gave a remarkable decrease (about

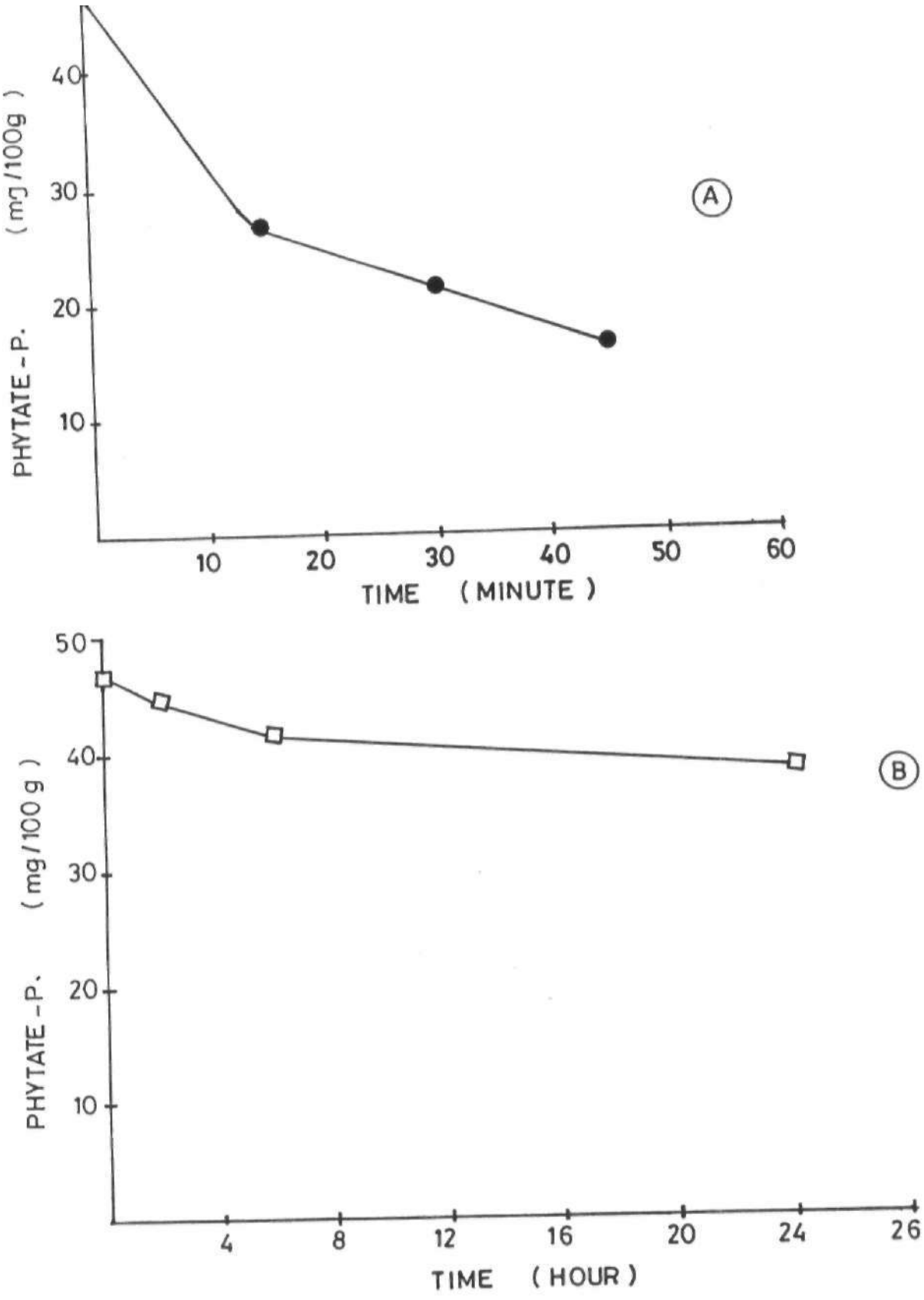


FIG. 3.6: The effect of cooking (●) and soaking (□) on Phytate-P. level in Tamarind seed.

56% after 30 minutes) in phytic acid level thus illustrating the thermolabile nature of phytic acid. Soaking of the seed sample did not however have much effect (Figure 3.6B). Only about 17% decrease was obtained after Soaking for 24 hours, meaning that, the amount of phytate ions leached into soaking water is low. The cultural practice of fermenting both the African locust bean and tamarind seeds has been reported to reduce their contents of hydrocyanic, phytic and oxalic acids (Chakraborty, 1976 and Eka, 1980), although the extent of reduction was not given. In this study, heating is obviously the preferred method of removing phytic acid.

3.7.2 Trypsin Inhibitor.

The toxicity of trypsin inhibitors is in their impairing protein digesting enzymes (Proteases)-trypsin and chymotrypsin (Duffus and Slaughter, 1980). Figure 3.7 shows the antitryptic activity of tamarind pulp and seed in water and 0.5M NaCl. It is clear that there is more trypsin inhibitor in the pulp than in the seed. The NaCl (0.5M, approx. 3%) extracts of both pulp and seed samples showed more inhibitory effect than the water extracts. This is because the concentration of inhibitors is more in the NaCl extracts than in the water extracts, as trypsin inhibitors being protein in nature are extracted more with strong salt solution (section 3.3.2.1). In addition, it has been reported that many types of proteases are strongly inhibited by 2-3% NaCl (Troller and Christian, 1978). Figure 3.7 also shows that antiproteolytic effect increases with increase in the concentration of the inhibitor in agreement with the report of Roy and Rao, (1971).

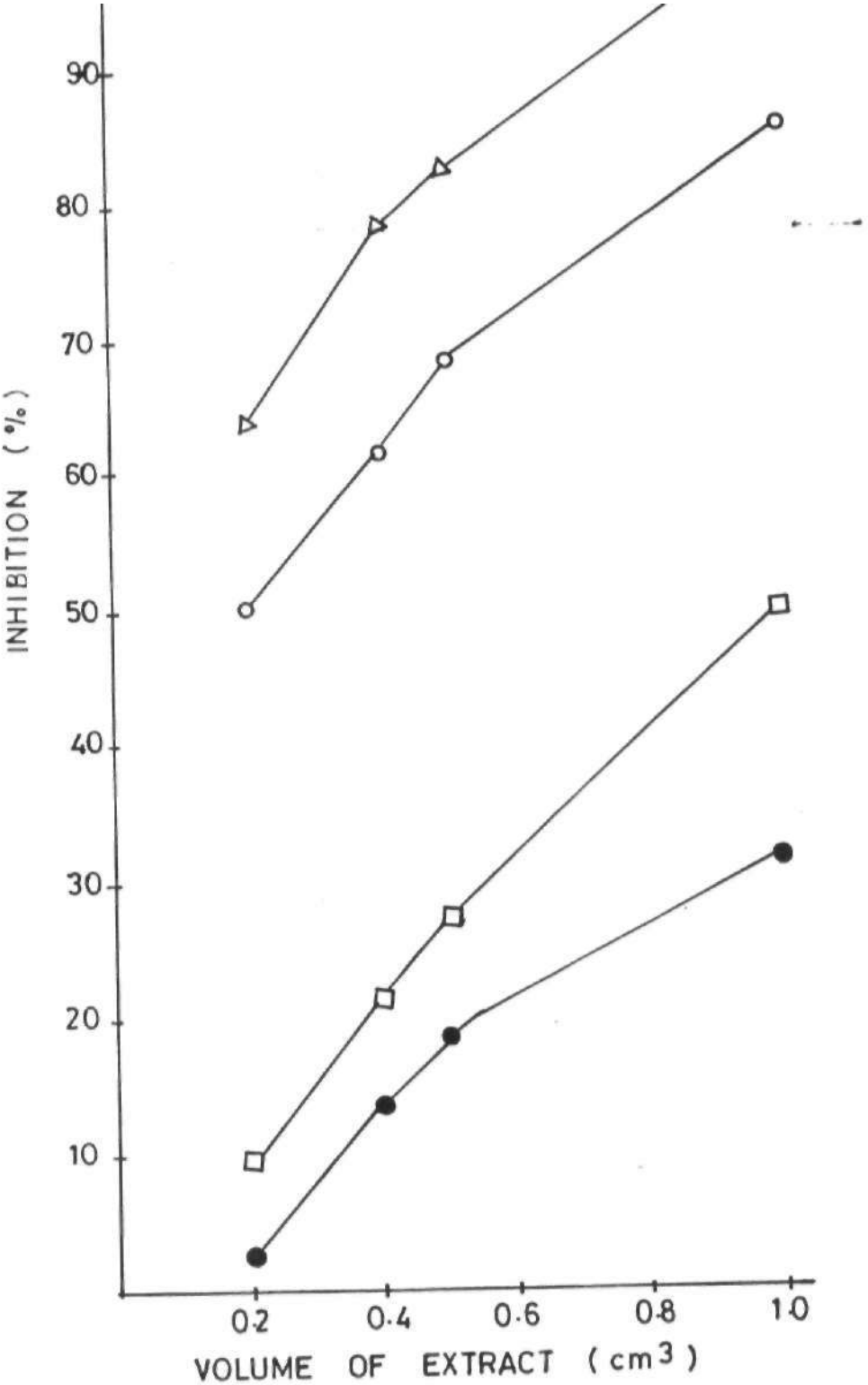


FIG 3.7: Antitryptic activities of Tamarind Pulp and seed in both sodium chloride (0.5M) and water extracts. Sodium Chloride extract of pulp (△); water extract of pulp (○); Sodium Chloride extract of seed (□); water extract of seed (●).

