DETERMINATION OF IODINE AND SOME ASSOCIATED PARAMETERS IN SOME FOODSTUFFS

BY

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DECLARATION

I hereby declare that this thesis contains the report of my research work and has not been presented in any previous application for higher degree. Information from other sources has been acknowledged by means of reference.

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CERTIFICATION

This thesis entitled "Determination of Iodine and some Associated parameters in some foodstuffs by Olayide Bolajoko Majekodunmi, 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 literary presentation.

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Dedication

This work is dedicated to my Lord Jesus Christ - my saviour and my shepherd.

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I am most grateful to the Almighty God who granted me the grace to go through this programme successfully. To Him alone be all the glory.

I wish to thank my supervisor, Professor S.A. Thomas for his guidance and helpful criticisms throughout the period of this work. May God continue to enlighten him.

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ABSTRACT

Fifty-two food items of various types (grains, seeds, fruits, vegetables, roots and tubers, fish, meats and egg and others} were analysed for their iodine contents. Moisture, starch, crude protein, lipid, carbohydrate, ash, minerals (Na, Ca, Fe, and Cu) and the known goitrogens, thiocyanate and phenol, were also determined in some of the food samples.

A preliminary study of the variation of absorbance with time for some of the samples in the determination of the iodine content by the Moxon and Dixon method (1980) showed that for certain foodstuffs colour development was complete before the recommended time. Finding the optimum time for the reaction and further dilution of some sample extracts were found to improve the determination of iodine.

The iodine content of the samples ranged from 0.24 ppm (Carrot) to 750 ppm (Sweet potato). The fish, meats, and egg were generally found to be richest in iodine. The grains on the other hand gave some of the lowest values for iodine. It was observed that the removal of the testa of brown beans led to a significant loss in iodine content.

As expected, the grains, roots and tubers gave the highest values for starch, while the fruits and vegetables showed the lowest values. Rased on their starch and iodine contents, the foodstuffs may be grouped into three categories, namely: (a) foods rich in iodine but low in starch, (b) foods relatively rich in iodine and starch, and (c) foods relatively

poor in iodine but rich in starch.

The crude protein content of the foodstuffs ranged from 4.1% (sweet potato) to 69.7% (olarias). Cabbage, unexpectedly was shown to be high in crude protein (21.4%). A comparison of the crude protein and iodine contents showed that a high crude protein is usually accompanied by a high iodine content in the samples analysed.

The lipid content of groundnut was found to be highest (51.4%) followed by those of beef (35.6%) and soyabeans (21.5%). The grains showed the lowest values (below 1%). A comparison of the lipid and iodine content for some of the foodstuffs showed that generally, a moderate to high lipid content (over 10%) was accompanied by a high iodine content (over 200 ug/g). Foods rich in both lipid and crude protein content tended to have high iodine content.

The carbohydrate content of the grains, roots and tubers were expectedly the highest (over 20%) while those of the fish and meat were lowest (below 4%). No distinct trend was observed between the iodine and carbohydrate contents of the foodstuffs.

For most of the samples studied, it. was found that the calcium content is much lower than the sodium content. The removal of the testa of brown beans led to a significant reduction in both the sodium and calcium contents. A comparision of the sodium, calcium, and iodine contents of some foodstuffs indicates that the iodine in foodstuffs may be

present in forms other than the iodide and iodate of sodium and calcium in which it occurs in nature, as there was no obvious correlation between these parameters.

Both copper and iron were found to be relatively high in plant materials while in the animal materials examined, high copper content was accompanied by low iron content. A comparision of the iodine content with the iron and copper contents showed the Fe: I ratio to be greater than 1 for most of the foodstuffs. Generally the Cu: I ratio was less than 1. However, millet, guinea corn, soyabeans, yam, and garden egg all have Cu:I ratios above 1 and may therefore be goitrogenous.

The known goitrogen thiocyanate, was not detected in the food items analysed but the goitrogen phenol, was observed in onion, garri, fluted pumpkin leaf, and millet. This seems to support the suggestions that onion, garri, and millet are goitrogenous. The proposition that millet particularly may be goitrogenous is supported by its low contents of iodine and protein, a high carbohydrate content, a *Cu:* T ratio above 1, and its phenolic content.

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CHAPTER 1

1.0 INTRODUCTION

1.1 Iodine

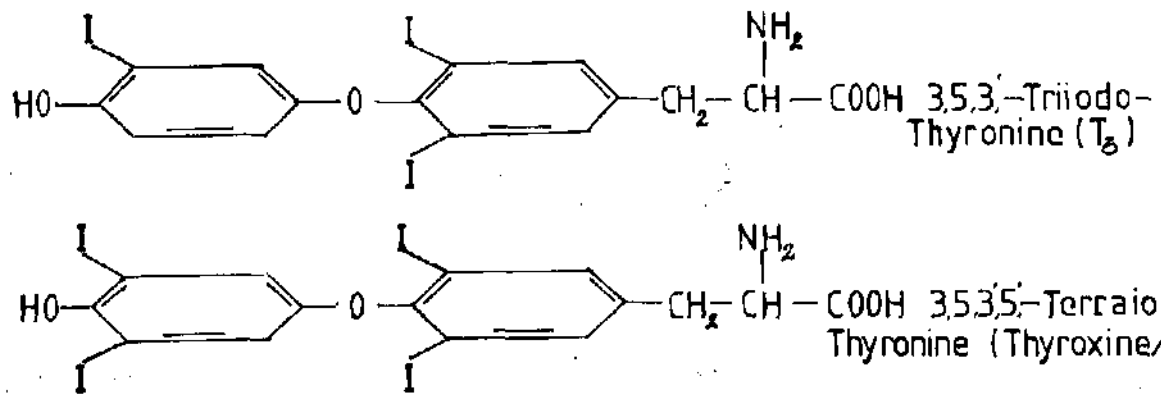
Iodine, as a trace element in human diet, is very important in the metabolic processes occurring in the human body. It was first discovered and isolated in 1811 due to it corroding action on vats, during the decomposition of calcium nitrate, in which seaweed (kelp) had been kept (Follis, 1958; Guiry and Blunden, 1991). In 1896, a high concentration of iodine was observed in thyroid tissue. Its presence in thyroxine, an active principle of the thyroid gland, has since been confirmed (Follis, 1958).

In terms of relative abundance in the earth's crust, iodine is considered to be about the sixty-first element (Snell and Ettre, 1971). Iodine does not exist in the elemental state in nature. Iodine occurs as iodide in brines and in the form of sodium and calcium iodates (Cotton and Wilkinson, 1972). The mineral lautarite is calcium iodate (Snell and Ettre, 1971). 60-70% of iodine in brown seaweed is inorganic, occurring mostly as free iodide (Shaw, 1962), with the rest found as monoiodotyrosine, diiodotyrosine, and triiodothyronine (Shaw, 1962; Ragan, 1981). Surprisingly little is known about the chemical form of iodine in the diet but is generally thought to be absorbed as iodide in the body (Small et al, 1961).

Iodine forms the well-known blue complex with starch (Keller and Seibold, 1971), hence it is used in the detection and determination of starch (Allen et al, 1974) and may itself be determined from this complex (Boltz, 1958).

1.2 The Thyroid gland

The thyroid gland is a member of the endocrine family and is located in the front part of the neck. It secretes the hormones thyroxine (T_4) and triiodothyronine (T_3) (Bondy and Rosenberg, 1974).



Thyroxine is about 60% iodine (Kordel, 1970) and accounts for approximately 98% of the hormone iodine in the blood while T_3 accounts for about 2% (Bondy and Rosenberg 1974). The thyroid gland contains the highest concentration of iodine (0.2-5% of dry weight) and the largest amount of total iodine (70-80%) in the body (Church and Pond, 1982).

The thyroid cell performs two parallel functions in producing its hormones: one is the synthesis of the protein substrate, thyroglobulin; the other is the accumulation of

iodine from the very small amount present in the blood. This is followed by iodination of tyrosine residues in thyroglobulin and the conversion of these into iodothyronines (T_3 and T_4) (Bondy and Rosenberg, 1974).

1.2.1 Iodine metabolism in the Thyroid gland

Iodine is present in the environment in limited quantities compared to the amount needed for normal thyroid hormone production. As a consequence, there is a highly efficient mechanism for accumulation and conservation of this element by the thyroid (Bondy and Rosenberg, 1974).

The metabolic transformations of iodine in the thyroid gland are summarised in scheme 1.1 (Follis, 1958).

It is in the thyroglobulin that the thyroid gland stores thyroid hormones, as well as iodine in forms which can be re-utilised for new hormone synthesis (Bondy and Rosenberg, 1974).

1.2.2 Importance of Thyroid hormones to the human body

The thyroid hormones have profound effects on both growth and development and the metabolic rates of many processes in the adult. These fundamental cellular effects are translated into a variety of physiological changes some of which are cardiovascular, central nervous system, and absorption effects (Bondy and Rosenberg, 1974).

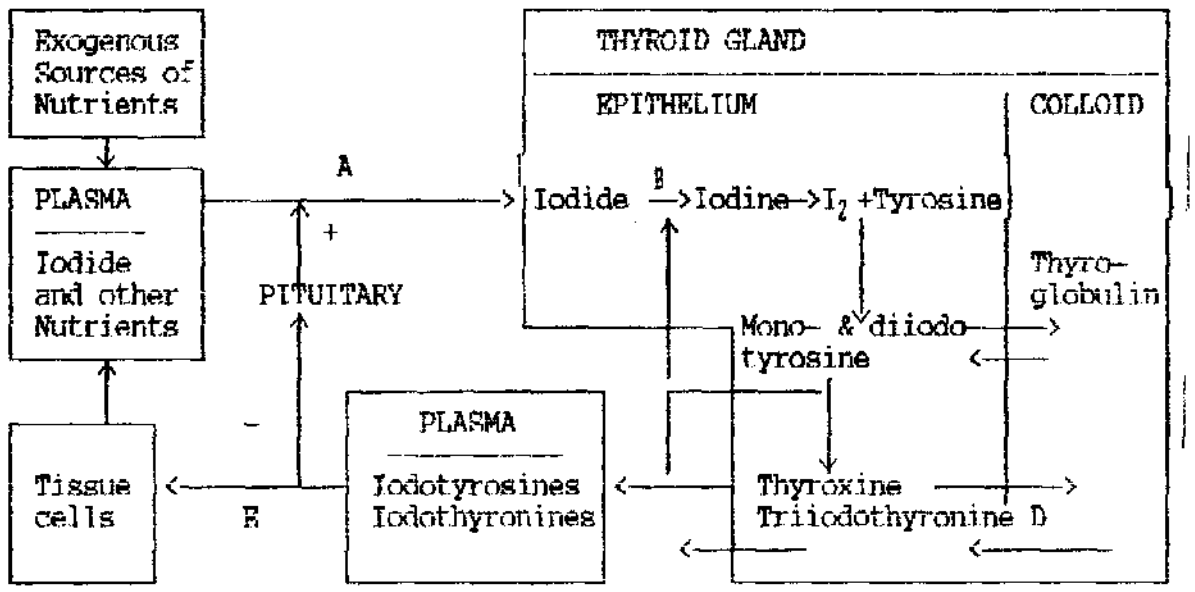
The chief task of the thyroid hormones is to determine

the speed at which a person lives - that is, the rate at which the body consumes its oxygen (Kordel, 1970). For this reason, the thyroid gland has the power to increase a person's sensitivity to all normal mental and physical stimuli and to an astonishing degree, the thyroid also governs the constantly changing flow of a person's emotions. When functioning normally, the thyroid helps to keep a person from becoming either too fat or too thin. The thyroid hormones also contributes to the regulation of the texture of the skin and hair (Kordel, 1970). It is also known that the thyroid hormones aid the synthesis of serum albumin, hence the synthesis of protein (Lewallen, Rall, and Berman, 1959). The thyroid hormones also controls body weight, determines the amount of energy a person possesses, and stimulates sexual powers (Kordel, 1970).

The thyroid hormones, being responsible for controlling the basal metabolic rate, are particularly important in growth (Robert, 1980). Inadequate secretion of particularly thyroxine during developmental years (hypothyroidism) causes arrested physical and mental development, a condition known as cretinism. A cretin, aged 14 or 15 years, is physically underdeveloped, pot-bellied, and so mentally retarded that he may not even be able to feed himself (Roberts, 1980). Cretinism is also characterised by deafness (Winter, 1991). Inadequate secretion of thyroxine in adults is not so serious, as growth by this time is complete. The condition is called myxedema, the symptoms

being a decreased metabolic rate, increase in the amount of subcutaneous fat, coarsening of the skin, and general physical and mental sluggishness (Roberts, 1980). Excessive production of *thyroxine (hyperthyroidism or thyrotoxicosis)*, leads to a condition called exophthalmic goitre - so called because of the characteristic swelling of the thyroid gland and protrusion of the eyeball that accompanies it. Other symptoms include, a greatly increased metabolic rate, loss of body weight, and accelerated heartbeat, all these being accompanied by a general physical and mental restlessness (Roberts, 1980).

The thyroid, through its hormones, is important for determining its owners's general, mental and emotional state, and it is interesting to speculate to what extent a person's personality may be altered by minor fluctuation in thyroid activity (Roberts, 1980).



Scheme 1.1 A Schematic representation of the Metabolism of Iodine in the Thyroid gland

A. Iodine trapping mechanism which is inhibited by SCN, ClO_4, and other compounds (Vanderlaan and Vanderlaan, 1947; Wyngaarden, Stanbury and Rapp, 1953) and Stimulated by the pituitary.

B. Oxidation of iodide to iodine which is inhibited by antithyroid compounds (Mackenzie and Mackenzie, 1943; Mackenzie, 1947) and stimulated by the pituitary.

C. Coupling reaction of iodotyrosines to form iodothyronines.

D. Protease reaction which liberates thyronines from thyroglobulin.

E. Liberation of thyronines and their action on tissue cells; inhibition of pituitary so that less thyroid stimulating hormone is formed.

A cellular effect of thyroid hormones involves ion transport. There is evidence that in most mammalian cells, maintenance of normal levels of intracellular ions, for example Na^+, K^+, Cl^- and HCO_3^-, requires 10 to 30% of the total energy expended by the cells. It is known that in kidney, liver, and diaphragm, the intracellular K^+ concentration is increased by thyroid hormone, being lowest in hypothyroidism and highest in hyperthyroidism (Ismail - Beigi and Edelman, 1970).

1.3 Iodine Deficiency Disorders (IDD)

Severe goitre, cretinism, low birth weight, high perinatal mortality, endemic cognitive disorders, and motor or intellectual subnormality and other iodine deficiency disorders (IDD) have their main cause in the inadequate availability of iodine from the soil lined to a severe limitation of food exchange (Thilly et al, 1992).

A dietary deficiency of iodine, less than approximately 107 ug/day (Bondy and Rosenberg, 1974), leads to a greatly enlarged thyroid gland, a condition called goitre (Kordel, 1970). Goitre is induced by an attempt of the thyroid gland to secrete more thyroxine in response to the thyroid stimulating hormone (TSH) secreted by the pituitary gland. TSH is released in response to a reduced thyroxine production. In the absence of adequate thyroxine for inhibiting TSH release, the thyroid gland becomes hyperactive and enlarged (Church and Pond, 1982). Goitre is known to be associated with defective development, high mortality and partial or complete absence of hair (Godden, 1931).

A deficiency of dietary iodine leads to a decreased production of thyroid hormones (hypothyroidism) which causes a reduction in the basal metabolic rate (Kordel, 1970; Roberts, 1980; Church and Pond, 1982). Severe iodine deficiency has been shown to be the cause of delay in bone maturation and transient hypothyroxinemia, in newly born babies (Thilly et al, 1992). Iodine along with iron, Copper

(Kordel, 1970) and Manganese (Godden, 1931) are concerned with keeping the blood up to its normal richness. They are essential, in haemoglobin formation (Godden, 1931; Kordel, 1970).