3.7.2.1 Effect of Heat on Anti-tryptic Activities

Figures 3.8 and 3.9 show the effect of ordinary heating on water bath and autoclaving respectively, on trypsin inhibitor activities in 0.5cm³ levels of sample extracts. Antitryptic activity decreased progressively with increasing heating periods, thus indicating the heat labile nature of trypsin inhibitor. While a boiling period of 30minutes was enough to remove all antitryptic activity in seed samples, a longer time period (60minutes for water extract and 120minutes for NaCl extract) was needed to remove all trypsin inhibitors from the pulp samples. Autoclaving involves heating to very high temperatures under reduced pressure. It is therefore not surprising that very short time periods (15 to 30minutes) were required to remove all anti-tryptic activities from the samples.

3.8 Sorption Isotherm of Tamarind Fruit

The concept of equilibrium moisture content (e.m.c) is important in the study of the preservation and storage potential of a food. This is because the e.m.c determines the minimum moisture content (m.c) to which the food should be dried under a given set of conditions. The main aim of drying any product is to lower the m.c to a level at which growth of spoilage micro-organisms is prevented.

When a product is exposed to an atmosphere of uniform relative humidity (r.h) and constant temperature for a defined period of time, the intergranular r.h. reaches a constant value known as the equilibrium relative humidity (e.r.h). A plot of the m.c of the product at equilibrium

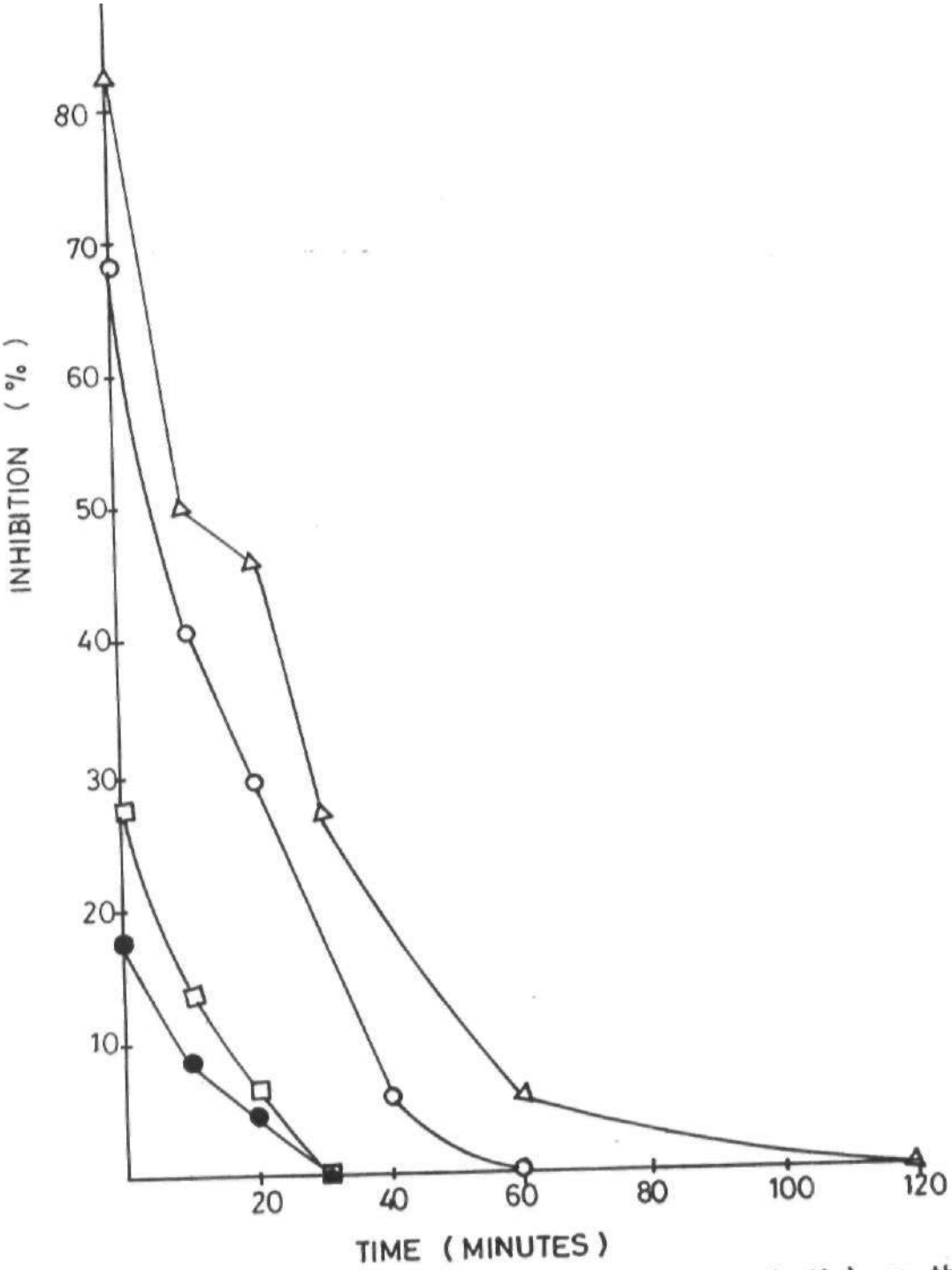


FIG 3.8: The effect of heat (water bath) on the Trypsin Inhibitor Activity in 0.5 cm^3 of sample extract. Sodium Chloride extract of pulp (Δ); water extract of pulp; (O); Sodium Chloride extract of seed; (□) water extract of seed (●).

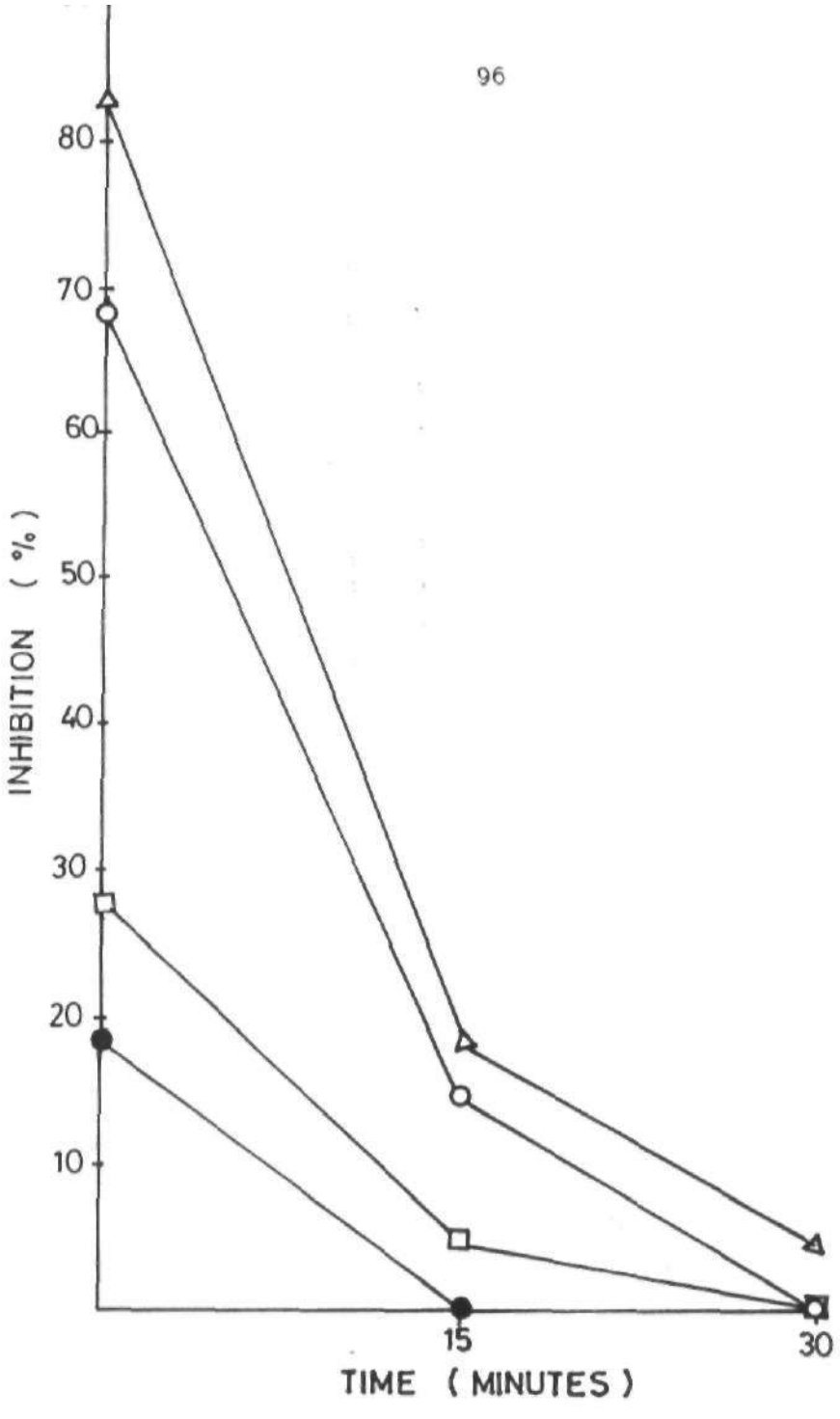


FIG 3.9: Effect of Autoclaving on the Trypsin Inhibitor Activity in 0.5 cm^3 of sample extract, Sodium Chloride extract of pulp (Δ); water extract of pulp (O); Sodium Chloride extract of seed (□); water extract of seed. (●).

against e.r.h or water activity (a_w = r.h/100) is known as the moisture sorption isotherm. Sorption isotherms of a product is therefore, essential in evaluating the storability potential of that product in this case tamarind pulp (Denloye and Ade-John, 1985).

Tamarind fruits are harvested around October to December. The fruits without the epicarp are stored in jute bags to ensure an all year round supply of the fruits. The minimum m.c. under which this fruit can be safely stored can be determined from the sorption isotherm which is given in Figure 3.10. The isotherm of the pulp has a J-shape and can be divided into three zones: the not very distinct, low water activity and low moisture content end (Zone 1); the increasingly high water activity but relatively low moisture region (Zone 11), and the high water activity and correspondingly high moisture content end (Zone 111). Zone 1 is the region where the intrinsic water in foods corresponds to the immobile water in food (Fennema, 1985). This region is however much narrower in tamarind pulp than what generally obtains in sigmoidal sorption isotherm of foods, which is normally referred to as the monolayer region. It appears therefore that tamarind pulp has such a small monolayer or none at all, as is found particularly in foods that consist largely of soluble low molecular weight components (Troller and Christian, 1978). The lack of monolayer value of tamarind pulp may be due to its high water-soluble solids (Table 3.1) while the J-shape of the isotherm can be attributed to the high sugar content of the sample (Wolfe et al, 1972 and Fennema, 1985).

Following this zone is the absorptive phase (Zone 11) where water is taken up and released by the food as the water activity increases. The

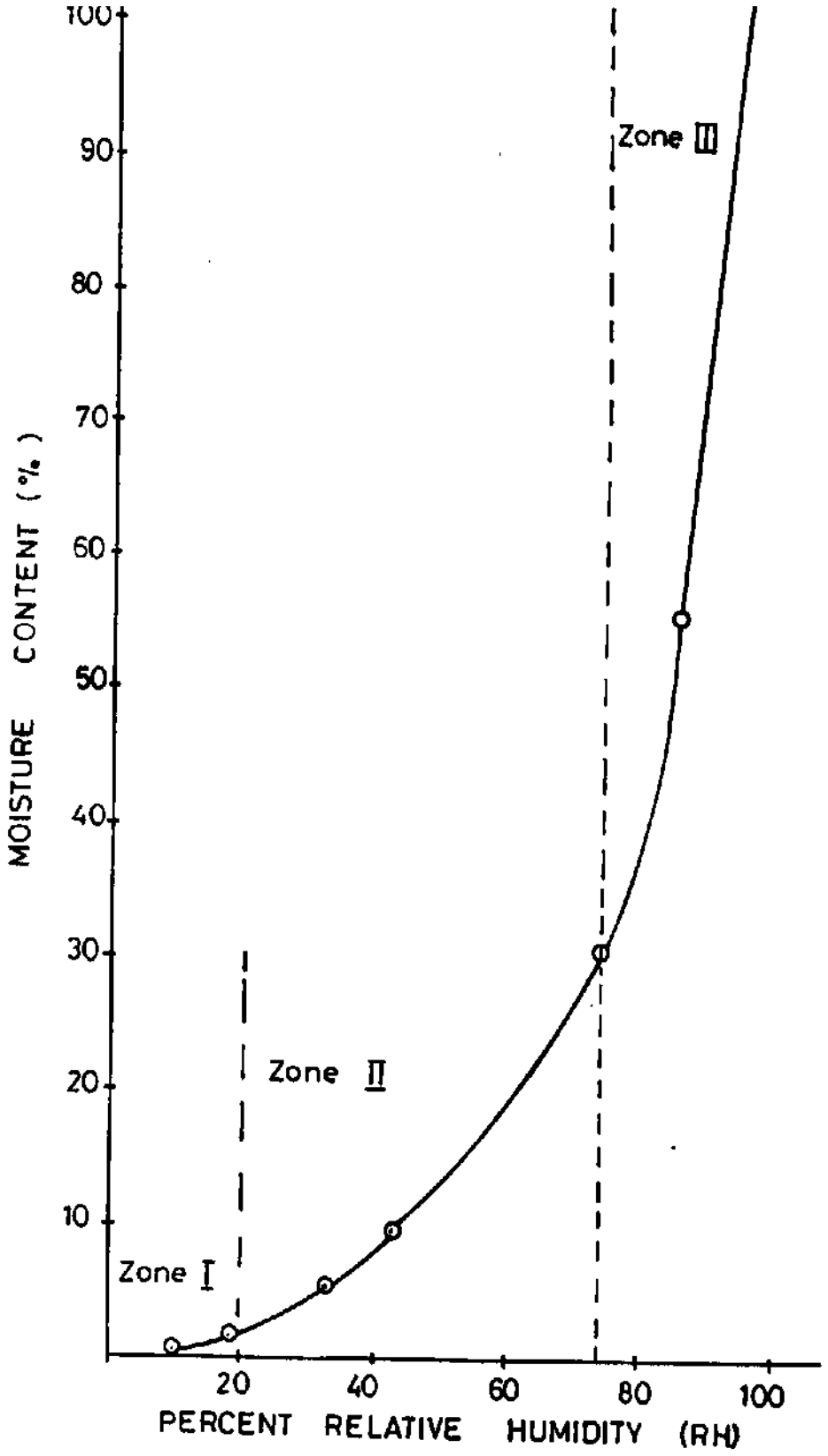


FIG 3·10: Sorption Isotherm for Tamarind pulp at 27°C

rate of chemical reaction increases in the food as it absorbs moisture up to the region of water activity 0.74. Water in Zone 111 is least strongly bound as is indicated by the steepness of the isotherm and this is the most mobile phase in terms of molecular water. At relative humidity of 74% (a_w = 0.74), the sample appears to absorb moisture more rapidly and this is because, the water holding capacity of food is no longer predictable, at high relative humidities (Fennema, 1985).

Mould growth on pulp samples was visible only at relative humidities greater than 74% after storage for about 80 days. This remarkable keeping qualities of the pulp can be attributed to its highly acidic nature (pH 2.7). A reduced pH retards the growth of many spoilage organisms especially bacteria (Liebrand, 1978). This explains the all-through the year supply of tamarind in the northern parts of Nigeria. Although the pulp is remarkably resistant to mould growth, it is susceptible to insect attack as is observed in fruits sold in the market from around June to October.

The brown pulp of tamarind becomes deeper in colour with a characteristic stickly feel and a decrease in sourness after long term storage. The fruit at this stage has obviously undergone some biochemical change with the absorption of moisture from the atmosphere, thus giving rise to a high moisture content. The browning reaction involves the carbohydrates in the fruit and is known to improve or impair the consumer acceptance and nutritional value of many foods (Stadtman, 1948).

It is generally accepted that mould becomes a problem at 70% r.h and above under warm conditions as obtained in the tropics (Pixton, 1967). Thus, the safest moisture content of a sample will be that at 70% r.h or

less. Indeed, many types of fruits are preserved by dehydration to water activity of 0.65-0.60 (65%-60% r.h) (Troller and Christian, 1978). Since it is generally more difficult to measure r.h than it is to determine m.c., it is the m.c that is usually taken as the criterion for safe storage (Pixton, 1967). Thus, in this study, to be safe from fungal attack and possibly insect attack, it is recommended that the tamarind fruit be stored at or below 20% moisture content at normal storage temperature.

3.9 Conclusion and Suggestions for Further Work.

Tamarind pulp with its high sugar content (41.2%), high calorie (327.30 Cal. per 100g), fair protein and lipid contents (8.79% and 2.53% respectively) and fairly good mineral composition, is a good food supplement especially when taken as porridge with high- protein foods like beans. Its soury taste also compensates for the sugary taste which is normally sort after in porridges and juice.

The seed protein content is quite high (26.93%) and may be a good source of protein concentrate for poults considering also that it has appreciable quantities of calcium, magnesium, phosphorus, potassium, zinc and copper which are necessary in their diets (appendix K). It is however essential to know the amino acid constituents of this protein and their bioavailability.

The seed is known to contain low quantities of oxalic and, hydrocyanic acids (Chakraborty, 1976) and is found in this work to contain low quantities of phytic acid (47mg/100g) and trypsin inhibitors. These antinutritional factors might otherwise affect the availability of the

mineral elements and the proteins contained therein. Again, for inclusion in a poultry feed, the seed's content of vitamins A, D, E, K_1, thiamine, riboflavin, pantothenic acid, niacin, biotin, choline, folacin, vitamin B_{12} and iodine would need to be investigated.

Thin layer and gas chromatographic separations of the sample lipids have shown that these contain several glyceride units which would require further investigation since they were not identified for lack of lipid standards. The seed has however been reported to contain 42% linoleic acid and 27% oleic acid which are essential to animal and human nutrition (Hilditch and Williams, Eds. 1964). The pulp lipid was found to contain high unsaponifiable matter and generally, unsaponifiable matter consists mainly of terpenes. This fraction should be further examined for it might contain the active ingredient responsible for the use of the pulp in folk medicine.

The antimicrobial action of the acidic pulp should also be investigated for use as preservative.

REFERENCES

Abede, A.A. (1981). Chemical analysis of some Nigerian foodstuffs. M.Sc Thesis, Ahmadu Bello University, Zaria. Nigeria.

Addy, E.O.H. (1978). Studies on the nutritive value of leaves and fruits of the Baobab tree Adansonia digitata M.Sc. Thesis, Ahmadu Bello University, Zaria. Nigeria.

Allen, S.E., Ed. (1974) Chemical analysis of ecological materials. 1st Ed. Blackwell Scientific Publications, Oxford.

Analysis Committee of oil Chemists' society, (A.C.O.C.S.). Oil and Soap (1940) 17:12.

Andrews, P. (1965). The Gel Filtration behaviour of proteins related to their molecular weights over a wide range. Biochem. J. 96:595-606.

Association of Official Analytical Chemists, AOAC (1980). Official Methods of Analysis. 13th Ed., Horwitz, W.,Ed. AOAC Washington D.C.

Baough, R.J. and Travis, J. (1976) Human Leukocyte Granule Blastase: Rapid Isolation and Characterization. Biochemistry 15:836.

Boulter, D. (1982). The composition and nutritional value of legumes in relationship to crop improvement by breeding Proc. Nutr. Soc. 41:1.

Braverman, J.B.S. (1963) Introduction to the Biochemistry of Foods 1st Ed. Elsevier Publishing Company, Amsterdam. pp.269-282.

Brouk, B. (1975) Plants consumed by Man Academic Press. pp. 148-203

Carnegie, P.R. (1965) A Peptide -mapping technique for the estimation of molecular size. Nature 206:1128-1130.