The frequency of cretinism and other IDD varies according to the levels of severity of the iodine deficiency. It is known that cretinism is only observed when the iodine, intake is less than 20ug or even less than 40ug per day. Severe iodine deficiency is generally defined by a high prevalence of goitre (about 30 to 50% of the total population), the presence of cretinism (between 2 and 10% of the population) and a median urinary iodine level below 25ug of iodine per littre of urine (Thilly et al, 1992). Selenium deficiency along with iodine deficiency and thiocyanate overload play complimentary roles in the etiopathogeny of IDD (Thilly et al, 1992). Severe iodine deficiency is particularly damaging during the foetal and early post-natal lives, which are crucial periods of thyroid adaptation as well as of brain, bone, and somatic maturity (Thilly et al, 1992).

IDD are typically problems of the whole populations, hence the present attention being given this problem in Nigeria by the government. Many preventive interventions have been suggested. These include iodised salt, iodine fortified water and baby foods, intramuscular and oral administration of iodised oil (Olin, 1924; Khazan, 1948; Roberts, 1980; Thilly et al, 1992; Lammand and Tressol, 1992), use of Seaweed as

vegetables and soil fertilizers (Booth, 1964; Guiry and Blunden, 1991), incorporation of iodine into the soil via iodine containing fertilizers (Balks, 1936), and the use of seawater for the production of dietary salt (McClendon, 1922).

The programme of salt iodisation is very much in place in Nigeria. According to a recent statement credited to the UNICEF project Officer for micronutrients in Nigeria, the country has achieved 93% salt iodisation which far exceeds the 8% mandatory universal recommendation to eliminate goitre in women and mental retardation in children (Nigerian Network News, 1995). Nigeria is thus one of the few countries in the world to have started salt iodisation for the prevention of goitre.

1.3.1 Goitrogens

Goitre, which is the most commonly encountered iodine deficiency disorder observed in Nigeria, has a multifactorial origin. Apart from inadequate dietary iodine, it can also be caused by the presence of goitrogens in food substances, water, and drugs.

Goitrogens are defined as a variety of simple compounds, which when administered to humans or animals, cause goitre by the inhibition of thyroid hormone synthesis (Rondy and Rosenberg, 1974). The hypothyroidism that ensues, causes increased thyroid stimulating hormone (TSH) secretion and thyroid growth.

Goitrogens are basically of two types: (1) agents which

inhibit concentration of iodide by the thyroid, and (2) agents which inhibit iodination (Bondy and Rosenberg, 1974).

Most of the agents which inhibit iodide concentration are monovalent anions of approximately the same size and shape as I^-. These include ClO_4^-, BF_4^-, At^-, Br^-, ReO_4^- and TeO_4^-. Of these, ReO_4^-, TeO_4^- and At^- clearly are concentrated by the thyroid. These compounds become goitrogenic when they are present in such large amounts in the blood that they competitively exclude iodide from being concentrated from the blood. Ordinarily, none of these ions are present in the diet in sufficient quantity to cause goitre formation (Bondy and Rosenberg, 1974).

Goitrogens which inhibit iodination include thiourea, methimazole, resorcinol and L-5-vinyl Thiooxazolidone (goitrin) (Bondy and Rosenberg, 1974). These substances, apart from resorcinol, contain the thiocyanate group - SCN. A number of plants known to contain goitrin include cabbage and other plants of the genus Brassica, turnip, brussels sprout, Kale, Swede roots, Cabbage Seeds, rape seeds, and mustard seeds (Sapieka, 1969). Other goitrogenous foods commonly consumed in Nigeria are cassava, millet (Thilly et al, 1992), and onion (Sapieka, 1969; Bondy and Rosenberg, 1974).

1.3.1.1 Thiocyanate as a goitrogen

It is believed that SCN has detrimental effect on the iodination of proteins (Sheela and Schenck, 1983) and iodide

transport (Bondy and Rosenberg, 1974). When thiocyanate was still in use to treat hypertension, occasional cases of thiocyanate goitre were reported (Barker, Lindberg and Wald, 1941; Wolff, 1964). The quantities of thiocyanate consumed in food are generally thought inadequate to produce goitre by themselves (Clements, 1960; Greer, 1962; Wolff, 1964) because the diet normally contains enough iodine to prevent their effects and the precursors of the goitrogenic oxazolidones are inactivated during cooking which destroys the enzymes necessary to convert them to goitrogens (Sapieka, 1969). However, there is a possibility that thiocyanate may occasionally be involved in combination with other naturally occurring goitrogens in the production of goitre (Gmelin and Virtanen, 1960; Langer, 1966).

A study of the goitrogenic role of chronic exposure to moderately elevated levels of thiocyanate originating from consumption of dried unfermented cassava was made in Eastern Nigeria (Ekpechi, 1967). In the highly goitrous areas, dried unfermented cassava was consumed in large quantities, and his experiments with feeding rats with this staple diet suggested that the goitrogenous action of unfermented cassava could be attributed to the presence of a goitrogen which action could not be prevented with the giving of iodine with the cassava (Ekpechi, 1967). Thiocyanate overload originating from consumption of poorly detoxified cassava is such that this goitrogenic factor aggravates a relative or severe iodine

deficiency (Thilly at al, 1992).

Thiocyanate overload, although it probably has no toxic effect as such at relatively low level, acts by increasing the iodine loss through the urine, thus aggravating a pre-existing severe iodine deficiency (Thilly et al, 1992). This was the finding in the study among the people of Ubangui in Zaire (Thilly et al, 1992) and in Eastern Nigeria (Ekpechi, 1967) where IDD were very much prevalent.

1.4 Toxic effects of Iodine

Not only a deficiency of iodine intake has unfavourable consequences, but also an overdose of it. The toxic effects of overdose of iodine includes skin problems like the aggravation of acne, and disorders of the thyroid gland (Winter, 1991). Goitre (due to hyperthyroidism) was demonstrated as a consequence of a high intake of kelp (seaweed) products both in children in Japan (Suzuki, 1965) and in adults in Finland (Liewendahl, 1972). Similar incidents have been reported from Australia and other countries (Wheeler et al, 1982). Seaweed is known for its high iodine content.

It is ironic that the only clearly substantiated goitre endemia caused by a goitrogen ingestion is that due to iodine itself (Bondy and Rosenberg, 1974). In Hokkaido, Japan, the consumption of large quantities of seaweed gives an iodine intake of 8 to 25mg per day or more and has heen shown to be the cause of goitre (Suzuki et al, 1965). Since most

inhabitants of the Hokkaido endemic area have equally high iodine intakes, but only 6 to 12% develop goitre, it is evident that other factors contribute to susceptibility (Bondy and Rosenberg, 1974).

1.5 Dietary sources of iodine and recommended dosage

Dietary sources of iodine include iodised salt, sea foods (fish, crab, shrimp, oyster, lobster, seaweeds, etc), milk, vegetables and grains grown in iodide rich soils (Winter, 1991). Apart from food, drinking water is another important source of iodine. In Nigeria (Wilson, 1954), it was found that people living and farming on basalt soil had the advantage of an increased food supply and higher iodine level in drinking water and there was no goitre or evidence of increased enlargement of the thyroid gland in adolescence. On the other hand, those living and farming for a long period on pre-cambrian granites had a high incidence of goitre and enlargement of the thyroid gland in adolescence. Goitre was absent from those living on the marine sediments of the Niger.

Iodine intake in human populations varies from 10 to 20ug per day in such varied locales as South America, Africa, and Greece, to as much as several thousand micrograms per day in areas of Japan where seaweed is consumed in the diet (Bondy and Rosenberg, 1974). The recommended dietary allowance of iodine for adults of all ages is 150ug per day (Wilson, 1954; Bondy and Rosenberg, 1974; Gerald, 1991; Winter, 1991); during

pregnancy, 175ug per day (Winter; 1991); while during breast feeding, 200ug per day. The human body requires an uptake of about 2.2ug of iodide per hour to synthesise optimal quantities of thyroid hormones and the thyroid control systems adjust thyroid activity to maintain this level.

1.6 Objectives of research

For most Nigerians, the iodine intake is derived almost entirely from food and water which is a translation of the natural iodine content of the soil. Noting the importance of iodine to the socioeconomic development of the nation, an investigation of the iodine content of some food materials was made. Since iodine is usually present as the calcium and sodium iodates (Cotton and Wilkinson, 1972), a determination of these elements was also carried out to see if there is any correlation among the sodium, calcium, and iodine contents. The starch, crude protein, lipid, carbohydrate, and moisture contents of various food items were also determined in search of any correlations and possible indication of goitrogenous action of the food items. Determinations were also made of the iron and copper contents of some of the food materials as these with iodine, are required for red blood cell formation. Attempts were also made to determine known goitrogens such as thiocyanate and resorcinol. The goitrogens inhibit iodination as already stated.

There is at present to the best of our knowledge no documented comprehensive report on the iodine content of food materials in Nigeria, hence this research is a step in this direction.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Iodine content of foods

The iodine content of foods vary greatly ranging from less than 1.5ug/g for corn (Bhorn, 1917) to as much as $487x10^6$ug/g dry matter for seaweed (Ripperton, 1934). Largely owing to the iodine content of soil and water in an area, the figures that are recorded for the iodine content of food materials show considerable variation for one and the same class of material as determined by different workers, or even by the same workers for materials from different sources (Godden, 1931). The iodine content also varies with the species and by district within the species (Jarvis et al, 1926). The analytical technique used in the determination can also affect the values obtained (Heckman, 1979).

The determination of the iodine content of food is sometimes carried out along with those of the soil and water. Some investigators have shown that the iodine content of food materials is proportional to that present in the soil from which they were obtained (Rasputin, 1961; Gurevich, 1962; Berezhnaya, 1976). Such investigations into the iodine content of soil, water, and foodstuffs have been carried out in different countries and regions of the world. These include the United State of America (Forbes and Beegle, 1916; Bhorn, 1917; Woodruff, 1923; Remington et al, 1936; Allegrini

et al, 1983), Brazil (Pechnik and Guimaraes, 1961; Pechnik et al, 1962), France (Bertrand, 1920; Lammand and Tressol, 1992), India (Patnaik, 1934), Japan (Takei, 1937), former USSR (Dragomirova, 1944; Zhukova and Radzivon, 1944), Switzerland (Fellenberg, 1923, 1938), Sweden (Jaegerstan et al, 1983), Finland (Virtanen and Virtanen, 1940), Britain (Moxon and Dixon, 1982). Table 2.1 summarises some of the literature values for the iodine contents of some foodstuffs (Bhorn, 1917; Jarvis, 1928; Ripperton, 1934; Wenlock et al, 1982).

In Nigeria, the determination of the iodine content of drinking water was carried out in the former Central Plateau, Niger, Zaria, Kano, and Borno provinces (Wilson, 1954). Goitre was found to be prevalent in areas with low iodine content in water. No report on the iodine content of Nigerian foodstuffs was found in the literature.

Seafoods like fish, oyster, shrimp and crabs have been shown to contain some of the highest values of iodine (Tressler and Wells, 1924; Jarvis et al, 1926; Jarvis, 1928; Ripperton, 1935; Wenlock et al, 1982). Of all marine products, seaweeds are the richest in iodine (Tressler and Wells, 1924; Ripperton, 1934). They are followed in iodine content by crabs and ocean fish (Tressler and Wells, 1924), then by shellfish (Tressler and Wells, 1924; Jarvis, 1928).

Milk is another food substance believed to be rich in iodine by many authors. It has been shown that the iodine content of milk varies with the animal species with ewe milk

giving the highest values when compared with the milk of cow and goat (Godden, 1931). Cow milk is thought to have the lowest iodine content. The content of iodine in milk has also been found to vary with the season-being higher in the winter than in the summer, and the iodine content in milk being lower in the mountainous regions, in France (Lammand and Tressol, 1992).

The level of iodine in the water supply of a community has been found to be inversely proportional to the prevalence of goitre (Fellenberg, 1923; Olin, 1924; Balks, 1936; Khazan, 1949; Wilson, 1954; Florinskii and Rogacheva, 1963).

Table 2.1 Iodine Content of some food substances obtained by different investigators in various countries.

Food Substance	Iodine Content (ug/g)
Grains	
Barley	2.5
Corn	1.5-5
Seeds	
Groundnut (roasted)	$220x10^3$
Groundnut (raw)	$<20x10^3$
Fruits	
Apples	$<30x10^3$
Oranges	$20x10^3$
Bananas	$80x10^3$
Grape fruit (canned)	$<20x10^3$
Fruit juices	$50x10^3$
Tomatoes	$20x10^3$
Carrots	$20x10^3$
Vegetables	
Spinach	$20x10^3$
Cabbage	$20x10^3$
Onion	$20x10^3$
Lettuce	1.5-5
Roots and tubers	
Irish potato	$30x10^3$
Fish	
Marine fish	$515-1972;1500-5300x10^3$
Anodrocous fish	698-1539
Fresh water fish	35-158
Shellfish (abalone, clams, oysters, and scallops)	3621-6875
Crustaceans	445-1456
Cod filler	$740x10^3$
Haddock, raw; grilled	$1200x10^3$; $2100x10^3$
Plaice, raw; grilled	$100x10^3$; $130x10^3$
Kippers, grilled	$860x10^3$
Tuna, canned	$300x10^3$
Salmon, canned	$850x10^3$

Table 2.1 (cont.)

<u>Meat and Egg</u>	
Beef	60×10^3
Lamb	20×10^3–90×10^3
Pork, leg	30×10^3
Chicken, raw; roast	100×10^3; 50×10^3
Liver; lamb and ox	120×10^3
Kidney, pig and ox	120×10^3
Eggs	510×10^3, 540×10^3
<u>Miscellaneous</u>	
Seaweeds	1000; 1980–487×10^3
Liquid milk (summer)	70×10^3
Liquid milk (winter)	370×10^3
Condensed milk	740×10^3
Evaporated milk	110×10^3
Yoghurt, natural	420×10^3
Salt; iodised salt	440×10^3; 31×10^6
Tea, infusion	$<10 \times 10^3$
Coffee, instant	50×10^3
Jam	70×10^3
Bread	60×10^3
Biscuits	150×10^3

2.2 Detection and determination of iodine

A number of methods are reported in the literature by which the presence of iodine in a substance can be detected. These include those reported by Lucas, 1922; Pamfil and Wonnesch, 1924; Wade, 1925; Miko, 1927; Murman, 1929; Agostini, 1929; Duschak and Sneed, 1931; Geistenzang, 1932; Palmeri and Giselda, 1932; Wilson, 1940; Kuznetsov, 1951; and Bognar and Sarosi, 1965.

In the determination of trace amounts of iodine, various techniques are available. These include spectrometry (Kirkbiright and Wilson, 1974; C.A. 94:155060j; Kahl and Bhide, 1978), gas liquid chromatography (Hasty, 1971; C.A. 95:23010d), neutron activation analysis (Lag et al, 1972; Johansen and Steinnes, 1976), X-ray fluorescence (Creceline, 1975; Hohimer and Hargis, 1979), Polarography and ion-selective electrode (Hoover et al, 1970), Colorimetry (Heckman, 1979; Moxon and Dixon, 1980; Jones et al, 1982), potentiometric titration (Mokhnach et al, 1971; Shkerman et al, 1978), radiometry (Golubstov and Ignatov, 1976), argentometry (Uzcl, 1935), volumetric analysis (Celsi and Copello, 1951), and gamma ray analysis (Yates et al, 1978).

2.2.1 Advantages and disadvantages of some of the various techniques of determination

Quantitative analysis by colorimetry is an extremely useful tool because it has wide applicability, high

sensitivity, moderate to high selectivity, good accuracy, and is easy and convenient to use (Skoog and West, 1980). Also, many colorimetric procedures are readily automatable, which leads to significant increases in throughput rates (Fishman and Friedman, 1989).