Chakraborty, R. (1976) Studies on hydrocyanic, oxalic and phytic acids

content of foodstuffs. M.Sc. Thesis, Ahmadu Bello University Zaria, Nigeria.

Chrispeels, M.J. and Sadava, D. (1977) Plants, Food and People. W.H. Freeman and company, San Francisco. pp 9-38.

Christie, W.W. (1973) Lipid Analysis: isolation, separation, identification and structural analysis of lipids. 1st Ed. Pergamon Press, Oxford. pp46-120.

Clegg, K.M. (1956) The application of the anthrone reagent to the estimation of starch in cereals. J.Sc. Fd. Agric. 7: 40-44.

Conn, E.E. (1973) Cyanogenetic glycosides In: Toxicants occuring naturally in foods. National Academy of Sciences, Washington D.C. pp. 303.

Cookerell, T.; Halliday, D. and Morgan, D.J. (1975) Quality control in the animal foodstuffs Manufacturing Industry, In: Tropical Products Report No 77, London.

Dalziel, J.M. (1948) The Useful Plants of West Africa: Crown Agents of the Colonies, London.

Dam's method In: Practical Laboratory Manual, Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.

Davadas, R.P., Devadoss, M.V and Rawlands, R.J. (1964). Nutr. Dietec. 1:34-39.

Denloye, A.O. and Ade-John, A. (1985) Moisture Sorption Isotherms of some Nigerian food grains. J. Stored Prod. Res. 21:53-58.

Deshpande, S.S. and Cheryan, M. (1984). Effects of phytic acid, divalent cations and their interactions on α- amylase activity.

J. Fd. Sc. 49:516.

de Wit, H.C.D. (1966) Plants of the World: The higher plant 1. 1st Ed. Thames and Hudson, London. pp.10-50.

Duckworth, R.B. (1966) Fruits and Vegetables. 1st Ed. Pergamon Press. pp.xiii-xxiv.

Duffus, C.M. and Slaughter, J.C. (1980) Seeds and their uses. 1st Ed. John Wiley and sons, New York. pp.78-100.

Duke, J.A.; Reed, C.F.; Weder, J.K.P. (1981) Tamarindus indica L. In: Handbook of Legumes of world economic importance. Plenum Press, New York. pp228-230.

Eastwood, M.A. (1974) Dietary fibre in human nutrition. J.Sc. Fd. Agric. 15: 1523-1527.

Ega, R.A. (1986). Potential of cocoyam, cassava, locust bean and tamarind as carbohydrate and protein sources in animal feed. Ph D. Thesis. Ahmadu Bello University Zaria, Nigeria.

Eggum, B.O. and Campbell, L.D. (1979) "Nutritional and antinutritional assay" In: Seed Protein Improvement in Cereals and Grain Legumes. vol.1. International atomic energy agency (IAEA) vienna.

Eka, O.U. (1980). Effects of fermentation on the nutritional status of locust beans. Food Chem. 5:303-308.

Ekpete, B.U. (1985). Chemical Analysis of Banana (Musa Paradisiaca) A study of compositional changes from maturation to ripening. M.Sc. Thesis. Ahmadu Bello University, Zaria, Nigeria.

Erdman, J.W. Jr. (1979) Oil seed phytates: nutritional implications. J. Am.

Oil chem. Soc. 56:736.

FAO, (1965) Protein Requirement: FAO Nutrition Meetings Report series No 37. Rome.

FAO/WHO (1973) Energy and Protein Requirements: World Health Organization Technical Report No 522 Rome.

Fennema, O.R. (1985) Water and Ice In: Food Chemistry 2nd Ed. Marcel Dekkar Inc. New York. pp. 23-67.

George, A.J. (1965) Legal status and toxicity of saponins. Food Cosmet. Toxicol. 3:85.

Green, T.R. and Ryan, C.A. (1972) Wounded-Induced Proteinase Inhibitor in Plant Leaves: A Possible Defense Mechanism against Insects. Science 175:776.

Gurr, M.I. and James, A.T. (1971) Lipid Biochemistry: An introduction. 2nd Ed. Chapman and Hall, London. pp. 7-22.

Hamman, R.L. (1976) Phytochemical evaluation of the plant drug Tamarindus indica. B.Sc. project. Ahmadu Bello University, Zaria, Nigeria.

Harris, R.S. (1955) Phytic acid and its importance in Human nutrition. Nutri. Revs, 13: 257.

Harvey, R.B. (1920) The relation between the total acidity, the concentration of hydrogen ion and the taste of acid solutions. J.Am. Chem.Sco. 42:712.

Hilditch, T.P. and Williams, P.N. (Eds.) (1963) The Chemical constitution of Natural Fats. 4th Ed. Chapman and Hall , London. p. 313.

Hodge, J.E. and Davies, K.A. (1952) Selected methods for determining

reducing sugars. USDA Bur. Agric. Ind. Chem. A.I.C. 333.

Hulume, A.C., Ed., (1970) The Biochemistry of Fruits and their products. 1st Ed. Academic Press, London. pp.8-20.

Jacobs, M.B., (1951) Chemical Analysis of Foods and Food Product 2nd Ed. D-Van Nostrand Company Inc. New York. pp 365-564.

Jacobs. M.B., Ed. (1951) The Chemistry and Technology of Food and Food Products 2nd Ed., Interscience Publishers Inc. New York. pp. 3-226.

Jardin, C. (1967) List of Foods used in Africa. FAO, Rome.

Khokhar, S. and Chauhan, D.M. (1986) Antinutritional factors in Moth bean (vigna aconitifolia): varietal differences and effects of methods of domestic processing and cooking. J.Fd. Sc. 51 (3) : 591.

Liebrand , J.T. (1978) Acids in Foods In: Encyclopedia of Food science edited by paterson and Johnson, Avi Publishing Company. Westport connecticut.

Liener, I.E. (1976) Legume toxins in relation to protein digestibility-A Review. J.Fd. Sci. 41: 1076.

Lowry, D.H.; Rosenbrough, N.J.; Farr, A.L.; and Randall, R.J. (1951). Protein measurement with folin-phenol reagent. J. Biol. Che. 103:265.

McCance, R.a. and Widdowson, E.M. (1946) The Chemical Composition of foods 2nd Ed. Med. Res. council special report series No 235. His majesty's stationary office (HMSO) London. pp. 70-91.

Mendeloff, A.I. (1978) Dietary Fibre and Gastro intestinal Diseases: some

facts and fancies. Medical clinics of North America 62 (1): 165-171.

Moore, J.c. (1964). Gel Permeation chromatography. I,A New Method for Molecular Weight Distribution of High polymers. J. Polym. Sci, A 2:835-843.

Oakenfull, D. (1981) saponins in Food- A Review. Food Chem. 6:19.

Obodo, G.A. (1982) Chemical Analysis of some leser known Nigeria savannah seeds. M.Sc Thesis Ahmadu Bello, University Zaria, Nigeria,

Ockerman, H.W. (1978) Source book for food scientists. Vol. 1. Avi publishing company, Westport connecticut.

Odilison, M.O.V. (1985) New Nigerian, 27th June.

Oke, O.L. (1966) The need for a new source of proteins. West African pharmacist 8 (6) : 112-116.

Oke, O.L. (1969) Role of hydrochanic acid in nutrition. World review of Nutr. Dietect. 11:170-198.

Oke, O.L. (1972) A nutritional policy for Nigeria. World review of Nutr. Dietec. 14:9.

Olakanmi, O. (1988) Personal communication. Dept. of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.

Owen, J. (1970) The medico-social and Cultural significance of Adansonia digitata (Baobab) in African communities.
African notes 6:24-36.

Oyeleke, O.A. (1984) Outlines of Food Analysis. 1st Ed. Ahmadu Bello University Press, Zaria. pp.29-30.

Oyenuga, V.A. (1968) Nigerian Foods and Feeding-stuffs : their chemistry and nutritive value. 3rd Ed. Ibadan University Press. pp. 1-70.

Oyenuga, V.A. and Fetuga, B.L. (1975) Dietary importance of fruits and vegetables (seminar topic 9) National Horticultural Research Institute, Ibadan, Nigeria.

Pearson, D. (1976) The Chemical Analysis of Foods. 7th Ed. Church-hill Livingstone, London. pp 488-497.

Pecsok, R.L., Shields, L.D, Cairns, T., McWilliam, I.G. (1976) Modern Methods of Chemical Analysis 2nd Ed. John Wiley and sons, New York. p.74.

Pharmacia Fine Chemicals (1969) Sephadex Gel Filtration in theory and Practice (Instruction Manual). Uppsala-Sweden. pp. 1-10.

Pixton, S.W. (1967) Moisture content - its significance and measurement in stored products. J. Stored Prod. Res. 3:35-47.

Platt, B.S. (1962). Tables of representative values of Foods commonly used in tropical countries. Med. Res. Council. special report series No 302. H.M.S.O., London pp. 2-16.

Pomeranz, Y., and Meloan, C.E. (1978) Food Analysis: theory and practice revised Ed. Avi Publishing Company Inc. Westport - Connecticut. pp.425-560.

Potter, N.N. (1968) Food Science Avi Publishing Company Inc. Westport - connecticut. p.483.

Purseglove, J.W. (1968) Tropicaal Crops. Dicotyledons. 1st Ed. John Wiley and sons New York.

Raganna, S., Ed. (1977) Manual of Analysis of Fruits and Vegetable Products Tat. Mc Graw-hill, New Delhi p.76.

Ranjhan, S.K. and Krishna, G. (1980). Laboratory Manual of Nutrition Research. Vikas publishing House PVT Ltd. pp. 56-63.

Raun et al (1956) J. Agr. Food Chem. 4:869 as cited by Chakraborty, R. (1976) Studies on hydrocyanic, oxalic and phytic acid content of foodstuffs M.Sc. Thesis. Ahmadu Bello University, Zaria, Nigeria.

Reinhold, J.G. (1971) High Phytate content of Rural Iranian Bread-a possible cause of human zinc deficiency Am. J.Clin. Nutr. 24:1204.

Roy, D.N. and Rao, P.S. (1971). Evidence, Isolation, Purification and Some Properties of a trypsin inhibitor in Lathyrus sativus. J. Agric. Food Chem. 19:257.

Scott, W.J. (1956) "Water Relations of Food Spoilage Micro-organisms. Advan. Food Res. 7:84-127.

Screedharan, V.P., Symon, B. and Narayanan, C.S. (1985). Moisture Influence on Fungal Growth in Black pepper J. Stored Prod. Res. 21:127-129.

Singh, M. and Krikorian, A.D. (1982) Inhibition of trypsin activity in vitro by phytate. J. Agric. Food Chem. 32:799.

Stadtman, E.R. (1948) Non-enzymic browning in fruit products. Advan. Food Res. 1:325-372.

Stanton, W.R. (1966) Grain Legumes in Africa. FAO-Rome. pp.1-26.

Stevens (1951) Handbook of Experimental Psycology. 1st Ed. John Wiley, New York. p.1148.

Thorpe, W.V.; Bray, H.G. and James, S.P. (1970) Biochemistry for Medical Students 9th Ed. J.A. Church-Hill, London. pp. 67-72, 409-421.

Tillman, A.D. and Brethour, J.R. (1958) Untilization of Phytin Phosphorus by sheep. J. Animal Sci. 17:104.

Tirah, S. (1981) Encyclopedia Britanica (Micropedia) IX. Encyclopedia Britanica Inc. p.792.

Troller, J.A. and Christian, J.H.B. (1978) Water Activity and Food. 1st Ed. Academic Press, New York. pp. 1-10.

Ulrich, R. (1970) In: The Biochemistry of Fruits and their Products. By Hulme, A.C. (Ed.) Acadamic Press, London. pp. 89-101.

Walker, A.R.P., (1976) Colon Cancer and Diet, with special reference to intakes of fat and fibre. Am. J.Clin. Nutr. 29 (12): 1417-1426.

Ward, A. T.; Marquardt, R.R.; Campbell, L.D., (1977). Further Studies on the Isolation of the Thermolabile Growth Inhibitor from the Faba Bean (Vicia faba L. var. minor). J. Nutr. 107:1325.

Wardlaw, C.W., (1937) Trop. Agric. Trin. 14:34.

Wilson, E.D., Fisher, K.H. and Garcia, P.A. (1979) Principles of Nutrition 4th Ed. John Wiley and sons New York. pp. 53-55.

Whitaker, J.R. (1963) Determination of Molecular Weights of Proteins by Gel Filtration on Sephadex. Anal. Chem. 35:1950-1953.

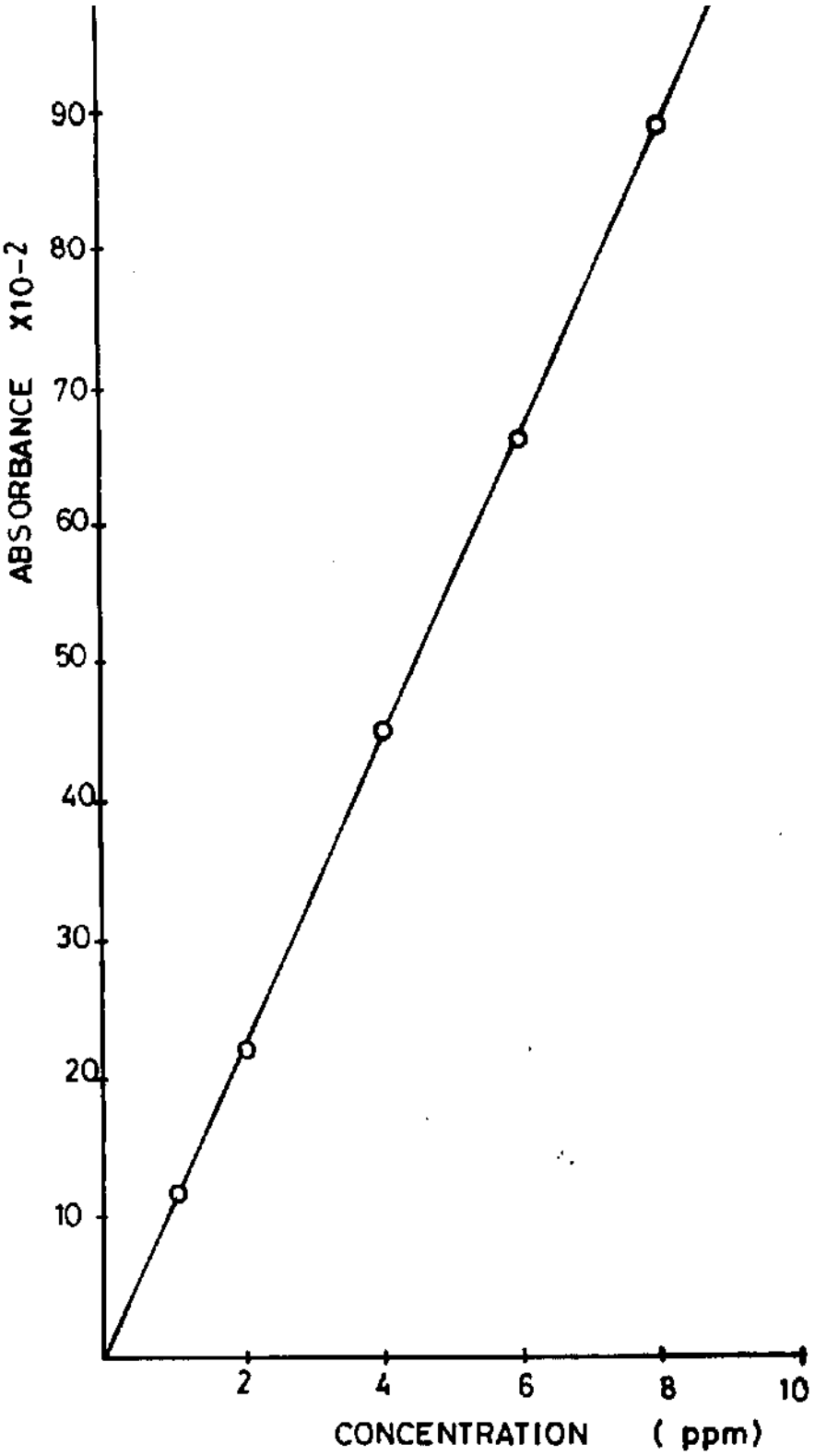
Whiteside, P.J., (1975) Pye Unicam Atomic Absorption Spectroscopy Data Book 1st Ed. Pye Unicam Ltd.

Whiting, G.C., (1970) In: The Biochemistry of Fruits and their Products, by Hulme, A.C. (Ed.) Academic Press, London . pp. 1-14.

WHO Expert Committee (1973). Trace Element in Human Nutrition. World Health Organisation Tech. Rep. series No 532.

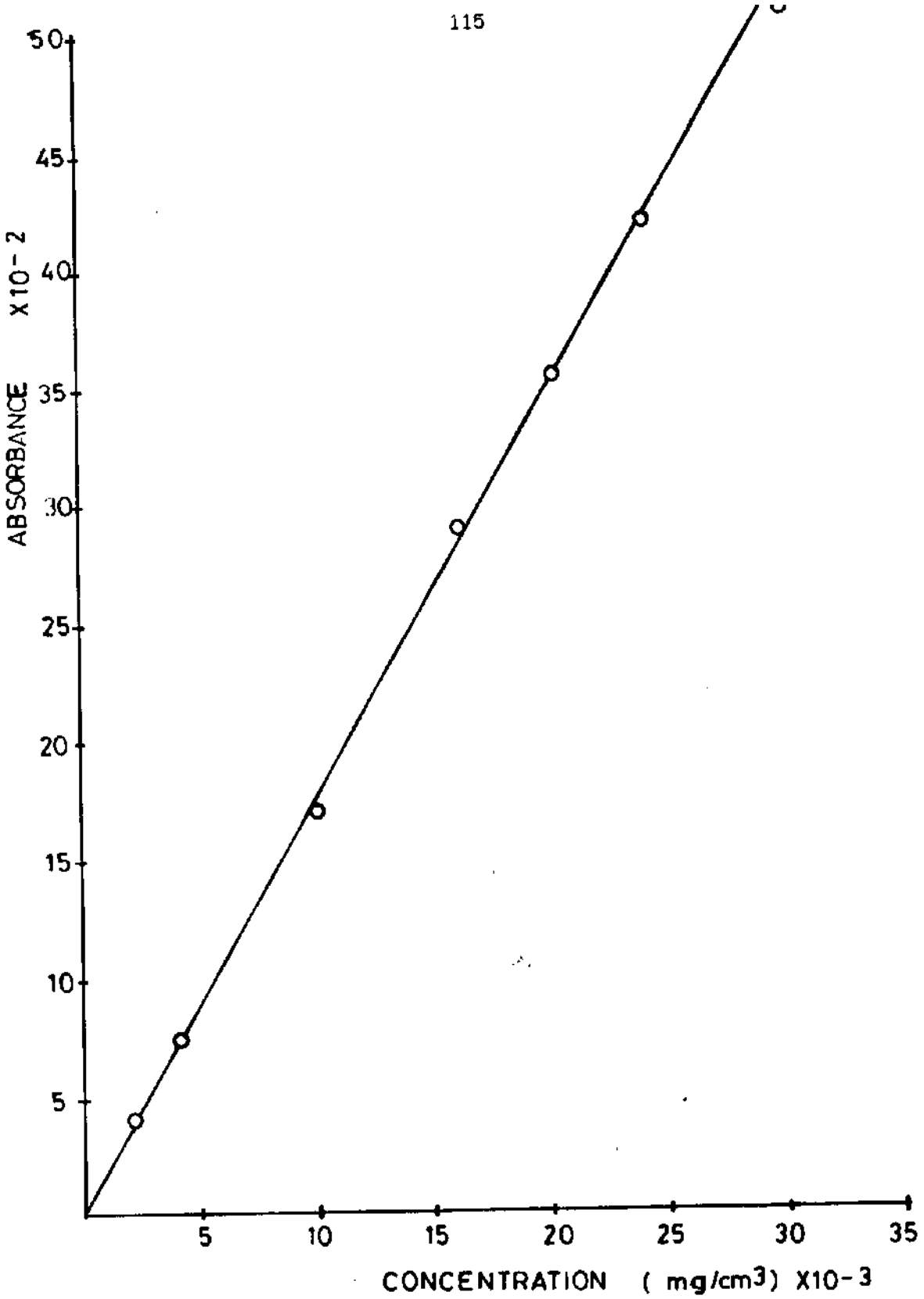
Whyte, P.L., and Selvey, N, (1969) Nutritional Qualities of Fresh Fruits and Vegetables 1st Ed. Future Publishing Company, New York. pp. 8-30.