Polarographic techniques can be very sensitive and rapid in operation but they are not as popular as colorimetric methods for biological nutrient analysis. This is partly due to the frequent necessity for a prior separation of interfering substances. Polarography has considerable value for special investigations particularly those concerning trace elements (Allen et al, 1974).

Ion-selective electrode has a difficulty arising from interference problems. In some cases the sample solution forms precipitates or complex compounds with the ion present in the membrane. The most serious problem is the response of some electrodes to ions other than that being measured, even though cation electrodes are generally free from anionic interferences and vice-versa (Allen et al, 1974).

X-ray fluorescence has the advantage of speed and precision. The sample is not destroyed during the test (Allen et al, 1974). Virtually any type of sample can be simply prepared for analysis by X-ray fluorescence. A problem arises though if different sizes of particles of the solid matrices are used. There arises the need to carefully control the

preparation of the samples and standards in chemical composition and physical nature (Winefordner, 1976).

Gas-liquid chromatography has the advantages of speed, good resolution, and it is quantitative. However, the apparatus is elaborate and non-volatile compounds cannot be analysed directly but must first be converted to volatile derivatives (Allen et al, 1974).

Neutron activation analysis is sensitive, widely applicable to different materials, though it is subject to interferences like self-shielding and nuclear reactions (Winefordner, 1976).

Gamma ray analysis is the most widely used method of measurement in activation analysis. The main errors arising in activation analysis are those due to unequal flux at sample and standard, in-accurate counting procedures, and counting statistics (Winefordner, 1976).

2.2.2 Colorimetric determination of Iodine

A number of absorptiometric procedures are available for the determination of trace amounts of iodine. This include the determination of iodine by its action as a catalyst, starch-iodine complex formation, its formation of the triiodide ion, and other methods.

The estimation of iodine as iodide by the measurement of its catalytic action on the reaction taking place between

cerium (IV) and arsenite (III) in acidic solution (Sandell and Kolthoff, 1934; Malmstadt and Hadjiioannou, 1963) is widely used. Iodide can also be detected and determined by its catalytic effect on cerium (IV) and antimony (III) reaction (Bogner and Sarosi, 1965). There is also the method of Sveikina (1975) in which iodide catalyses the destruction of thiocyanate by nitrite. In the presence of iodide, the rate of decrease of colour increases in proportion to the amount of iodide present which enables one to effectively determine amounts of iodide in the range of 0.005 to 1.0ug depending on the method, with a high degree of accuracy (Boltz, 1958). One theory which may explain the mechanism of reaction is the Schaffer equivalence change theory (Schaffer, 1947) which postulates that there is only a possibility of certain valence states existing which may be represented by known compounds. If the redox change is not equivalent, then a bimolecular reaction could not occur even though bimolecular collision had taken place. Therefore, successful reaction is involved with the factor of the equivalence of valence change and this must determine that portion of the collisions which achieve favourable results. Since reaction velocity can be inhibited by unequivalent valence changes, the introduction of a third substance able to exist in two valence states required for mediation of the reaction into consecutive even steps should markedly catalyze the reaction (Boltz, 1958).

The starch-iodine procedure for the determination of

iodine is possible because it is capable of undergoing formation of an absorption compound. Here, an organic substance functions as the absorption compound (Feigl, 1954). Many studies have been carried out on the iodo-starch complex (Euler and Myrback, 1921; Tadokoro and Sato, 1923; Simeri and Browning, 1939; Rundle and French, 1943; Swanson, 1948). The analysis of iodine by starch was used as early as 1851 by Grange (Boltz, 1958).

One of the more sensitive methods for the determination of iodine involves the measurement of the triiodide ion in the ultra-violet region of the spectrum (Boltz, 1958). Studies have been made which indicate that iodine in various solvents shows a varied state which depends entirely on the nature of the solvent (Brode, 1947). Iodine dissolved in solvents where it does not undergo solvation, such as CCl_4,CS_2 or $CHCl_3$, is purple and has a spectrum similar to that of the vapour state of iodine (Brode, 1947). Brown colours, however, are obtained in solvents which form complexes with iodine, such as water, potassium iodide, or ethanol, yielding spectra of solvated or molecular addition compounds of iodine and solvent and exhibiting visible and strong ultraviolet peaks (Boltz, 1958).

There are a multitude of procedures apart from those already described which are available to be used in the absorptiometric determination of iodine. Alpha-naphtolflavone reacts with iodine to give a spectrophotometrically measurable blue compound (Reith, 1929). Iodine forms nitrous acid when

reacted with hydroxylamine which then diazotises sulphanilic acid and the latter couples with alpha-naphthylamine to produce a red dye (Endres and Kaufman, 1936). O-Tolidine reacts with iodine to yield a blue-green colour (Lange and Ward, 1925). Iodide has been determined after reaction with dioxane (Saifer and Hughes, 1937). In acidic solution, iodate oxidises pyrogallol to pupurgallin forming a sensitive reddish-brown colour (Snell and Snell, 1949). The decrease in fluorescence of fluorescin has been used since the diiodo derivative is no fluorescent and its formation is controlled by the P^H of the solution (Harlay, 1947). The measurement of the turbidity of silver iodide has been successfully used for small amounts of iodide (Scott and Hurley, 1934). The absorption of mercurous iodide on mercuric chloride (Klein, 1954) and the formation of palladous iodide (Martins, 1930) have also been used in the determination of iodine.

2.2.3 Destruction of organic matter

Various methods are available by which the iodine containing substance, in this case food materials, can be destroyed thereby leaving the iodine in a form suitable for quantitative analysis. One of such methods is bomb calorimetry (Stole and Knopp, 1963; Mitchell, 1965), but it is time consuming for a large number of samples (Moxon and Dixon, 1980). It has also been reported that combustion is often incomplete and that the ash residue can interfere with catalytic finish techniques (Fioravanti and Halmi, 1971; Moxon

and Dixon, 1980).

Acid combustion (Rogers and Poole, 1958; Zak, 1952; Hach and Lewellen, 1969; Joerin, 1975) has been widely used for foods and chemical specimens, but it requires the distillation of iodine into a suitable medium and so is only applicable to small numbers of samples (Moxon and Dixon, 1980).

Alkaline dry ashing has been widely used for foods, clinical specimens and animal feedingstuffs. It is simple, adequately sensitive and has a productivity potential that makes it suitable for use on a large number of samples (Moxon and Dixon, 1980). Alkaline dry ashing (alkaline fusion) can be carried out using sodium carbonate (Barker, 1948; Caraway, 1952; Klugerman, 1954; Meyer et al, 1955; Moxon and Dixon, 1980), Sodium carbonate-sodium hydroxide (Salter and Johnson, 1948)., potassium hydroxide (McCullagh, 1934; Waleszek Piotrowski and Koch, 1952), sodium hydroxide-potassium nitrite (Salter and McKay, 1944) or potassium hydroxide/potassium carbonate-zinc sulphate (Moxon and Dixon, 1980). These are capable of destroying organic matter when a muffle furnace is used with the recommended temperature varying from 400-480^{0}C (Lapin and Reis, 1961; Sveikina, 1975; Moxon and Dixon, 1980), to 600-650^{0}C (Boltz, 1958; Krylova, 1963; Stole, 1963; Lauber, 1975). The alkali iodide formed will not be lost because it has not reached its melting point (Boltz, 1958). It was confirmed that the use of a lid on the crucible during the ashing procedure was essential to prevent loses of iodine

(Moxon and Dixon, 1980). The use of radioactive iodine has borne out the fact that the alkaline fusion technique does not lead to loss (Decker and Hayden, 1951).

Following the destruction of organic matter, the ash can be identified to give a clear medium capable of being used for the determination of iodide (Boltz, 1958). Alternatively, the ash residue can be dissolved in distilled water and any trace of carbonaceous matter removed preferably by centrifugation as some batches of filter paper were found to adsorb iodide (Moxon and Dixon, 1980). Subsequent determinations can then be made on the solution without prior removal of the iodine. However, some procedures for aqueous media exist where the iodine is neither removed nor the medium destroyed but the determination is carried out by direct treatment of the solution being tested (Klein, 1954; Boltz, 1958).

2.3 The role of iodine and other substances in goitre formation

It is recognised that a deficiency in dietary iodine is the major cause of goitre (Sherman, 1924; Remington and Levine, 1936; Kolomoiitseva, 1962; Baimusaev, 1977). It is also recognised that with an inadequate intake of iodine, diets with high fat content, high carbohydrate or low protein content (Wilson, 1954), or extensive consumption of foods containing substances chemically related to thiocyanate or to thiourea, may have goitrogenic effects (Wilson, 1954; Ekpechi, 1967; Thilly et al, 1992). Extensive investigations into the

effects of iodine deficiency and thiocyanate overload especially from the consumption of poorly detoxified cassava have been carried out in Nigeria (Ekpechi, 1967) and in Zaire (Thilly et al, 1992). The thiocyanate which is a goitrogen was found to aggravate a relative or a severe iodine deficiency.

The effect of the role of other trace elements along with iodine deficiency in the diet has also been studied. Very recently, a severe selenium deficiency has also been associated with iodine deficiency disorder (IDD) in the human population (Thilly et al, 1992). Inconsistencies exist in the findings of various investigators as to the role of copper in the etiology of endemic goitre (Florinskii and Rogacheva, 1963). In a goitre endemic region, it was found that the iodine, cobalt, copper, and molybdenum content of the soil water and food products of the regions were low (Gurevich, 1962). It was also discovered that the greatest incidence of goitre was present with the least iodine content in foodstuffs, and also increased with the copper: iodine and cobalt; iodine ratios. A copper: iodine ratio in the water greater than one was accompanied by over 10% goitre (Kolomiitseva, 1962). It was also found that the iodine and manganese concentrations were low in both the soil and water of a region where goitre was endemic (Baimusaev, 1977).

The role of calcium and phosphorus along with the presence or absence of vitamin D in rats was investigated and

found not to significantly affect the degree of goitre produced (Remington and Levine, 1930). In another investigation, the addition of calcium carbonate to diets of rats deficient in iodine resulted in a greater and more rapid increase in the size of the thyroid gland, as well as in a more marked arcinar hyperplasia. The experimental evidence was considered indicative of an intimate inter relationship between iodine and calcium in the production of goitre. On this basis, the theories of the incitation of goitre by deficiency of iodine and an excess of calcium appeared to be correlated (Thompson, 1933).

2.4 Moisture content of foodstuffs

The available water present in foodstuffs is determined as moisture content. The appearance, keeping quality, and dry matter content of agricultural products, for example foodstuffs are closely related to the moisture content (Whyte and Selvey, 1969). Generally, stored products are dried to lower their moisture content in order to prevent the growth of spoilage micro-organisms and also reduce Biochemical reactions.

2.5 Starch content of foodstuffs

Starch is the major storage polysaccharide in plant materials, and is widely distributed in the plant. It is present in reasonably large amounts in roots, seeds, and some fruits but the concentration in other parts of the plant,

especially the leaves, are subject to daily variation, falling to a minimum at night (Allen et al, 1974). In animals, starch is stored in muscle tissues in a form called glycogen. Literature values of starch for some foodstuffs are given in table 2.2.

2.6 Crude protein content of foodstuffs

Crude protein is the major compound containing nitrogen in food items. Minor nitrogenous ingredients of food include amino acids, purines, ammonium salts and vitamin B_1. Nitrogen is therefore used as an index of the protein termed 'crude protein' as distinct from true protein (Oyeleke, 1984).

Proteins are the most complex biological molecules and probably the most important structures of the biological system. Every living cell has protein as one of its principal constituents. A large variety of proteins are found in animal and vegetable life. Plants make protein from the carbondioxide of the air and water and nitrogen containing material derived from the soil in which they grow. Animals and man obtain proteins by eating plants or the flesh of other animals. The protein of the diet are the body's only source of nitrogen, an essential constituent of all living cells (Church and Pond, 1982). It is recognised that with an inadequate iodine intake, foods low in protein content have goitrogenic action (Wilson, 1954). Literature values of protein for some foodstuffs are given in table 2.3.

2.7 Lipid content of foodstuffs

Lipids (fats and oils) are materials soluble in certain organic solvents. Lipids constitutes a high source of energy providing 9 Kcal/g compared to 4Cal/g from carbohydrate and protein (Oyeleke, 1984). Lipids are derived from both animal and vegetable sources. Diets high in fats as well as inadequate intake of iodine are said to have goitrogenic action (Wilson, 1954). Some literature values of lipids are given for some foodstuffs in table 2.3.

Table 2.2 Summary of some literature values for starch for some foodstuffs (Pearson, 1976; Harris and Loesecke, 1971).

Foodstuff	Starch content (%)
Grains	
Corn	50-70
Brown rice	85
Polished rice	>90
Soyabeans	Trace
Root and Tuber	
Sweet Potato	25.68
Irish potato	20.30
Seed	
Groundnut	10
Fruits	
Banana	3
Apples	0.3
Carrot	Trace
Egg plant	-
Tomato	0.15
Vegetable	
Runner beans	0.2
Cabbage	-
Onion	-
Miscellaneous	
Bread	50
Chocolate	2-7

2.8 Ash content of foodstuffs

The ash content is a term for the inorganic residue that remains after the organic matter has been burnt away. The importance of the ash content is that it gives an idea of the quantity but not the content of mineral elements present in the sample.

2.9 Carbohydrate content of foodstuffs

The value of carbohydrate refers to both available carbohydrate (Starch and soluble sugar) and the unavailable carbohydrates such as cellulose, lignin, and hemicellulose (Oyeleke, 1984).

Like the other bulk organic nutrients required for life in relatively large amounts (proteins and lipids), carbohydrates are also absolutely essential for life. Carbohydrates are oxidised in the body to give heat and energy for all forms of body activity.

Diets high in carbohydrate and deficient in iodine are considered to have goitrogenic action (Wilson, 1954). Literature values for some foodstuffs are given in table 2.3.

2.10 Mineral Elements

The four mineral elements studied are sodium, calcium, iron, and copper. While sodium and calcium are macronutrients, iron and copper are micro nutrients required by the body. They are obtainable from the diet in various amounts and generally help the body to function as it should.

2.10.1 Sodium

Sodium contributes to the ionic balance of the cell fluid and is essential for humans and animals. Two of the forms in which sodium occur in nature are sodium iodide and sodium iodate (Cotton and Wilkinson, 1972). Table 2.4 summarises some of the literature values of sodium for some foodstuffs.

The determination of sodium by flame emission techniques are particularly suitable as its arc line (589nm) is the most intense of any element at normal flame temperature (Allen et al, 1974). Atomic absorption spectrometry can also be used for the determination of sodium (Allen et al, 1974); Fishman and Friedman, 1989). The gravimetric technique for sodium uses uranyl zinc acetate, but it is not very suitable owing to the low levels of sodium in most organic materials (Allen et al, 1974).

2.10.2 Calcium

Calcium is essential in vegetation for apical and root tip development and accumulates in cell walls as calcium pectate. It is a major constituent in the skeleton of many animal groups and humans. Apart from bone formation, it is also necessary in the human body for tooth formation, growth, blood-clotting, catalysing biological reactions, maintenance and function of cell membrane, regulation of muscular contraction and transmission of nerve impulses, and regulation of strontium uptake (Guthrie, 1979). Milk is an important

source of calcium, especially for growing children. Table 2.5 summarises the calcium content for some foodstuffs.

One form in which calcium occurs in nature is as calcium iodate (lautarite) (Snell and Ettre, 1971; Cotton and Wilkinson, 1972). Calcium also exists as dolomite ($CaCO_3$. $MgCO_3$) and as limestone ($CaCO_3$).

Several procedures are available for the estimation of calcium. A method is based on the precipitation of calcium as the oxalate but this has been largely replaced by EDTA titration. Few colorimetric techniques for calcium are completely satisfactory (Allen et al, 1974). A suitable technique for calcium determination is atomic absorption spectrometry (Fishman and Friedman, 1989).