APPENDICES



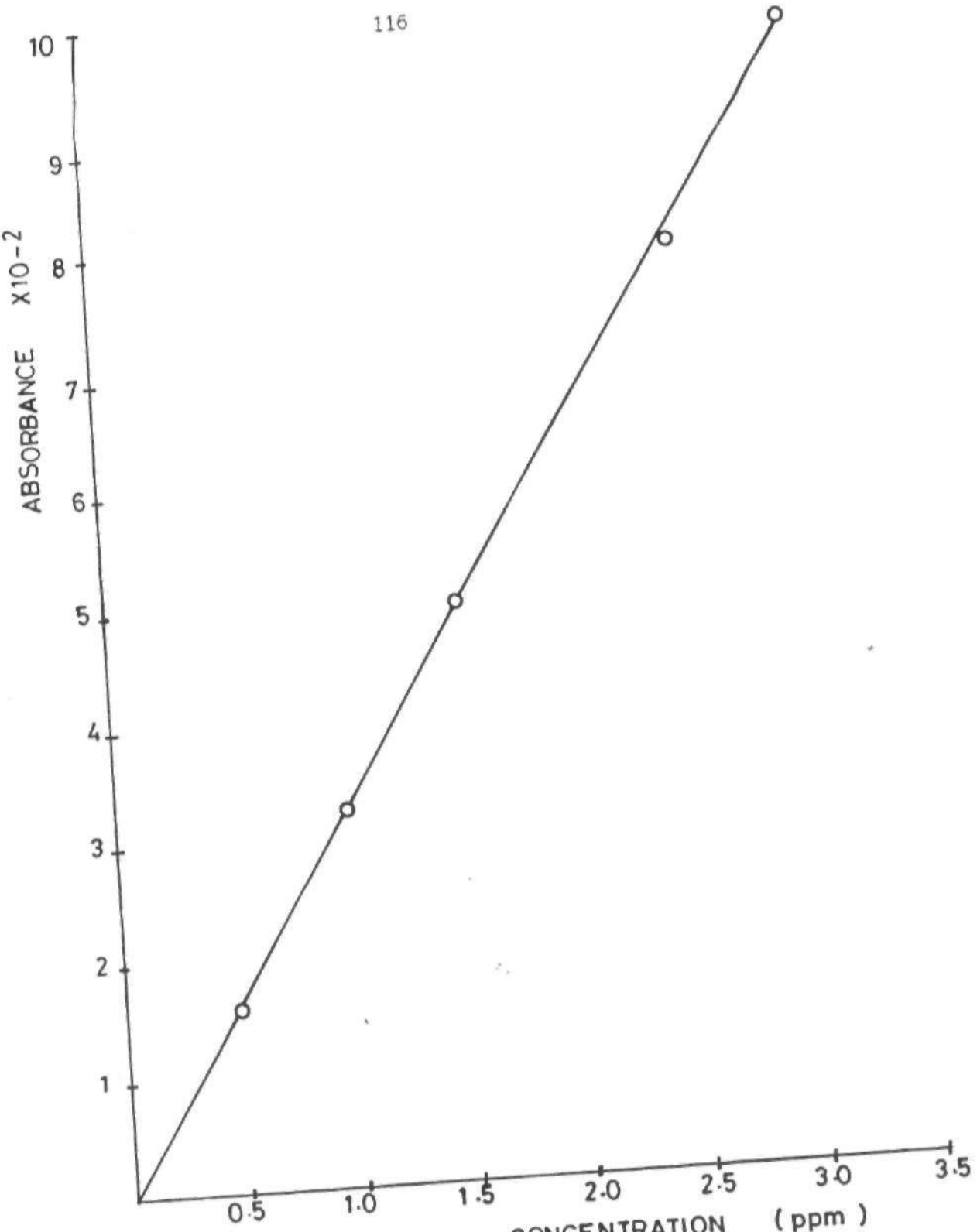
Calibration Curve for the determination of Calcium

Appendix A



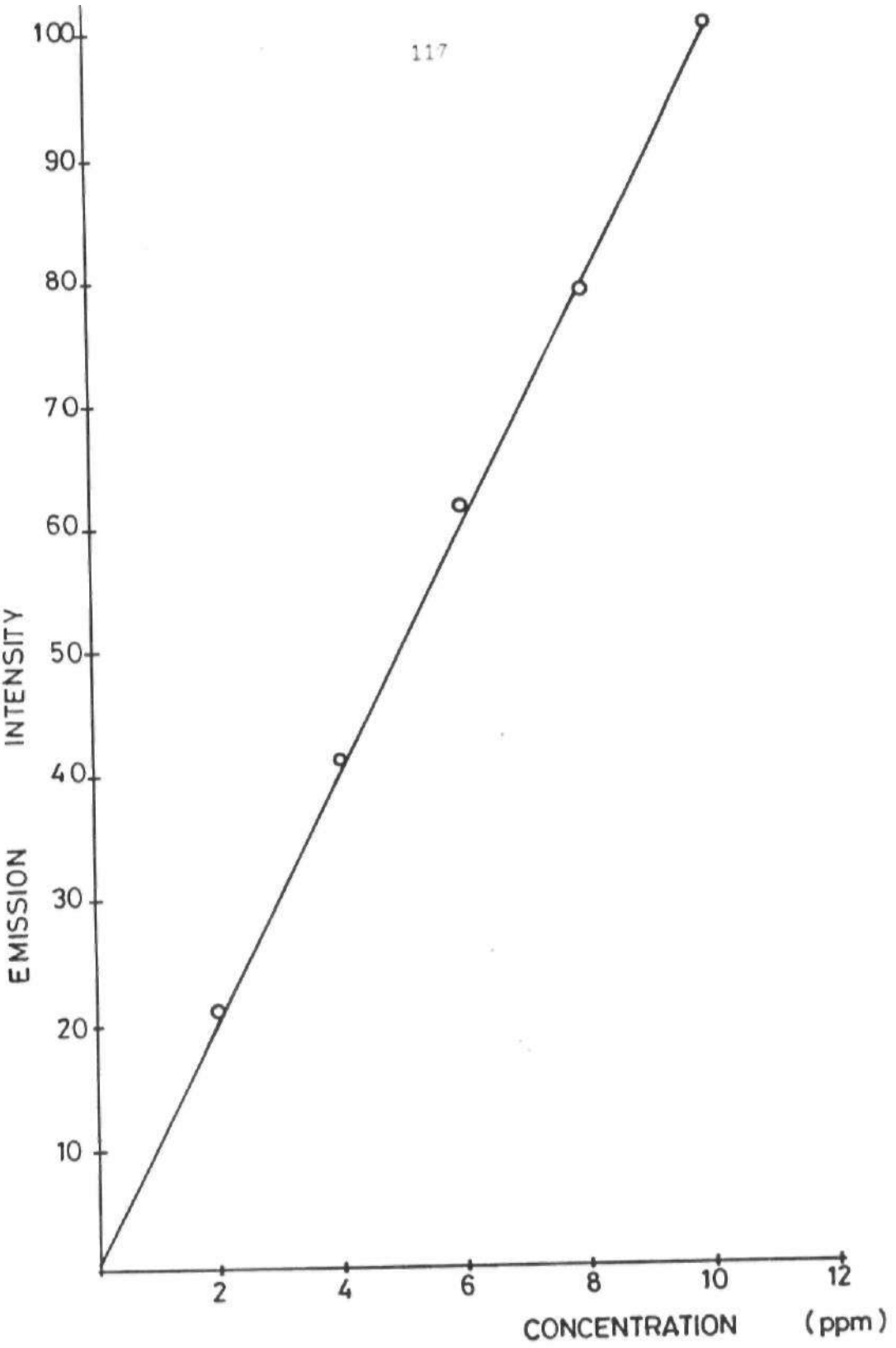
Calibration Curve for the determination of Phosphorus