2.10.3 Copper

Copper was first recognised as a dietary essential in 1928 when it was observed that anaemia could be prevented only if both copper and iron were available. The role of copper in preventing anaemia has been attributed variously to its effectiveness in facilitating iron absorption, in stimulating the synthesis of heme or globin fractions of the haemoglobin molecule, or in releasing stored iron from the ferritin in the liver. It appears that as part of a multi-functional enzyme known as ceruloplasmin or ferroxidase I or II, copper plays an important role in the oxidation of ferrous to ferric iron (Beaton and McHenry, 1964; Guthrie, 1979). Copper exists in the human body as homocyanins - an oxygen carrying protein.

Copper is an essential component of many enzymes.

Copper can be determined by polarography (Allen et al, 1974), atomic absorption spectrometry and atomic emission spectrometry (Fishman and Friedman, 1989). Table 2.6 gives some literature values of copper for some foodstuffs.

2.10.4 Iron

Iron functions as a carrier of oxygen and carbondioxide and in bond formation. Iron occurs in the respiratory pigment, porphyrin, which is required in electron transfer (cytochrome) process in plants and animals. It activates some oxidases and is considered to be necessary for chlorophyll synthesis (Allen et al, 1974). Both iodine and iron are considered to be important in red blood cell formation in the human body (Kordel, 1970). About 70% of the iron in the body is considered functional iron. The majority of this is present in the haemoglobin molecule of the red blood cell. A small portion exists as part of myoglobin, the iron containing protein in muscle. Though the body can absorb both Fe^{2+} and Fe^{3+}, the absorption of Fe^{2+} form is greater. The ferric iron (Fe^{3+}) that predominates in food is usually reduced to the ferrous (Fe^{2+}) form prior to absorption. Liver is the richest source of iron (Guthrie, 1979). Table 2.7 summarises some of the literature values for iron in some foodstuffs.

Methods used for the determination of iron includes colorimetry, atomic absorption spectrometry, polarography, and atomic emission spectrometry (Allen et al, 1974; Fishman and

Friedman, 1989).

2.10.5 Flame photometry (Atomic Emission spectrometry)

This technique is based on the principle that when an element is introduced into the flame, the atoms of the element are excited by the flame. On returning to the ground state, the atoms emit radiations characteristic of the element concerned. The intensity of the radiation emitted is directly proportional to the concentration of the element present.

In order for the emission to be used in quantitative chemical analyses, the following are necessary : (1) the sample must be atomised (constituents of interest converted to atoms in the gas phase) and the resulting atoms or ions excited; (2) the resulting characteristic emission lines must be spectrally separated and their relative intensities measured by a suitable dispersion-detection system (spectrometer or spectrograph); and (3) the resulting intensities must be compared with standards of known elemental composition. Solid samples are usually analysed by arc or spark excitation sources that atomise and excite elements directly from the sample. Solutions are generally analysed with flame or plasma excitation sources into which the sample is aspirated (Fishman and Friedman, 1989).

Table 2.3 Summary of some literature values for lipid, protein, and carbohydrate content of some foodstuffs (Fox and Cameron, 1972; Pyke, 1972; Guthrie, 1979; (Inglett and Charalambus, 1979).

Foodstuff	Lipid (%)	Protein (%)	Carbohydrate (%)
Beef (fresh)	7	20-25	
Fish "	5-13	14-18	
Millet	4.5	16	
Soyabeans		40	
Guinea corn	4	10	68
Cabbage			5.4
Pawpaw	0.06	0.36	12.18
Groundnut	51	28	
Yam tuber		8.5	
Sweet potato	<1	2	21.1

Table 2.4 Summary of some literature values for sodium for some foodstuffs (Guthrie, 1979)

Low Na (ppm)$x10^{-5}$		Moderate Na (ppm)$x10^{-4}$		High Na (ppm)$x10^{-3}$	
Pineapple	1	Chicken	9	Salmon	5
Grape fruit	1	Celery	10	Cornflakes	10
Sweet potato	1	Egg	12	Bacon	17
Carrot	50	Tomato juice	20		
Milk	50	Sardines (Canned)	50		

Table 2.5 Summary of some literature values for calcium for some foodstuffs (Fox and Cameron, 1972; Pyke, 1972; Guthrie, 1979; Inglett and Charalambus, 1979).

Foodstuff	Calcium content (ppm)x10^{-5}
Beef	8
Sardine	40
Millet	50
Guinea corn	20
Cabbage	80
Pawpaw	30
Sweet potato	8
Rice	4
Spinach	200
Egg	60
Milk	120
Bread	90

Table 2.6 Summary of some literature values for copper for some foodstuff (Pennington and Calloway, 1973).

Rich sources (>8ppm)	Intermediate sources (2-8ppm)	Poor sources (<2ppm)
Liver	Leafy vegetables	Milk
Shell fish	Eggs	Butter
Nuts	Muscle meat	Cheese
Cocoa	Fish	Sugar
Cherries	Poultry	Fresh fruits
Mushrooms	Peas	Vegetables
Whole grain cereals	Beans	
	Fresh fruit	
	Refined cereals	

Table 2.7 Summary of some literature values for iron for some foodstuffs (Guthrie, 1979; Inglett and Charalambus, 1979).

Foodstuff	Fe content (ppm)x10^{-5}
Pawpaw	10
Lima beans	2
Spinach	2
Beef	8
Egg	3

2.10.6 Atomic Absorption Spectrometry (AAS)

This is a very sensitive and highly selective method of analysis for most metallic elements (Walsh, 1995).

Each element absorbs energy at a series of wavelengths that constitutes the unique atomic spectrum of that element. Selectivity is achieved by careful choice of the wavelength of radiant energy passed through the sample atomic vapour using either a hollow cathode lamp or an electrodeless discharge lamp. Because the atomic absorption occurs in extremely narrow (approximately 0.002nm) intervals of the electromagnetic spectrum a wavelength can usually be selected that is absorbed by only one constituent of a sample. The atoms of the sample aspirated into the flame remain in the ground state. On passing the radiant energy of a characteristic wavelength through the flame, the atoms absorb some of this radiant energy and become excited. The decrease in the intensity of the radiation is then measured using a monochromator and detector system. The decrease in intensity

is therefore related to the concentration of the element.

Under deal conditions, the extent of absorption at a specific wavelength is related to the concentration c, of a given element in the vapour and to the length, b, of the path that the beam traverses through the vapour, according to Beer's law: A = abc

Where a is a constant of proportionality and A, the absorbance is defined as:

A = Log Po/P

Where Po is the radiant power of the unabsorbed beam (measured in the absence of atomic vapour) and P is the radiant power of the beam after it has passed through the atomic vapour (Fishman and Friedman, 1989).

2.11 Determination of Goitrogens (SCN^- Phenol)

2.11.1 Determination of SCN^-

It is known that ferric iron reacts with thiocyanate to give a series of intensely red-coloured compound (Vogel, 1978). The reaction can be represented as follows:

$Fe^{3+} + SCN^- \longrightarrow (Fe(SCN))^{2+}$

This complex formed is the predominant coloured species at low concentration. The presence of a strong acid is recommended in order to suppress the hydrolysis $Fe^{3+} + 3H_2O \rightleftharpoons Fe(OH)_3 + 3H^+$

The acid should either be HCl or HNO_3 and not H_2SO_4 which has the tendency to complex with ferric ions (Vogel 1978). Fe^{3+} in concentrated HCl in the presence of SCN^- yields a

bright orange colour which can be measured in a colorimeter (Sheela and Schenck, 1983). Utsumi et al (1975) also describes a photometric method for SCN^- determination using sodium azide.

2.11.2 Determination of phenols

The goitrogen, resorcinol, is a phenol. Colorimetric procedures for the determination of phenols are based either on diazotization and coupling with phenols, or on the formation of an indophenol dye. All the methods suffer the disadvantage that the colour formed varies according to the phenolic component (Allen et al, 1974).

Colorimetric procedures for the determination of phenols are described by Allen et al, 1974, and Wershaw et al, 1987. Phenols can also be determined by high performance liquid chromatography (Wershaw et al, 1987).

CHAPTER 3

3.0 EXPERIMENTAL

3.1 APPARATUS

Muffle furnace (0-1000^{0}C), porcelain crucibles with lids, centrifuge, centrifuge tubes (100ml capacity), colorimeter-corning 253, flame photometer-EEL model A, atomic absorption spectrophotometer-shimadzu AA-650, thermostatically controlled oven, watch clock, analytical balance, soxhlet apparatus, thimbles, Kjeldahl flasks, and distillation apparatus.

3.2 REAGENTS

Potassium hydroxide solution (1M), Zinc sulphate solution (10%m/v), Potassium thiocyanate solution (0.023%m/v), Sodium nitrite solution (2.07% m/v), Ammonium iron (III) sulphate reagent, Potassium iodide, Perchloric acid (70%), Phenol red indicator in ethanol (0.1% m/v), Sodium hydroxide solution (1M), Potassium iodate, Acetic acid (10%v/v), Nitric acid (concentrated), Calcium carbonate, Lanthanium oxide, Hydrochloric acid (concentrated), Sulphuric acid (98%), Copper (II) sulphate, Iron wire, Sodium chloride, Phenol, 4-aminophenazone, Sodium bicarbonate (5%m/v), Potassium ferricyanide solution (10% m/v), Carbon tetrachloride, Ferric chloride solution in concentrated HCl (40% m/v), Potassium thiocyanate, Petroleum spirit (60^0-80^0, Sodium sulphate, Phenolphthalein (1% m/v), Boric acid (4% m/v), Alcoholic

methyl red (0.2% m/v),

Alcoholic methyl blue (0.2% m/v),

3.3 Samples

Fifty-two food samples were collected mainly from Samaru - Zaria market. They are made up of 13 grains, 4 seeds, 8 fruits, 6 vegetables, 5 roots and tubers, 4 fish, 5 meats and egg, and 7 miscellaneous food items.

The foodstuffs with their botanical names are shown in table 3.1.

3.3.1 Sample preparation

Each of the food samples was properly homogenised by thorough mincing or grinding. The fruits, vegetables, some of the roots and tubers (yam and potatoes), and meats were examined fresh - Dried samples of the grains, seeds, other roots and tubers (yam flour and dried fermented cassava), and fish were analysed. The eggs analysed were boiled. Contamination was avoided by keeping all the apparatus and work surfaces clean.

Table 3.1 Common and botanical names of foodstuffs analysed (Macmillan, 1935).

Common name	Botanical name
Corn	Zea mays
Guinea corn	Sorghum Durra
Millet	Panicum miliaceum
Rice	Oryza sp.
Beans	Vigna unguiculata
Soya beans	Glycine soja
Locust beans	Pakia biglobosa
Melon seeds	Cucumeropsis edulis
Groundnut	Arachis hypogaea
Carrot	Daucus carota
Garden egg	Solanum incanum
Pawpaw	Carica papaya
Mango	Magnifera indica
Water melon	Citrullus lanatus
Tomato	Lycopersicon esculentum
Pepper	Capsicum annum L.
Dried pepper	Capsicum frutescens L.
Spinach	Amaranthus cruentus
Water leaf	Talinum triangulare
Cabbage	Brassica oleracea
Fluted pumpkin leaf	Telfoira occidentalis
Onion	Alium ascalonicum
Garlic	Alium Sativum L.
Sweet potato	Ipomoea batatas
Cassava	Manihot esculenta B.
Yam	Dioscorea Spp.
Irish potato	Solanum tuberosum
Ogbono	Irvingia gabonensis

The food items are henceforth referred to by their common names.

3.4 Moisture content determination

This was carried out by the direct heating method (Jacobs, 1951).

A definite amount of food sample was weighed into a dry dish. The dish was then placed in an air oven thermostatically controlled at 105^{0}C and heated until successive weighings after cooling showed no further loss in weight. The loss in weight equals the moisture content. The % moisture content was calculated using the expression:

$$\% \text{ moisture content} = \frac{\text{Loss in weight of sample}}{\text{weight of original sample}} \times 100$$

3.5 Determination of Iodine

The method used is that described by Moxon and Dixon (1980) which is a colorimetric procedure for the determination of total iodine in food, based on the catalytic destruction of thiocyanate by nitrite in the presence of iodide. The reaction involves the addition of potassium thiocyanate solution and iron (III) ammonium sulphate reagent to the sample solution to give an orange solution which colour is due to the formation of iron (III) thiocyanate complex. The destruction of this colour complex on the addition of sodium nitrite solution is catalysed by the presence of iodide in the solution. The higher the concentration of the iodide, the lower the absorbance of the solution at the end of a period of

about twenty minutes. The method has a detection limit of 0.01ug/g of food.

3.5.1 Preparation of reagents

All the reagents were used without further purification. Analytical grade reagents were used for the preparation of standards. Distilled water was used throughout for preparing the reagents.

3.5.1.1 Preparation of IM KOH solution

56g of potassium hydroxide was weighted, dissolved in water, and diluted to 100ml in a volumetric flask.

3.5.1.2 Preparation of 10% (m/v) $ZnSO_4$ solution

10g of Zinc sulphate heptahydrate was dissolved in water and diluted to 100ml in a volumetric flask.

3.5.1.3 Preparation of 0.023%(m/v) KSCN solution.

0.23g of potassium thiocyanate was dissolved in water and diluted to 1l in a volumetric flask.

3.5.1.4 Preparation of 2.07% (m/v) $NaNO_2$ solution

2-07g of sodium nitrite was dissolved in water and diluted to 100ml in a volumetric flask. This solution was prepared when needed as it is stable for one day only.

3.5.1.5 Preparation of ammonium iron (III) sulphate reagent

77g of ammonium iron (III) sulphate ($NH_4\ Fe(SO_4)_2\ 12H_2O$)

was dissolved in 400ml of water. 167ml of concentrated nitric acid (S.G. 1.42) was added and diluted to 1l in a volumetric flask. The solution was warmed until all traces of the solid dissolved.

3.5.2 Preparation of standard iodide solutions standard iodide solution (4g/l):

0.5232g of previously dried potassium iodide was dissolved in water and diluted to 100ml in a volumetric flask. The concentration of this solution is 4g/l.

Standard iodide solution (40mg/l): 10ml of the stock iodide solution (4g/l) was diluted to 1l with water in a volumetric flask. This solution has concentration of 40mg/l.

Standard iodide solution (200ng/ml): 5ml of the standard iodide solution (40mg/l) was further diluted to 1l with water in a volumetric flask. This standard iodide solution has a concentration of 200ng/ml and was the working solution.

Into a series of 100ml volumetric flasks, 10,8,6,4,2, and 0ml of the standard iodide solution (200ng/ml) were pipetted. To each solution, 2ml of IM potassium hydroxide solution was added so that they were of the same alkalinity as those of the sample solutions when diluted to 100ml. These were stored in glass bottles away from light.

3.5.3 Preparation of samples for iodine determination

Each food sample was accurately weighed into a clean, dry

crucible the mass of food taken depended on the moisture content of the sample and on the anticipated iodine content. This mass ranged from 0.5g to 4g. 2ml of 1M potassium hydroxide solution was then added followed by 1ml of the 10% (m/v) zinc sulphate solution. The mixture was then slurried with a glass rod and any residue left on the glass rod was washed back into the crucible with a jet of distilled water. The crucible was then placed in an oven at 95^{0}C until dry, then covered with a lid and placed in a muffle furnace at 100^{0}C. The temperature was raised evenly to 550^{0}C in approximately 90 minutes and maintained at this temperature for 1 hour. The crucible was then immediately removed and allowed to cool to room temperature.

1ml of the 10% (m/v) zinc sulphate solution was added and the charred residue slurried. The drying and ashing procedure was repeated. Three reagent blanks were also taken through the whole procedure.

Following the destruction of organic matter, the residue was dissolved in 50ml of water. This was placed in a centrifuge tube and centrifuged to separate any carbonaceous matter. About half of the solution was decanted and stored in a clean container.

3.5.4 Procedure for colorimetric reaction

4 ml of each standard, sample, and reagent blank solution were pipetted into respective 15x1.5cm test tubes. To each tube, 1 ml of water, 1 ml of 0.023% (m/v) potassium

thiocyanate solution, and 2 ml of ammonium iron (III) sulphate reagent were added. The orange solutions in the tubes were mixed well. At exactly 90s intervals, 1ml of 2.07% (m/v) sodium nitrite solution was added and again the solution in the tubes were mixed. The absorbance of each solution was measured at a wavelength of 450nm in a colorimeter after 20 minutes still at exactly 90s intervals.

By using the range of standards, a calibration graph was prepared. From the absorbance values of the sample and blank solutions, the iodine content of the solutions were determined, in ug/ml, from the calibration graph. The iodine content of the sample in ug/g was calculated using the following equation:

$$\text{I content (ug/g)} = \frac{(A-B)x5}{W \times 100}$$

Where

A is the iodine content of the sample solution (ng/ml),

B is the mean iodine content of the blank solution (ug/ml)

W is the amount of sample in grams.

3.5.4.1 Variation of absorbance with time for some food substances based on the Moxon and Dixon method (1980)

A study was made to check whether the 20 minutes specified for optimum colour development in the Moxon and Dixon method would be sufficient for the various groups of food samples. The following food items were used: water leaf,

fluted pumpkin leaf, garlic, egg (whole), guinea corn, sweet potato, and onion.

After the development of the colour by the procedure described in section 3.5.4 and the addition of the sodium nitrite solution, the absorbance of each food item was taken after a time interval of 0,2,5,8,10,15,20, and 25 minutes. A graph of the absorbance against the time was then plotted.

3.6 Determination of starch

The method used for the determination of the starch content of the food samples is that described by Allen et al, 1974.

3.6.1 Preparation of reagents

3.6.1.1 Preparation of 0.1% (m/v) phenol red indicator

0.05g of phenol red indicator was weighed, and dissolved in ethanol in a 50ml volumetric flask, making it up to the mark with ethanol.

3.6.1.2 Preparation of IM sodium hydroxide

40g of sodium hydroxide was weighed and immediately transferred into a volumetric flask, dissolved with water, and diluted to 1l with water.

3.6.1.3 Preparation of 10% (m/v) potassium iodide

10g of potassium iodide was weighed and dissolved in water. It was diluted to 100ml in a volumetric flask.

3.6.1.4 Preparation of 0.0125M potassium iodate

0.669g of potassium iodate was weighed and dissolved in water. It was then diluted to 100ml in a volumetric flask.

3.6.1.5 Preparation of 10% (v/v) acetic acid

10ml of acetic acid was pipetted into a 100ml volumetric flask and was diluted to the mark with water.

3.6.2 Preparation of food samples for starch determination.

250mg (or more) of ground food material was weighted into a suitable test tube. 200mg of fine sand was added to it followed by $5cm^3$ of water and mixed well with a stirring rod. The tube was then heated in a boiling water bath for 15 minutes to gel the starch. It was then cooled to between 20 and 35^0C and 5ml of 70% perchloric acid was rapidly added while stirring. The tissue was ground against the side of the tube intermittently with a glass rod for 20 minutes. It was then transferred to a 100ml volumetric flask and diluted to volume with water. It was mixed well and allowed to settle. An appropriate aliquot was then transferred into a 50ml volumetric flask (Allen et al, 1974).

3.6.3 Preparation of standard starch solution

0.05g of pure starch was weighed and placed in $5cm^3$ of water, followed by the addition of $5cm^3$ of 70% perchloric acid. The solution was stirred. It was allowed to stand for

20 minutes, then transferred to a 50cm^3 volumetric flask and diluted to volume with water. 1cm^3 of this solution contains 1mg of starch. Into a series of 50cm^3 volumetric flask, 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, and 2.5 cm^3 of the standard was transferred respectively. These contained from 0.1 to 2.5mg starch.

3.6.4 Procedure for colorimetric determination of starch

To each of the standard and sample solutions, a few drops of indicator solution was added. Sodium hydroxide solution (1M) was then added until the solution turned red. Acetic acid (10% v/v) was added drop wise until the colour disappeared and then a further 2.5cm^3 of the acid was added. 0.5cm^3 10% (m/v) potassium iodide solution and 5cm^3 0.0125M potassium iodate solution were added and the solution shaken well. It was then diluted to volume and mixed properly. The absorbance was measured at 680nm using water as a reference.

Blank determinations were carried out in the same way.

A calibration graph was prepared from the standards and used to obtain the content (mg) of starch in the sample aliquot. The % starch content was calculated from the expression.

$$\% \text{ starch} = \frac{Z(\text{mg}) \times \text{solution volume (cm}^3)}{10 \times \text{aliquot (cm}^3) \times \text{sample weight (g)}}$$

Where Z is mg starch obtained from the calibration graph.

3.7 Determination of crude protein

This was carried out by the Kjeldahl method (AOAC, 1980). The wet samples were dried before grinding.

3.7.1 Preparation of reagents

3.7.1.1 Catalyst mixture

This was prepared by mixing sodium sulphate and copper sulphate in the ratio 10:1.

3.7.1.2Preparation of 1% (m/v) Phenolphthalein

1g of phenolphthalein was dissolved in ethanol and made up to 100ml with ethanol in a volumetric flask.

3.7.1.3 Preparation of 40% (m/v) sodium hydroxide

40g of sodium hydroxide was dissolved in water and made up to 100ml with water in a volumetric flask.

3.7.1.4 Preparation of 4% (m/v) boric acid

4g of boric acid was dissolved in water and made up to 100ml with water in a volumetric flask.

3.7.1.5 Mixed indicator

Two volumes of 0.2% alcoholic methyl red was mixed with one volume of 0.2% alcoholic methyl blue. The 0.2% alcoholic solutions was prepared by dissolving 0.2g of each indicator in ethanol and making it up to 100ml with ethanol in a volumetric flask.

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3.7.1.6 Preparation of 0.5N sulphuric acid

3.4 ml of concentrated sulphuric acid was added to water and diluted to 250ml in a volumetric flask.

3.7.2 Procedure for crude protein determination

0.5g of ground sample was weighed in Kjeldahl digestion flask (100ml capacity). 5g of digesting catalyst mixture was added. About 25ml of concentrated sulphuric acid was then added. The flask was placed in an inclined position and heated gently until frothing ceased. It was then boiled briskly until the solution cleared and then for about 30 minutes longer. It was allowed to cool and diluted with water to 250ml and poured into 500ml Kjeldahl distillation flask. 70ml of 40% (m/v) sodium hydroxide solution was added. The flask was then connected to the distillation apparatus with the tip of the condenser immersed in the 4% boric acid to which 2 drops of mixed indicator had been added. The flask was heated and the ammonia evolved trapped in the boric acid solution. The receiver was then removed, the tip of the condenser washed with water and the ammonia boric acid solution was titrated with 0.5N sulphuric acid. The percent nitrogen was calculated as:

$$\% \text{ Nitrogen} = \frac{0.014 \times N \times V}{\text{weight of sample (g)}} \times 100$$

Where N = Normality of H_2SO_4 used

V = titre value of H_2SO_4 used (ml)

% crude protein = % Nitrogen x y

Where the factor y used = 6.25 with the exception of soyabeans

y for soya beans = 5.71.

3.8 Determination of lipid content of foodstuffs

The well ground, dried sample (5g) was weighed into a pre-weighed fat-free thimble. Petroleum spirit (60^0-80^0) (about 350ml) was poured into a previously weighed 500ml round bottom flask. The soxhlet extraction apparatus was then fitted into the flask and the extraction carried out for 7 hours. The petroleum spirit was then distilled off. The oil left in the flask was dried in a hot air oven at 70^0C to a constant weight. The percentage lipid was calculated as:

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

This was carried out by the ACOCS method, (1942).

3.9 Ash content determination

This was carried out by the AOAC (1980) procedure. The dried ground sample (1g) was weighed into a pre-weighed crucible. This was then placed in a muffle furnace at 550^0C for 3 hours or more until a grey or white residue was obtained. The crucible was then transferred to a desiccator to cool after which it was weighed. The percent ash was calculated as follows:

$$\% \text{ Ash} = \frac{\text{weight of ash (g)}}{\text{weight of sample(g)}} \times 100$$

3.10 Carbohydrate determination

The carbohydrate content of the foodstuffs was determined by difference that is the lipid content, ash content and protein content were subtracted from 100.

% carbohydrate = 100 - (% lipid + % ash +% protein)

3.11 Metal determination

Sodium was determined by flame photometry while calcium, iron, and copper were determined by atomic absorption spectrometry. The food samples were prepared for the determination by the procedure described in section 3.5.3.

3.11.1 Determination of sodium

The procedure used is that described by Allen et al, 1974.

3.11.1.1 Preparation of sodium standard

The stock solution (100 ppm Na) was prepared by dissolving 0.2542g dry sodium chloride in water and made up to 1l in a volumetric flask.

The working standards were prepared by diluting the stock solution 100 times. From this, appropriate volumes were taken to given a range of 0 to 15 ppm sodium. The standard were stored in plastic containers.

3.11.1.2 Procedure for determination of sodium

A sodium filter was used. The standard solutions were

used to prepare a calibration curve after setting the top standard to a suitable scale deflection and the 0ppm standard to 0. The sample solution was aspirated into the flame under the same condition as the standards. Blank determinations were carried out in the same way.

The calibration curve was used to determine the concentration of sodium in the sample solution. The sodium concentration (ppm) for each sample was calculated as follows:

$$\text{Na (ppm)} = \frac{C \times \%\ E \times \text{Dilution factor} \times \text{solution volume}}{\text{weight of sample}}$$

Where C = Concentration of sodium (ppm) from the graph.

% E = % emission for the sample.

3.11.2 Determination of calcium, iron, and copper.

The procedures used are those described by Fishman and Friedman, 1959.

3.11.2.1 Preparation of Calcium Standard

Phosphate, sulphate, aluminium, and silicon interfere in calcium determination but their presence is masked by the addition of lanthanium.

Calcium standard solution: 1.25g of dried calcium carbonate was suspended in water and dissolved cautiously with a minimum of dilute hydrochloric acid. It was diluted to 1l in a volumetric flask with water . 1 ml = 0.5mg Ca.

Calcium working standards: The standard solution was diluted

to give a range of standards from 0 to 8 ppm Ca. For each working standard, 1 ml of lanthanium chloride solution was added for each 10ml of standard. A blank solution was prepared in a similar way.

Lanthanium chloride solution (87g/l): 29g of lanthanium oxide (La_2O_3) was mixed with a few millilitres of water to form a slurry. 250ml of concentrated hydrochloric acid was slowly added while stirring. The solution was diluted to 500ml with water.

3.11.2.2 Preparation of Iron Standard

Iron standard solution 1 (400ug/ml Fe): 0.4g analytical grade iron wire was weighed. It was dissolved in 5ml concentrated nitric acid and diluted to 1l in a volumetric flask with water.

Iron standard solution II (1 ml = 4 ug Fe): 10ml of iron standard solution I and 1 ml of concentrated nitric acid were diluted to 1l with water.

Iron working standards: A range of standards containing 0 to 4 ppm Fe was prepared from iron standard solution II.

3.11.2.3 Preparation of copper standard

Copper standard solution 1 (1000 ug/ml Cu): 1.252g of copper (II) oxide was dissolved in a minimum amount of nitric acid. The solution was heated to increase the rate of dissolution. 10ml of concentrated nitric acid was added and diluted to 1l with water in a volumetric flask.

Copper standard solution II (5 ug/ml Cu): 5 ml copper standard solution I and 1 ml concentrated nitric acid was diluted to 1l in a volumetric flask with water.

Copper working standards: A range of 0 to 4 ug/ml Cu standard solutions were prepared from the copper standard solution II.

3.11.2.4 Procedure for determination of Ca, Fe, and Cu.

The blank was aspirated to set the automatic zero control. The automatic concentration control was then used to set the concentrations of the standards. The instrument was calibrated each time a set of samples was analysed and calibration was checked at intervals. The instrumental conditions are summarised in table 3.2

The concentration of each metal in ppm was calculated as follows:

$$M(ppm) = \frac{C \times \% A \times \text{Dilution factor} \times \text{solution volumes}}{\text{weight of sample}}$$

Where M is the metal

C is the concentration of metal (ppm) obtained

From the calibration graph

% A is the % absorbance.

Table 3.2 Instrumental conditions for the determination of Ca, Fe, and Cu.

Element	Grating	Wavelength (nm)	Hollow Cathode Lamp	Oxidant	Fuel
Calcium	Visible	422.7	Calcium	Air	Acetylene
Iron	Ultra-violet	248.3	Iron	Air	Acetylene
Copper	Ultra-violet	324.7	Copper	Air	Acetylene

3.1.2 Determination of goitrogens (SCN^- and phenol)

The extracts of the food substances - Cabbage, onion, fluted pumpkin leaf, cassava, and millet were determined for the goitrogens. These food items were chosen because except for fluted pumpkin leaf, the others have been cited in literature to likely contain goitrogens (section 1.4.1).

3.12.1 Preparation of food samples for goitrogen determination

2g (or more) of ground or minced food material was weighed into a suitable test tube. 5ml of water was added and the solution mixed well with a stirring rod. The tube was then heated in a boiling water bath for about 15 minutes. It was then cooled to between 20 and 35^{0}C and 5ml of 70% perchloric acid was added. The solution was stirred. The tissue of the sample was ground against the side of the tube intermittently with a glass rod for 20 minutes. It was then

transferred to a 50ml volumetric flask and diluted to volume with water. It was mixed well and allowed to settle. The clear solution was decanted and stored (Allen et al, 1974).

3.12.2 Determination of Thiocyanate (SCN^-)

This was carried out according to the procedure described by Sheela and Schenck, 1983.

3.12.2.1 Preparation of SCN^- standard

0.097g of potassium thiocyanate was dissolved in water and made up to 1l in a volumetric flask. This solution has a concentration of 1 ug/ml.

0 to 1 ml of the standard solution respectively was taken and each diluted to 5 ml to give a range of 0 to 0.2 ug/ml SCN^- standard.

3.12.2.2 Preparation of 40% (m/v) $FeCl_3$ in concentrated HCl

20g of ferric chloride was weighted and dissolved in concentrated hydrochloric acid. The volume was made up to 50ml in a volumetric flask with concentrated hydrochloric acid.

3.12.2.3 Procedure for SCN^- determination

To each standard solution and 5ml of each sample, 0.02ml of the 40% ferric chloride solution was added. The absorbance was taken at 450nm. A calibration curve was plotted from the range of working standard solutions.

3.12.3 Determination of Phenol

The procedure used for the determination is that described by Allen et al, 1974.

3.12.3.1 Preparation of phenol standard

0.25g of phenol was weighted and dissolved in water. It was made up to 250ml with water in a volumetric flask. 1 ml of this solution was diluted to 100ml with water in a volumetric flask. A range of 0 to 30ml of this solution was pipetted into separating funnels to give a range of 0 to 300ug phenol.

3.12.3.2 Preparation of 4-amino/phenazone solution

0.17g of 4-aminophenazone was weighed, dissolved in water, and made up to 100ml in a volumetric flask.

3.12.3.3 Preparation of 5% (m/v) $NaHCO_3$

5g of sodium bicarbonate was weighed, dissolved in water, and made up to 100ml with water in a volumetric flask.

3.12.3.4 Preparation of 10% (m/v) potassium ferricyanide

10g of potassium ferricyanide was weighed, dissolved in water, and made up to 100ml with water in a volumetric flask.