Appendix B



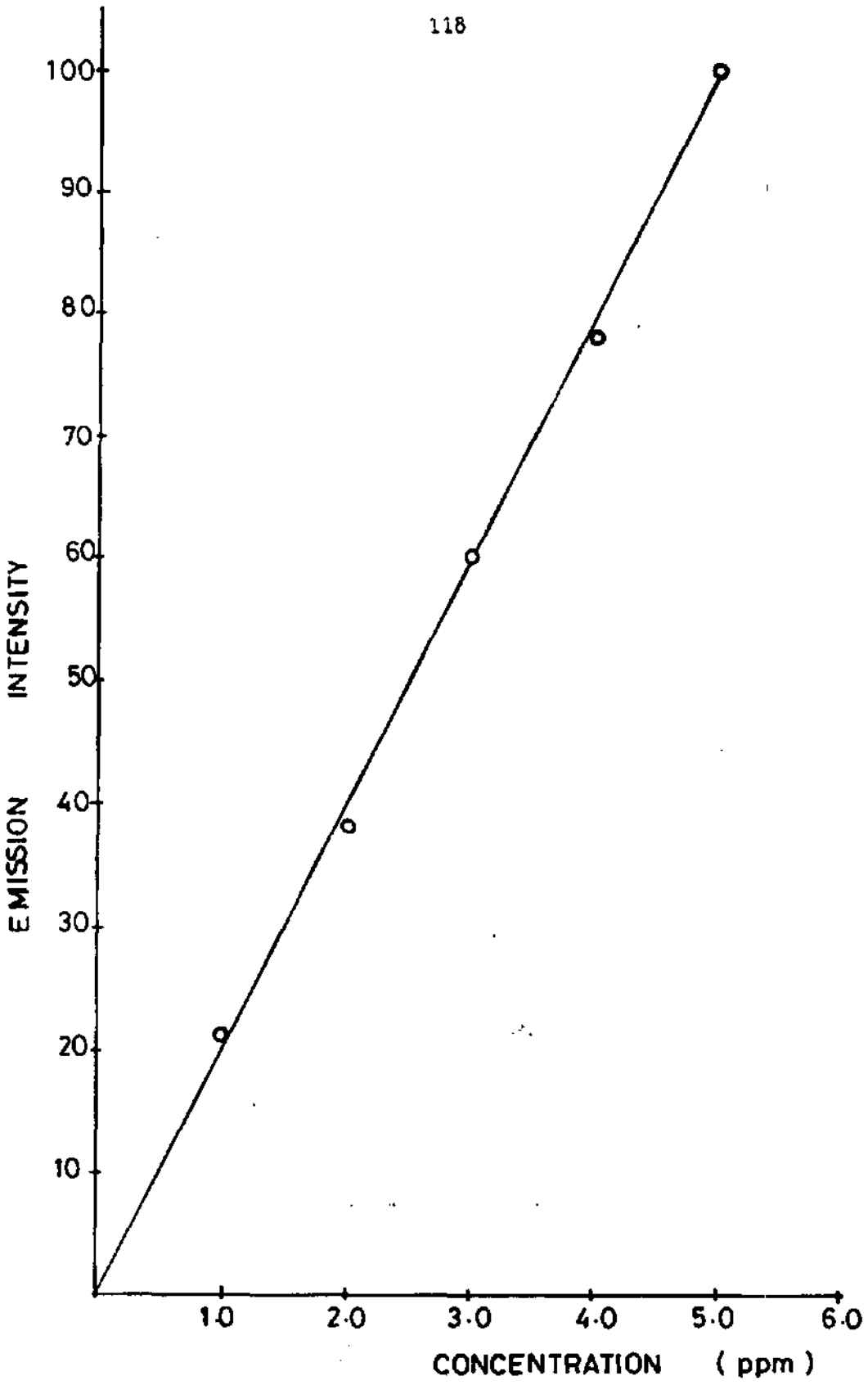
Calibration Curve for the determination of Magnesi

Appendix C



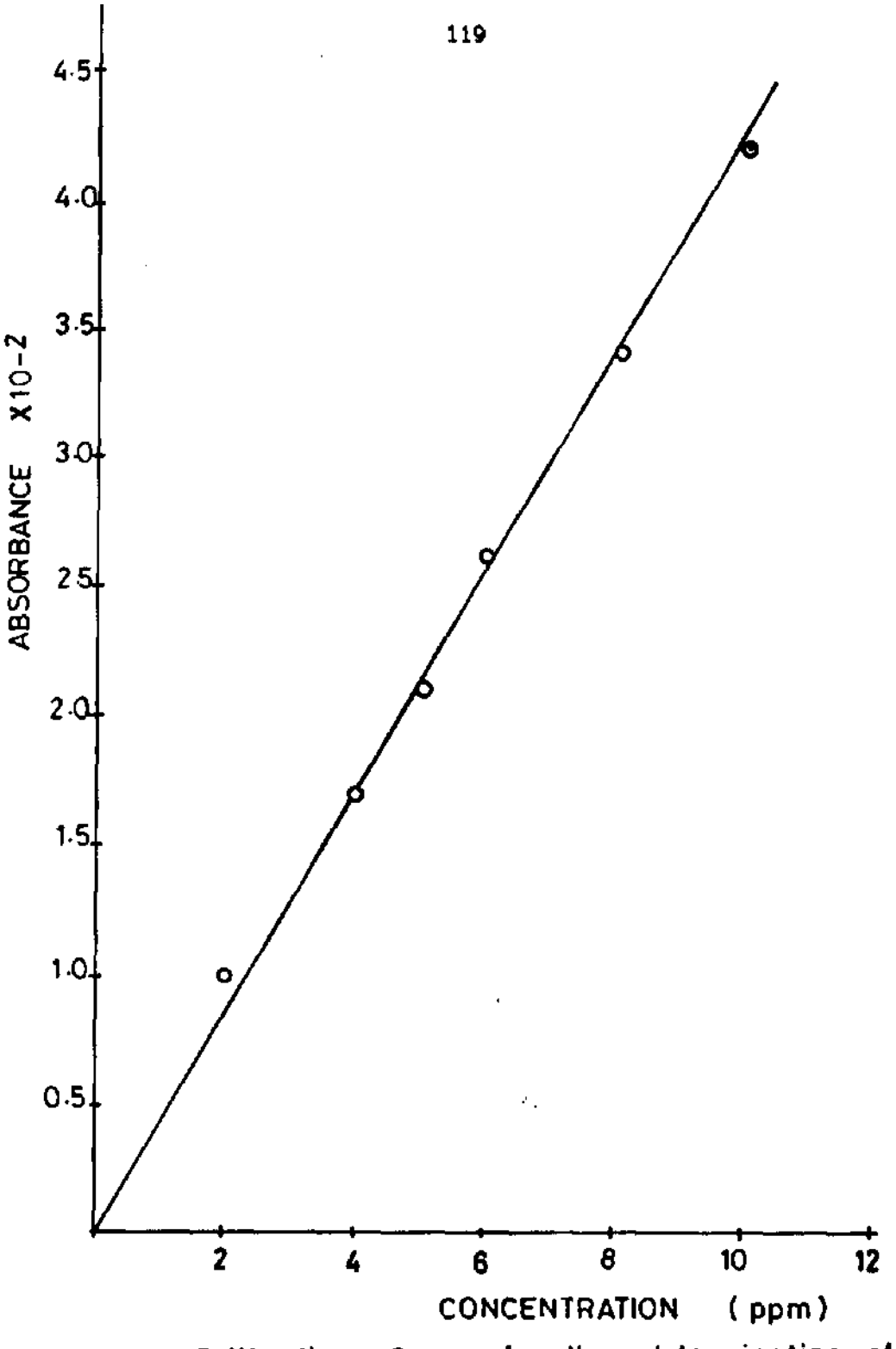
Calibration Curve for the determination of Potassium

Appendix D



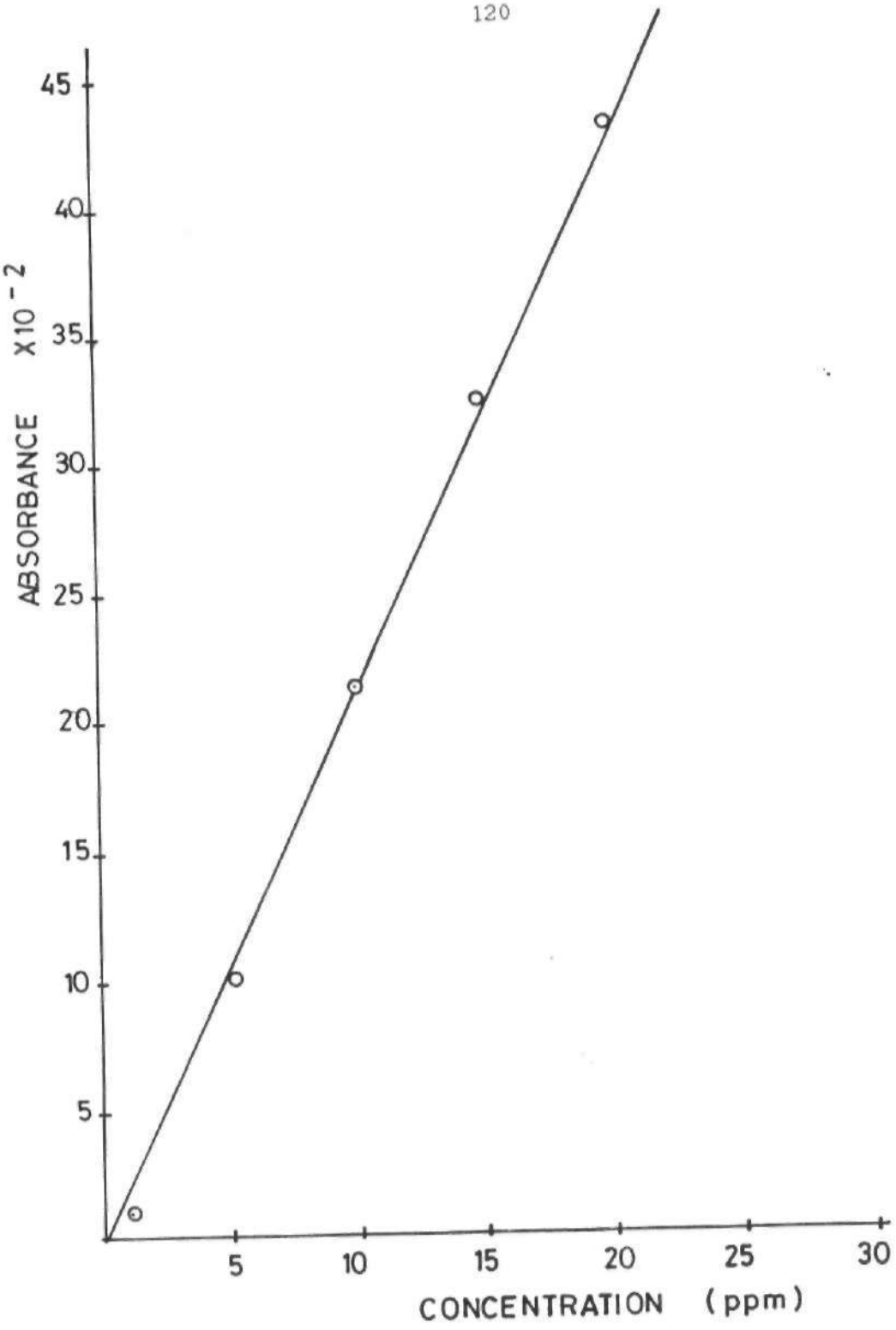
Calibration Curve for the determination of Sodium