3.12.3.5 Procedure for phenol determination

10ml of sample extract was measured into a separating funnel. To each standard and sample solution, 4ml of the 5%

(m/v) sodium bicarbonate solution, 5ml of the 4-aminophenazone solution, and 3 ml of the 10% (m/v) potassium ferricyanide solution were added. The solution was mixed well and allowed to stand for 15 minutes. 10ml of carbon tetrachloride was then added and the solution swirled well in the separating funnel. It was allowed to stand and the lower carbon tetrachloride layer was collected. This extraction with 10ml of carbon tetrachloride was repeated twice and the extracts were combined.

The absorbance of the extract was measured at 605nm. A calibration curve was prepared from the standard readings and used to calculated the concentration of phenol.

Phenol (ug/g) = x/weight of sample

Where X is the phenol concentration from the calibration graph.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

The values reported below are generally the mean of three determinations in each case.

4.1 Moisture content of the food substances

The moisture contents determined for some foodstuffs are given in table 4.1.

Long storage foods have their moisture contents below 20% (Church and Pond, 1982). The grains, seeds, dried fish, and processed foods like garri, yam flour, salt, sugar, and milk powder all fall into this category of food items. The other food items are high in moisture content and usually need to be stored either by refrigeration or freezing, except the roots and tubers which can keep for an appreciable length of time.

The moisture content of the foodstuffs was determined to see if any relation exists between it and the iodine content and also to give some idea of the mass of each substance to be used in iodine determination.

4.2 Iodine content determination

4.2.1 Variation of absorbance with time for some food substances in the determination of iodine

The plot of absorbance against time in the determination of iodine for representatives of the various classes of

foodstuffs, showed that none had an appreciable absorbance after 20 minutes of colour development (Figs. 4.1 - 4.7). The absorbance for the extracts from water leaf, fluted pumpkin leaf, garlic, egg (whole), guinea corn, sweet potato, and onion - were generally too low (below 0.11) to be accurately used in determining the iodine content from the calibration curve (See Appendix 1). For most of the food items (except fluted pumpkin leaf and garlic), the optimum absorbance that could be accurately read from the calibration curve was obtained after only 5 minutes of colour development (Figures 4.1 to 4.7).

A modification to the method of Moxon and Dixon, which suggests leaving the reaction to proceed for 20 minutes was judged more appropriate. This could be effected by:

(i) taking the absorbance at the lowest readable absorbance on the calibration curve;

(ii) preparing standards that will give very low absorbance values so that these can be read on the calibration curve. The problem with this is that an absorbance value below 0.1 is usually subject to error, or

iii) diluting the food extract to a known volume with distilled water and leaving the reaction to proceed for the suggested 20 minutes. This gave readable values on the calibration curve.

This third option was chosen for most of the food substances that did not give readable values initially. The

first option was applied to the water leaf extract.

The graphs in figures 4.1 to 4.7 show that the required time for optimum colour development ranges from 8 minutes (figures 4.1, 4.5 and 4.7) to 15 minutes (figures 4.3 and 4.6). Figures 4.2 and 4.4 indicate an optimum colour development time of 9 and 10 minutes respectively.

Table 4.1 Moisture content of some food substances (Mean value ± standard deviation)

Food substance	% Moisture content
Grains	
Yellow corn	8.2 ± 0.2
White corn	7.2 ± 0.1
Guinea corn	11.0 ± 0.1
Millet	8.3 ± 0.2
Local rice	2.0 ± 0.1
Polished rice	8.7 ± 0.1
White beans (unpeeled)	9.9 ± 0.2
White beans (peeled)	11.2 ± 0.1
Brown beans (unpeeled)	10.1 ± 0.2
Brown beans (peeled)	10.3 ± 0.3
Soyabeans (untreated)	6.9 ± 0.2
Soyabeans (treated)*	7.0 ± 0.4
Dried fermented locust beans	12.4 ± 1.0
Seeds	
Melon seeds	3.9 ± 0.2
Ogbono	5.2 ± 0.1
Salted roasted groundnut	3.0 ± 0.1
Fruits	
Garden egg	91.6 ± 0.8
Pawpaw	90.8 ± 1.2
Mango	87.8 ± 2.8
Water melon	96.8 ± 1.2
Tomato	93.8 ± 0.1
Fresh pepper	89.9 ± 0.8
Dried pepper	3.7 ± 0.1

Table 4.1 cont.

Food substance	% Moisture content
Vegetables	
Spinach	88.7 ± 0.3
Water leaf	91.5 ± 0.5
Cabbage	90.8 ± 0.2
Fluted pumpkin leaf	88.5 ± 0.4
Onion	88.7 ± 0.8
Garlic	70.9 ± 0.6
Roots and Tubers	
Sweet potato	71.9 ± 1.3
Garri (dried, fermented cassava)	6.4 ± 0.1
Yam tuber	63.4 ± 0.4
Yam flour	6.7 ± 0.1
Fish	
Alestes (dried)	5.7 ± 0.1
Clarias "	7.3 ± 0.1
Crayfish "	8.0 ± 0.2
Meat and egg	
Beef	78.0 ± 0.5
Goat meat	78.2 ± 1.0
Egg (whole)	79.6 ± 0.3
Egg (albumen)	84.4 ± 0.5
Egg (Yolk)	51.9 ± 0.2
Miscellaneous	
Salt	0.0 ± 0.0
Whole milk powder	3.6 ± 0.1
White sugar	0.0 ± 0.0
Bread	27.9 ± 1.7

* Raw soyabeans contains a trypsin inhibitor (antitrypsin factor) (Sapieka, 1969). To remove this, the soyabeans was boiled for a minimum of 20 minutes in water containing potash. The testa was then peeled off.

4.2.2 Iodine content of the food substances

The values obtained for the iodine contents of the various food materials analysed are given in table 4.2.

As can be seen from table 4.2, the grains show some of the lowest values of iodine as evidenced by the results for the corns, rice, and millet samples. The value obtained for the yellow corn falls in the range of some reported literature values for corn (2.5-5 ug/g). Of the beans, the dried fermented locust beans has the highest iodine content. This may in part be due to the processing which involves the addition of common salt. For the white and brown beans (15.5 and 7.8 ug/g for peeled, and 22.3 and 24.5 ug/g for the unpeeled samples respectively) the difference in the iodine contents of the peeled and unpeeled varieties is indicative of the fact that the testa contains a significant amount of iodine. This is especially so for the brown beans in which the bulk of the iodine seem to be in the testa. Investigations have shown that the outer layers of grains are richer in iodine than the inner layers. Thus bran is richer in iodine than flour (Glimm, 1930). For soyabeans, there does not seem to be much difference between the treated (which involves peeling the testa) and the untreated samples. The yellow corn showed a lower value of iodine than the white variety. The reason for this is not clear. Samples may have originated from soils with different iodine contents.



Figure 4·1 Variation of absorbance with time for water heat extract

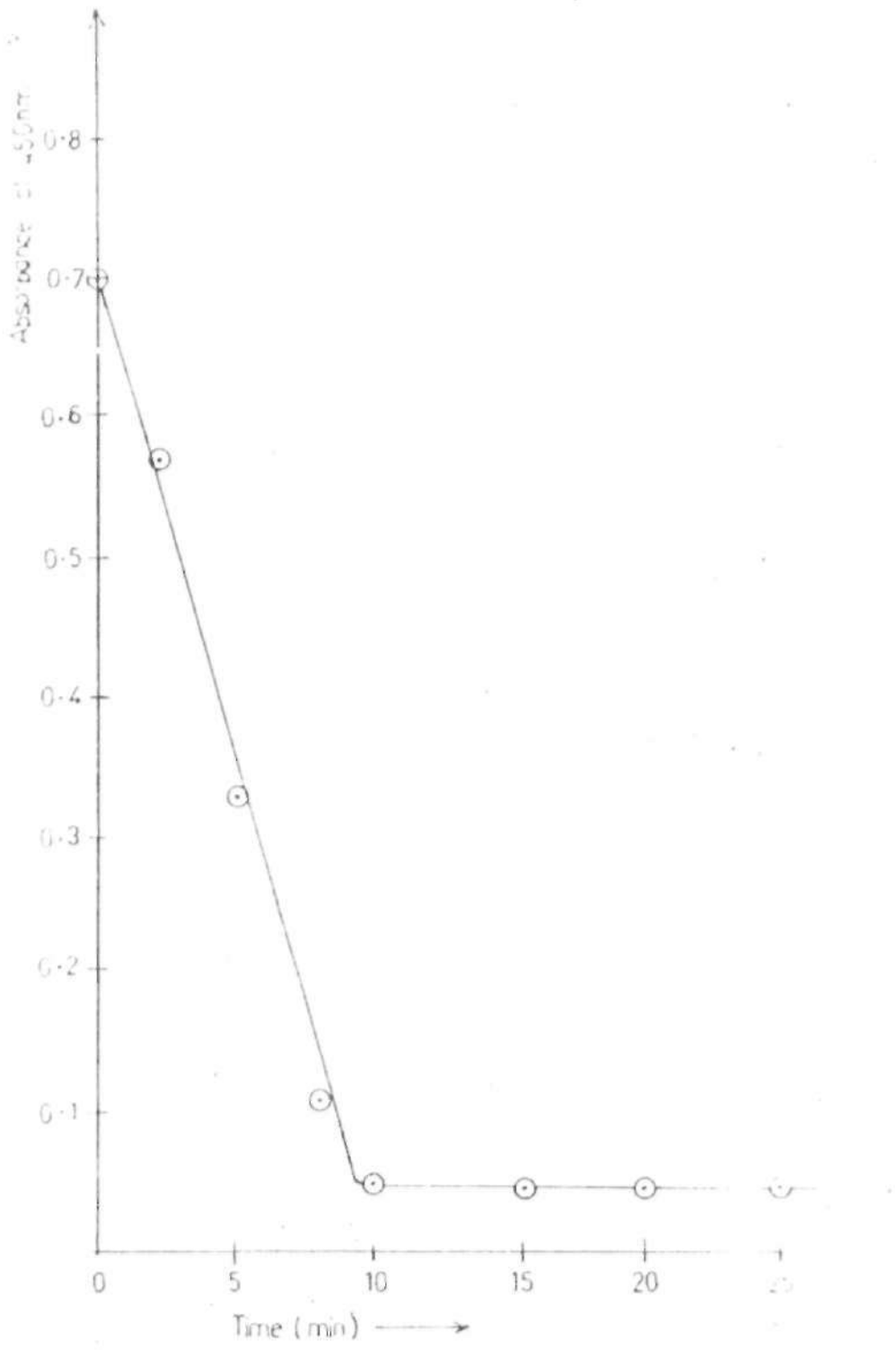


Figure 4·2 Variation of absorbance with time for fixed pentavalent vanadium extract

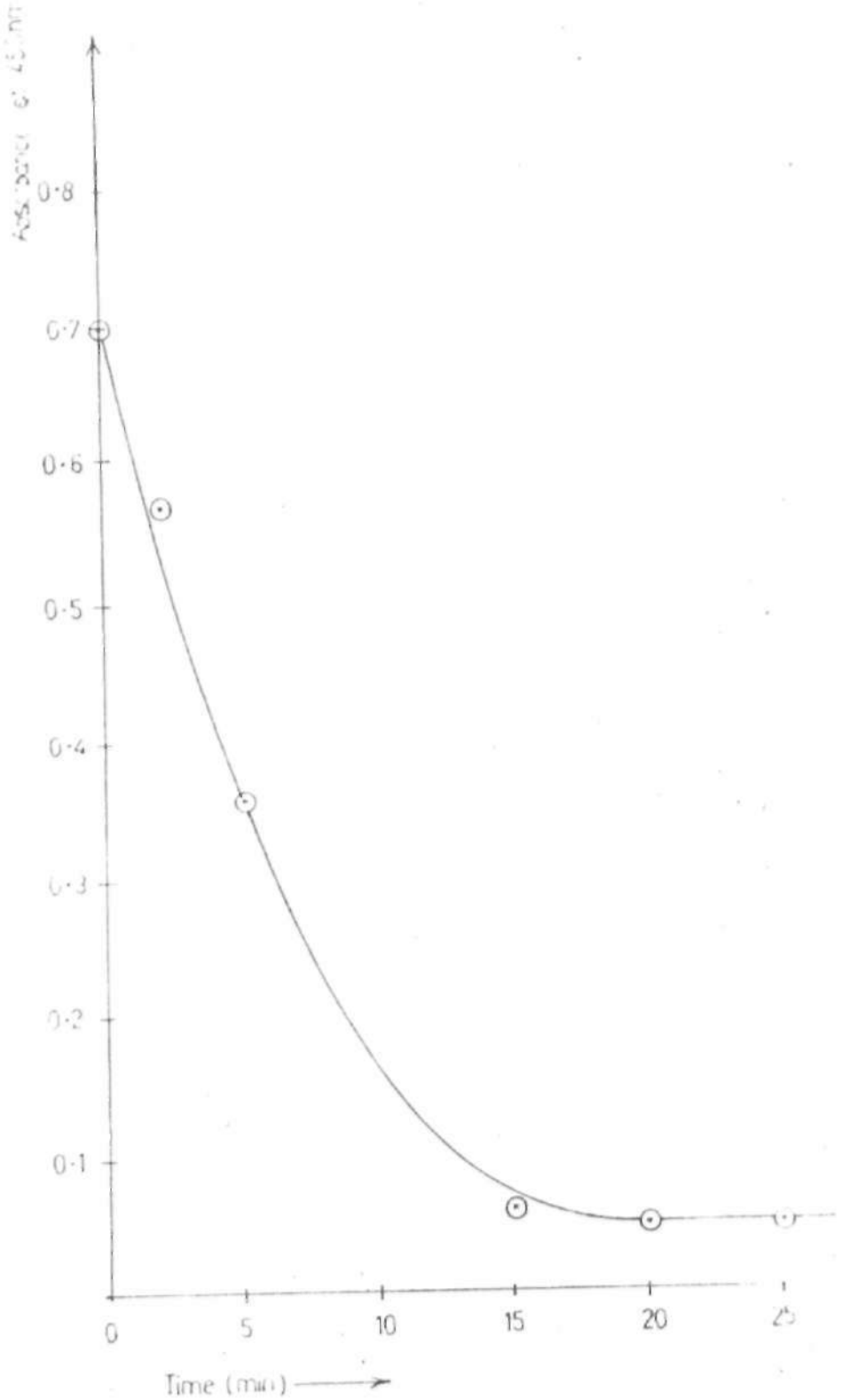


Figure 4·3 Variation of absorbance with time for yeast extract

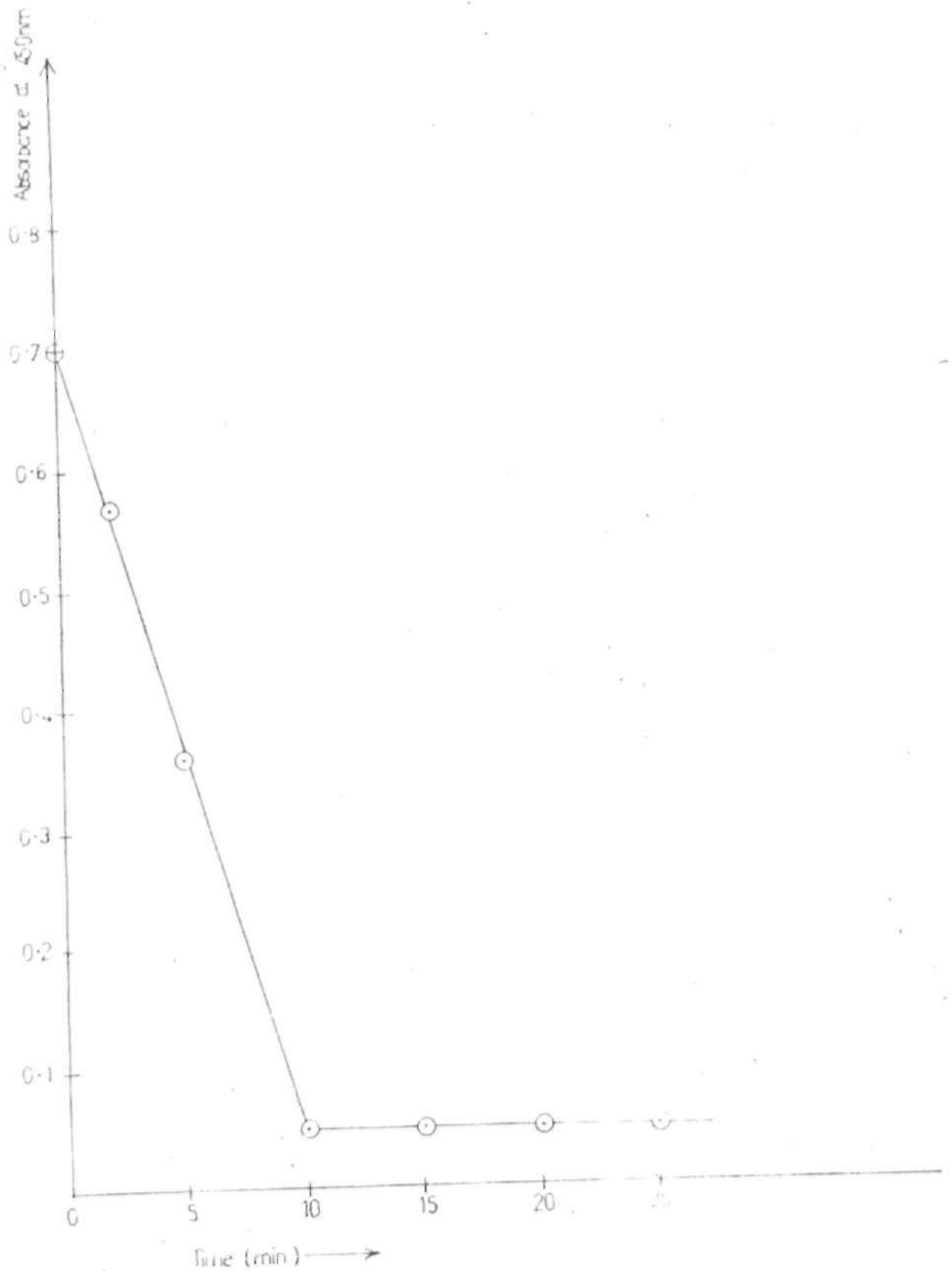


Figure 4·4 Variation of absorbance with time for egg (white) extract

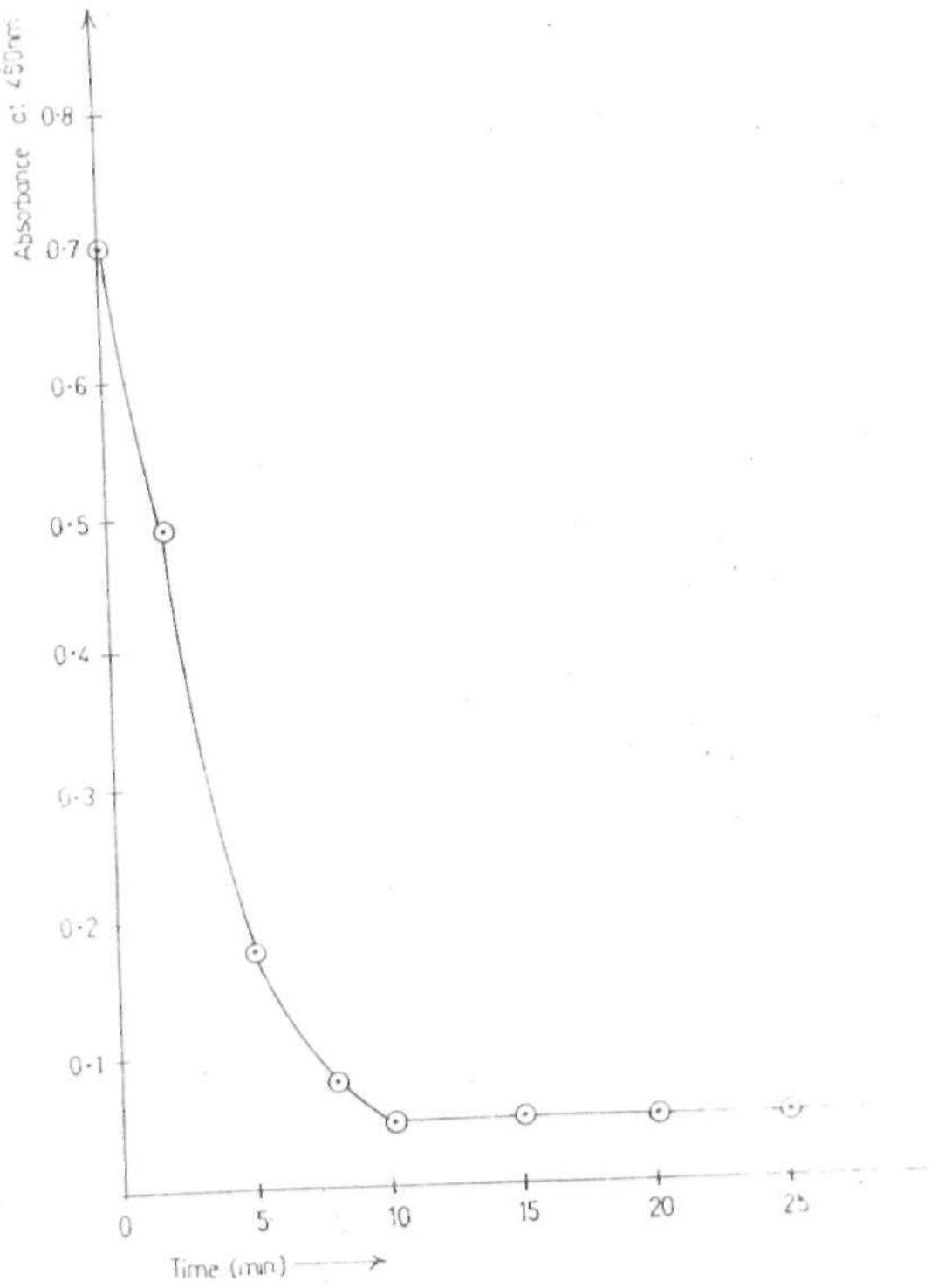


Figure 4·5 Variation of absorbance with time for guinea corn extract

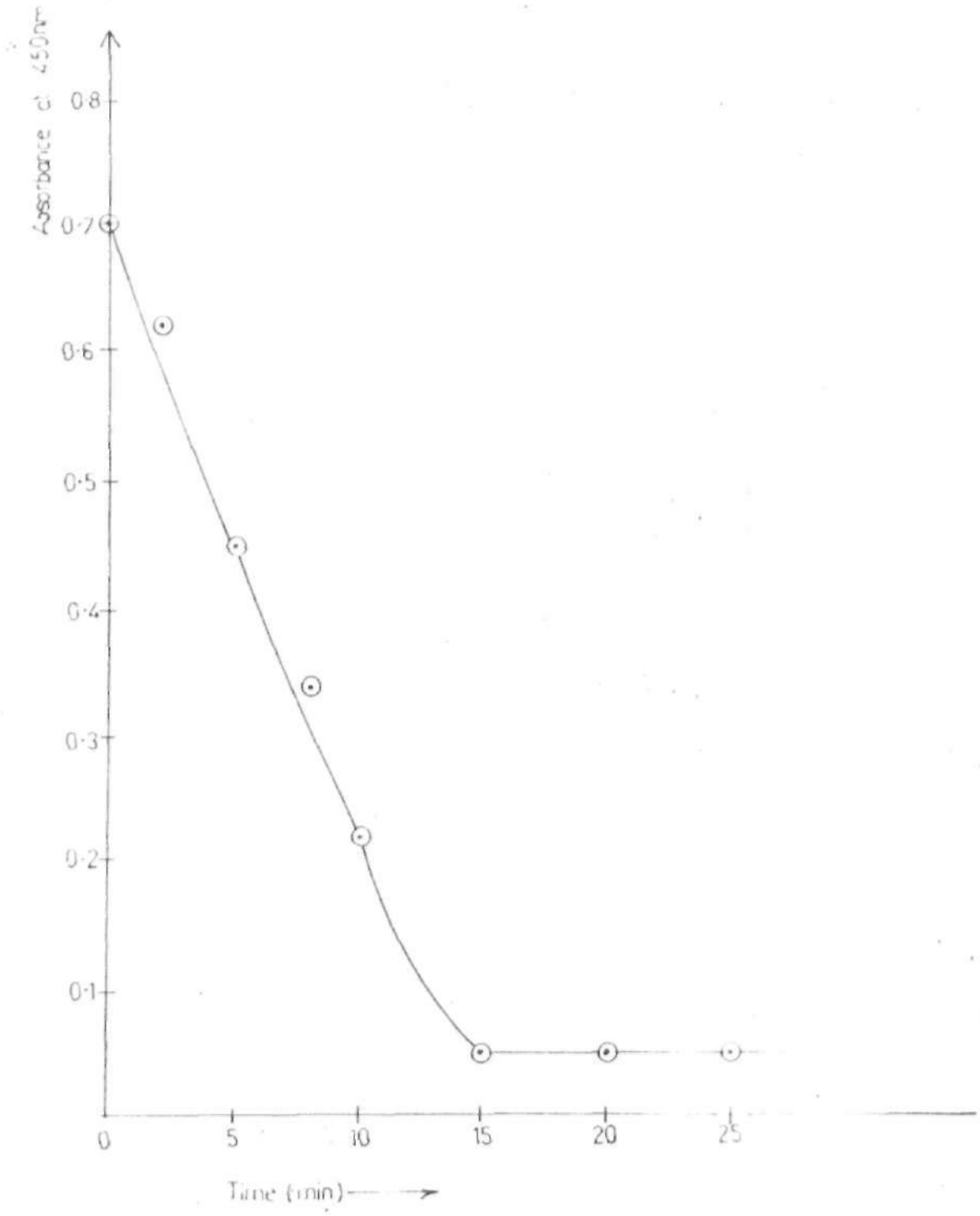


Figure 4·6 Variation of absorbance with time for sweet potato extract

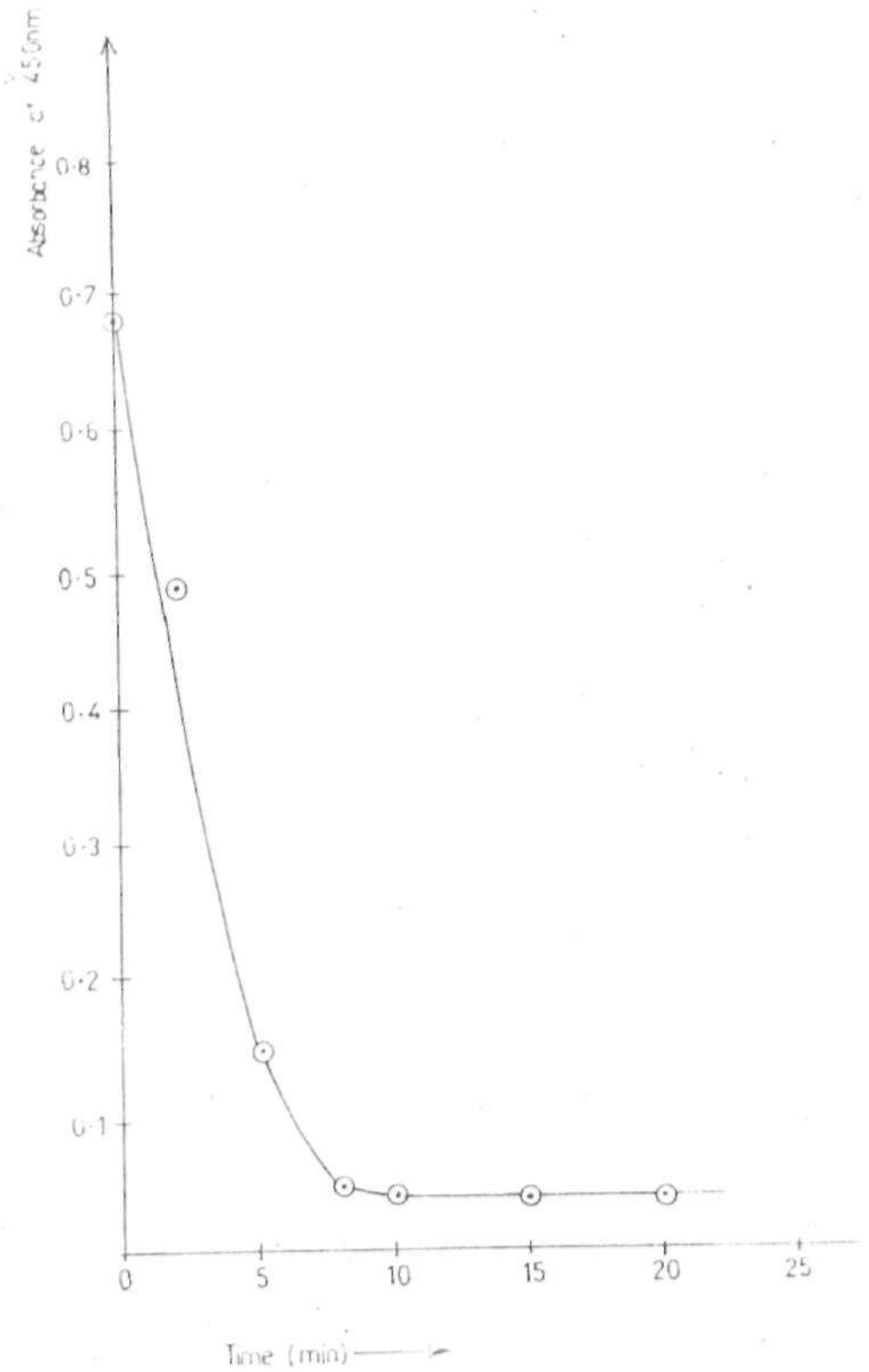


Figure 4·7 Variation of absorbance with time for [illegible] extract

The seeds give an appreciable value for iodine. As would be expected, salted roasted groundnut gave a much higher value for iodine than the unsalted variety. This is of course due to the iodine content of the salt, and provide some partial check for the validity of the analysis.

Fruits and vegetables like pawpaw, spinach, and cabbage are very rich sources of iodine. This may indicate that they were grown on iodide rich soils (Balks, 1936) or that they are able to accumulate iodine better than other foodstuffs. Of the roots and tubers, sweet potato has the highest value of iodine.

As expected (Tressler and Wells, 1924; Tressler, 1925), some of the richest sources of iodine with the fish showing iodine contents of over 145 ug/g. The iodine contents of the fish are somewhat above the range reported in the literature for some fresh water fish (Jarvis, 1928). The eggs and meats are also good sources of iodine, but these values reported in the literature (Wenlock et al, 1982) were generally much higher. The high iodine content of bread may be due to salting during preparation or the addition of potassium oxy-halide (usually potassium bromate) to strengthen the gluten of the flour (Fox and Cameron, 1972).

4.3. Comparison of the iodine and moisture contents of some foodstuffs.

A comparison of tables 4.1 and 4.2 shows that though the grains and seeds have low moisture contents this does not

translate to a high iodine content except for the dried fermented locust beans and the salted roasted groundnut which have had salt added to them during their processing.

Table 4.2 Iodine content of some food substances
Mean value ± standard deviation.