Appendix E



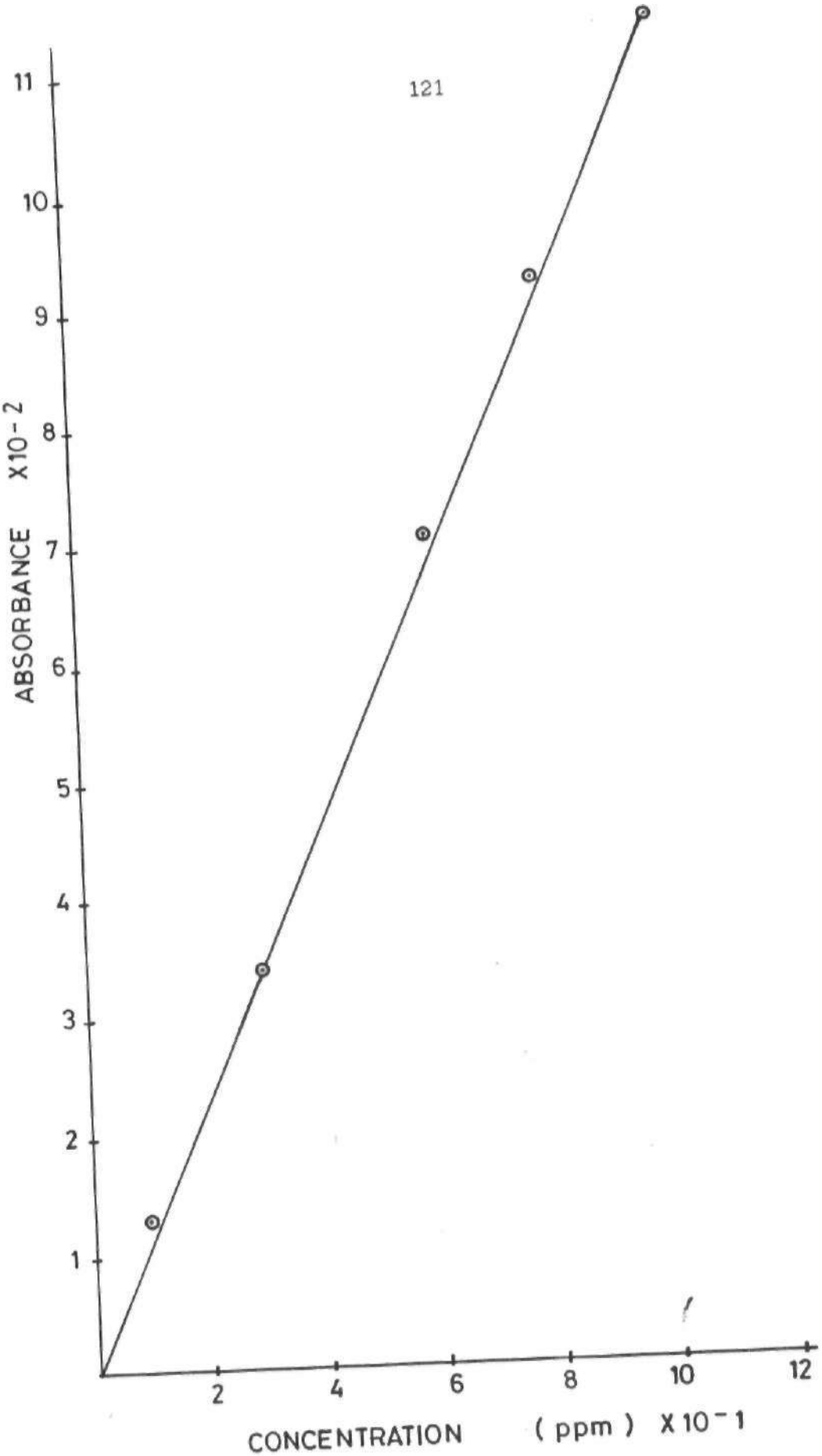
Calibration Curve for the determination of Copper.

Appendix F



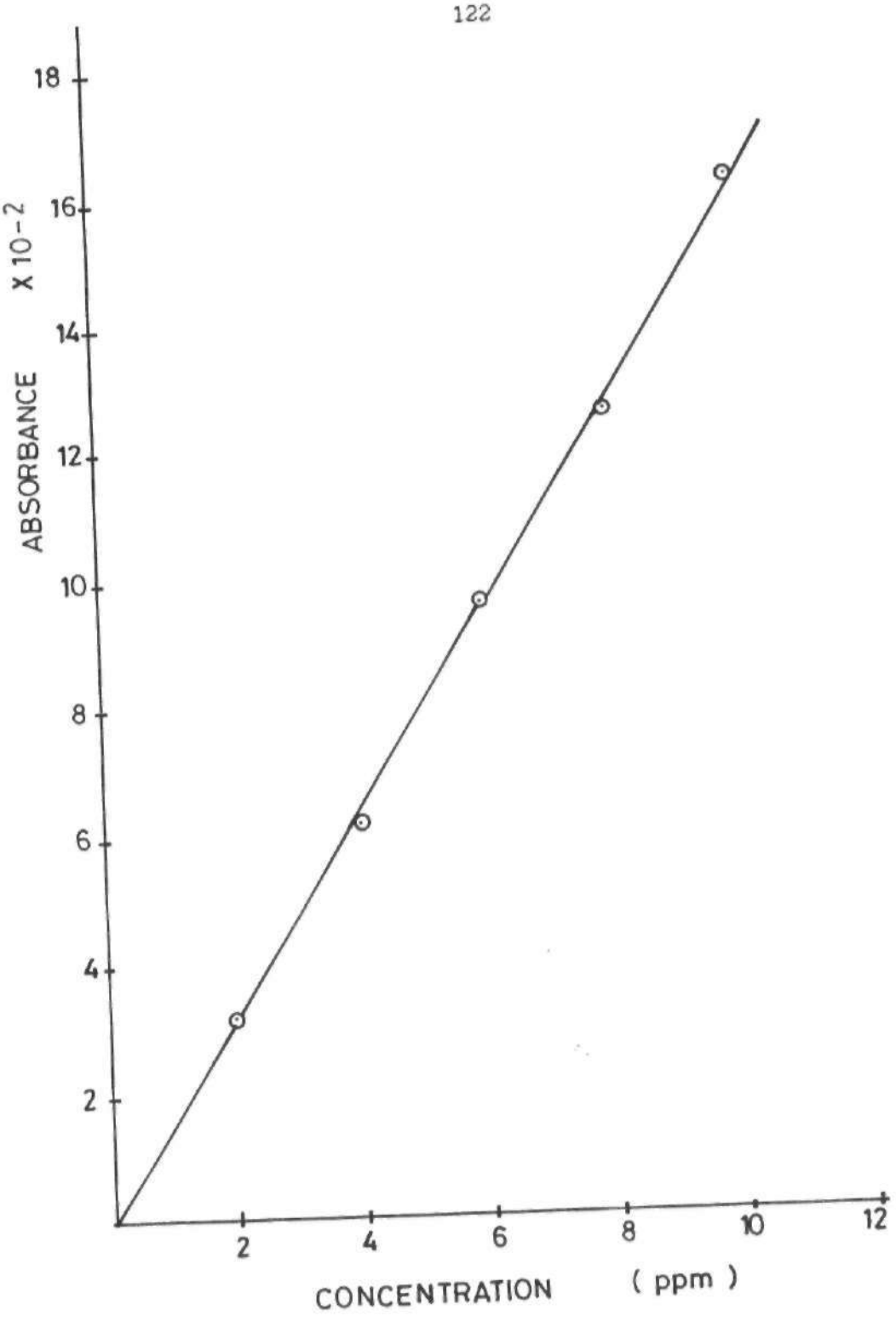
Calibration Curve for the determination of Iron.

Appendix G



Calibration Curve for the determination of Zinc.

Appendix H



Calibration Curve for the determination of Nickel.

Appendix 1

Appendix I

Concentrated Sulphuric acid - Water Mixtures for the attainment of various relative humidities.

Volume of concentrated H_2SO_4 S.G 1.84 (cm^3)	Concentration of solution in Molarity (M)	Relative humidity (r.h).
12.25	1.10	96
27.67	2.49	85
39.41	3.55	74
65.51	5.90	43
69.77	6.28	38
74.03	6.66	33
89.48	8.05	19
103.86	9.35	11

Volumes of the acid taken were made up to 200cm^3 with water.

Adopted from:

Raganna S. (1977) (Ed.) Manual of analysis of fruits and vegetable Products. 1st Ed. Tat. McGraw-Hill New Delhi p. 76.

Appendix K

Nutrient Requirements of Chicken.

Nutrient	0-8 weeks Chicks
Total protein	20%
Calcium	1000mg/100g food
Phosphorus	700 〃
Sodium	150 〃
Potassium	200 〃
Iron	4.0-8.0 〃
Copper	0.40 〃
Zinc	3.5-5.0 〃
Magnesium	40-50 〃

Ref: National Academy of science/National Res. Council - National requirements of poultry 5th Ed. (1966).