Food substance	Iodine Content (ug/g)
Grains	
Yellow corn	4.8 ± 0.2
White corn	9.0 ± 0.3
Guinea corn	2.0 ± 0.2
Millet	8.3 ± 0.4
Local rice	8.3 ± 0.7
Polished rice	15.3 ± 1.1
White beans (unpeeled)	22.3 ± 1.8
White beans (peeled)	15.5 ± 1.2
Brown beans (unpeeled)	24.5 ± 2.0
Brown beans (peeled)	7.8 ± 0.5
Soyabeans (untreated)	13.3 ± 0.7
Soyabeans (treated)	14.0 ± 0.6
Dried fermented locust beans	132.5 ± 2.7
Seeds	
Melon seeds	11.8 ± 0.6
Ogbono	58.8 ± 1.2
Unsalted roasted groundnut	46.0 ± 5.1
Salted roasted groundnut	210.0 ± 21.0
Fruits	
Carrots	0.2 ± 0.1
Garden egg	8.5 ± 0.2
Pawpaw	145.0 ± 16.0
Mango	35.0 ± 3.2
Water melon	4.6 ± 0.6
Tomato	6.4 ± 0.2
Fresh pepper	8.3 ± 0.1
Dried pepper	26.5 ±0.5

Table 4.2 Cont.

Food substance	Iodine Content (ug/g)
Vegetables	
Spinach	160.0 ± 6.4
Water leaf	70.1 ± 2.1
Cabbage	152.2 ± 9.1
Fluted pumpkin leaf	60.0 ± 4.2
Onion	7.0 ± 0.3
Garlic	6.3 ± 0.3
Roots and Tubers	
Sweet potato	750.0 ± 52.5
Garri (dried fermented cassava)	66.0 ± 3.3
Yam tuber	12.8 ± 0.5
Yam flour	6.8 ± 0.4
Irish potato	3.5 ± 0.4
Fish	
Alestes (dried)	210.0 ± 10.5
Clarias "	280.0 ± 16.8
Tilapia "	148.4 ± 6.0
Crayfish "	145.0 ± 8.7
Meat and Egg	
Beef	237.5 ± 14.3
Goat meat	112.5 ± 7.9
Egg (whole)	280.0 ± 14.0
Egg (albumen)	530.0 ± 20.0
Egg (Yolk)	625.0 ± 25.0
Miscellaneous	
Salt	95.0 ± 1.0
Whole milk powder	5.0 ± 0.3
Cocoa beverage	4.0 ± 0.1
White sugar	11.5 ± 0.2
Bread	95.0 ± 1.9
Biscuit	13.3 ± 0.1
Jam	18.0 ± 0.5

The fruits (except for dried pepper), vegetables, unprocessed root and tubers (sweet potato and yam), meat and eggs are all high in moisture content (Over 50%) with a moderate to high level of iodine (Over 10 ug/g to 750 ug/g). Exceptions in these groups with low iodine content but high moisture are carrots, garden egg, water melon, tomato, fresh pepper, onion, and garlic. The wide difference in the iodine contents of fresh and dried pepper may not only be due to the lower moisture content of the latter but also to the soil on which it is grown. The drying process may also have resulted in a higher content of iodine per unit mass in the dry pepper.

The fish have low moisture content (less than 10%) and a correspondingly high iodine content (Over 145 ug/g). This probably shows that the drying process together with a possible salt treatment helped to concentrate the iodine with little or no loss.

4.4 Starch content of some food substances

The starch contents of the food substances analysed are given in table 4.3. A comparison with reported values (table 2.2) shows that the determined values for the grains compared well with the reported values. For sweet potato, the reported value was significantly higher than the observed value. The reported starch content of the groundnut was also significantly higher. These differences in values may be due to differences in species. The fruits and vegetables compared well with the reported values. The reported starch content

for bread was only slightly higher than the observed value and this may be attributed to differences in the composition of the bread.

As would be expected, the grains show the highest starch content followed by the roots and tubers. The other groups of samples gave low values (less than 10%) for starch.

4.4.1 Comparison of the iodine and starch contents of some foodstuffs.

Table 4.4 compares the iodine and starch contents of some of the food samples analysed. The table shows that the food items may be broadly grouped into three categories namely: (a) foods rich in iodine but low in starch; (b) foods relatively rich in iodine and starch; and (c) foods relatively poor in iodine and rich in starch.

The first category (a) is made up of the fish, meats, and egg. Others are groundnut, dried fermented locust beans, melon seeds, soyabeans, mango, pawpaw, sweet potato, salt, white sugar, and vegetables like cabbage, spinach, water leaf, and fluted pumpkin leaf. These have iodine contents of over 10 ug/g and starch contents below 10%. The fish, meats, egg, groundnut, locust beans, melon seeds, and soya beans are also known to be good sources of protein.

Foodstuffs in the second category (b) include garri, unpeeled brown and white beans, and peeled white beans. These have iodine contents of between 16 and 66 ug/g and starch contents of between 28 and 58%.

The last category (c) is made up of white and yellow corn, millet, local rice, guinea corn (all grains), peeled brown beans and yam flour. These have starch contents of over 40% and iodine contents below 10 ug/g.

4.5 Protein content of food substances

Table 4.5 gives the crude protein for some of the samples.

The grains, except soyabeans, have relatively low values of crude protein. Foods high in protein have values greater than 20% (Church and Pond, 1982).

Table 4.3 The starch content of some food substances

Food substance	Starch content (%)
Grains	
Yellow corn	56.0
White corn	60.0
Guinea corn	40.0
Millet	50.0
Local rice	70.0
Polished rice	68.0
White beans (unpeeled)	42.0
White beans (peeled)	28.0
Brown beans (unpeeled)	44.0
Brown beans (peeled)	44.0
Soyabeans (untreated)	0.03
Soyabeans (treated)	0.03
Dried fermented locust beans	1.0
Roots and Tubers	
Sweet potato	9.0
Garri (dried fermented cassava)	58.0
Yam tuber	22.0
Yam flour	62.0
Seeds	
Melon seeds	1.6
Ogbono	10.0
Salted roasted groundnut	1.7
Fruits	
Garden egg	0.04
Pawpaw	0.03
Mango	1.0
Water melon	0.01
Tomato	0.0
Fresh pepper	0.1
Dried pepper	7.2
Vegetables	
Spinach	0.06
Water leaf	0.03
Cabbage	0.04
Fluted pumpkin leaf	0.05
Onion	0.03
Garlic	0.0

Table 4.3 cont.

Food substance	Starch content (%)
Fish	
Clarias (dried)	0.3
Crayfish "	0.4
Meat and Egg	
Beef	0.06
Goat meat	0.1
Egg (whole)	0.05
Egg (albumen)	0.0
Egg (Yolk)	0.07
Miscellaneous	
Salt	0.0
Whole milk powder	0.6
White sugar	4.0
Bread	44.0

Table 4.4 Comparison of the starch and iodine contents of some foodstuffs (a) foods rich in iodine but low in starch (b) foods relatively rich in iodine and starch, (c) foods relatively poor in iodine and rich in starch.

Food substance	Iodine content (ug/g)	Starch content (%)
	(a)	
Sweet potato	750	9.0
Egg (Yolk)	625	0.1
Egg (albumen)	530	0.0
Egg (Whole)	280	0.1
Clarias (dried)	280	0.3
Beef	238	0.1
Salted roasted groundnut	210	1.7
Spinach	160	0.1
Cabbage	152	0.1
Crayfish (dried)	145	0.4
Pawpaw	145	0.1
Locust beans	133	1.0
Goat meat	113	0.1
Salt	95	0.0
Water leaf	70	0.1
Fluted pumpkin leaf	60	0.1
Mango	35	1.0
Soya beans (treated)	14	0.1
Soya beans (untreated)	13	0.1
Melon seeds	12	1.6
White sugar	12	4.0
	(b)	
Garri (dried fermented cassava)	66	58.0
Brown beans (unpeeled)	25	44.0
White beans (unpeeled)	22	42.0
White beans (peeled)	16	28.0
	(c)	
White corn	9	60.0
Millet	8	50.0
Local rice	8	70.0
Brown beans (peeled)	8	44.0
Yam flour	7	62.0
Yellow corn	5	56.0
Guinea corn	2	40.0

Table 4.5 Crude protein content (%) of some foodstuffs

Foodstuff	Crude protein (%)
Grains	
Millet	11.4 ± 0.5
Guinea corn	11.4 ± 1.0
Soya beans (untreated)	47.2 ± 1.8
Seed	
Salted roasted groundnut	26.8 ± 0.7
Fruit	
Pawpaw	4.3 ± 0.5
Vegetable	
Cabbage	21.4 ± 0.7
Root and tuber	
Yam tuber	8.1 ± 0.2
Sweet potato	4.1 ± 0.5
Meat and fish	
Beef	66.8 ± 1.0
Clarias	69.7 ± 1.6

The protein content of millet is lower than the reported value (16%) but the guinea corn value is slightly greater than the value of 10% obtained elsewhere. The protein, content of soyabeans determined is higher than the reported value of 40% (Table 2.3). The groundnut sample analysed has a crude protein content which is comparable to the literature value of 28%.

The fruit, pawpaw, gave a low value for crude protein (4.3%) while the vegetable, cabbage, has a relatively high value (21.4%). The protein content of pawpaw determined is significantly higher than the reported value of 0.36% (Table 2.3).

The yam and sweet potato samples gave relatively low values for crude protein (8.1% and 4.1% respectively). The value for yam is comparable to the literature value of 8.5% while the value for sweet potato is slightly higher than the literature value (2%).

Both the fish and beef show relatively high crude protein contents (Beef 67% and fish 70%). As would be expected, the foodstuffs usually classified as good protein sources all gave values well above 20% (26-70%). The cabbage sample, a vegetable, indicates a protein content of over 20% suggesting that it is a good protein source.

It has been observed that with an inadequate iodine intake, foods low in protein content could be goitrogenic (Wilson, 1954). A comparison of tables 4.2 and 4.5 displayed

on the bar chart of figure 4.8 shows that the grains millet and guinea corn, and yam tuber are relatively low in both their iodine and protein contents. Diets derived principally from these foodstuffs would require iodine supplementation to ameliorate possible goitrogenic effects. Although soyabean has a relatively low iodine content, its protein content appears sufficiently high to mitigate goitrogenous activity of soya bean based diets. Sweet potato and pawpaw on the other hand have low protein content but high iodine contents and would not be expected to be goitrogenic. Cabbage with relatively high protein and iodine contents, is also not likely to be goitrogenic. The meats (beef and clarias) are good sources of protein and iodine.

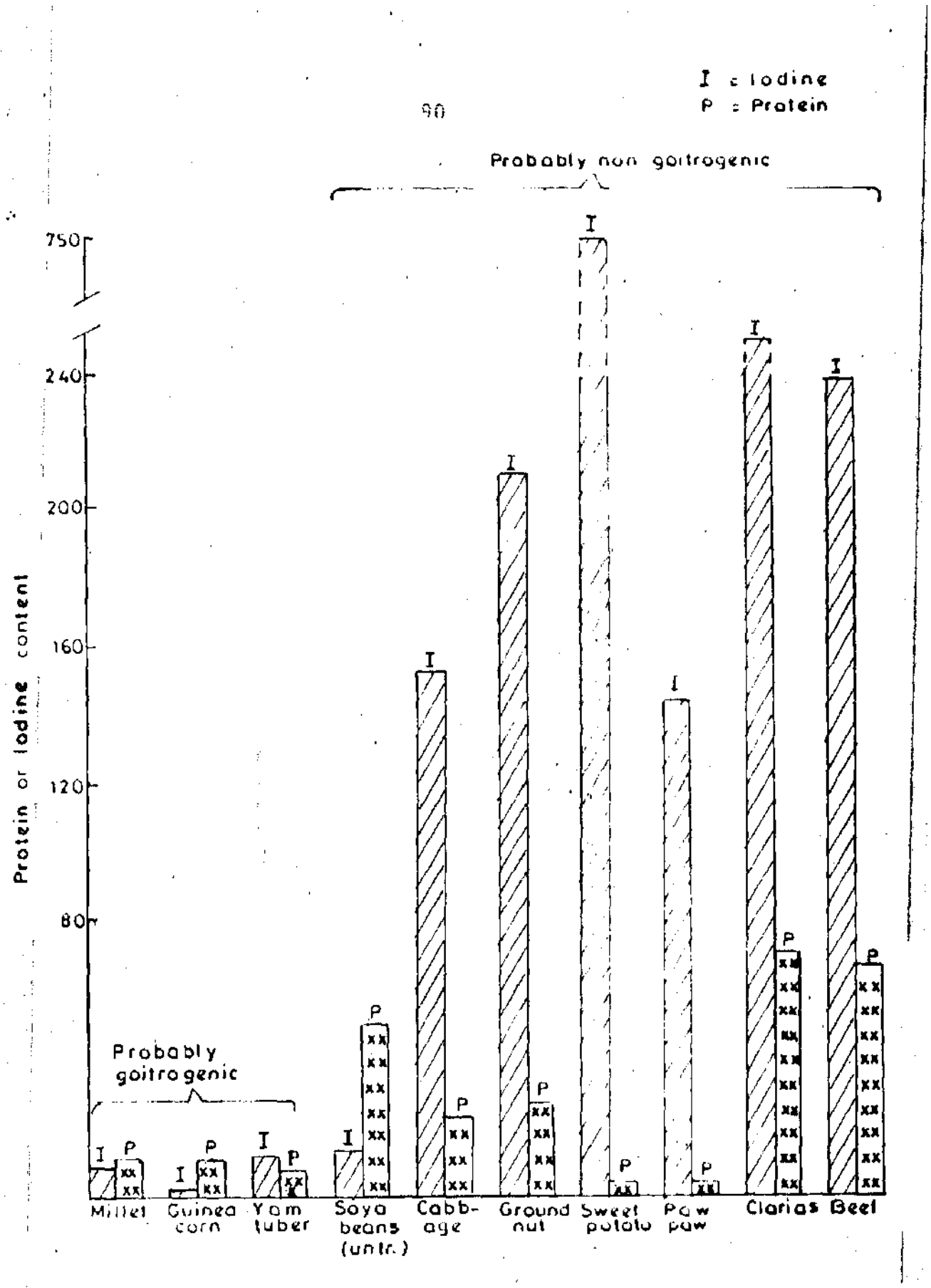


FIG. 4.8: COMPARISON OF THE IODINE (ppm) AND PROTEIN (%) CONTENTS OF SOME FOOD STUFFS

4.6 Lipid content of food substances

Table 4.6 gives the lipid contents of some of the samples. As would be expected the grains (except soyabeans), fruit, cabbage, root and tuber all have lipid contents below 10% (2.9 - 6.6%).

The seed, groundnut, gave the highest value for lipid content (51.4%), followed by the beef (35.6%), untreated soyabeans (21.5%) and clarias (11.2%). The values for the lipid contents of the grains, groundnut, and fish are in agreement with those reported for some samples in the literature (See table 2.3). However, our values for beef, pawpaw, and sweet potato are somewhat higher than those reported in table 2.3. Differences in beef part and varieties of pawpaw and sweet potato analysed may account for this observation.

Wilson (1954) suggested that diets high in fats and inadequate in iodine have goitrogenic action. As can be seen by the comparison of tables 4.2 and 4.6 displayed in the bar diagram of figure 4.9, groundnut, beef, and soyabean (untreated) have high lipid contents. However, these also have relatively high iodine contents which may mitigate the goitrogenic effect of the lipid. Clarias, cabbage, and sweet potato have relatively low lipid contents and high iodine contents and would not be expected to be goitrogenic a conclusion which was also reached by comparison of the iodine and protein contents.

Table 4.6 Lipid content of some foodstuffs

Foodstuff	Lipid content (%)
Grains	
Millet	6.5 ± 0.6
Guinea corn	4.6 ± 0.3
Soyabeans (untreated)	21.5 ± 0.4
Seed	
Groundnut (salted and roasted)	51.4 ± 0.7
Fruit	
Pawpaw	6.1 ± 0.6
Vegetable	
Cabbage	6.6 ± 0.8
Root and tuber	
Yam tuber	2.9 ± 0.8
Sweet potato	3.5 ± 0.5
Meat and Fish	
Beef	35.6 ± 0.2
Clarias	11.2 ± 0.03

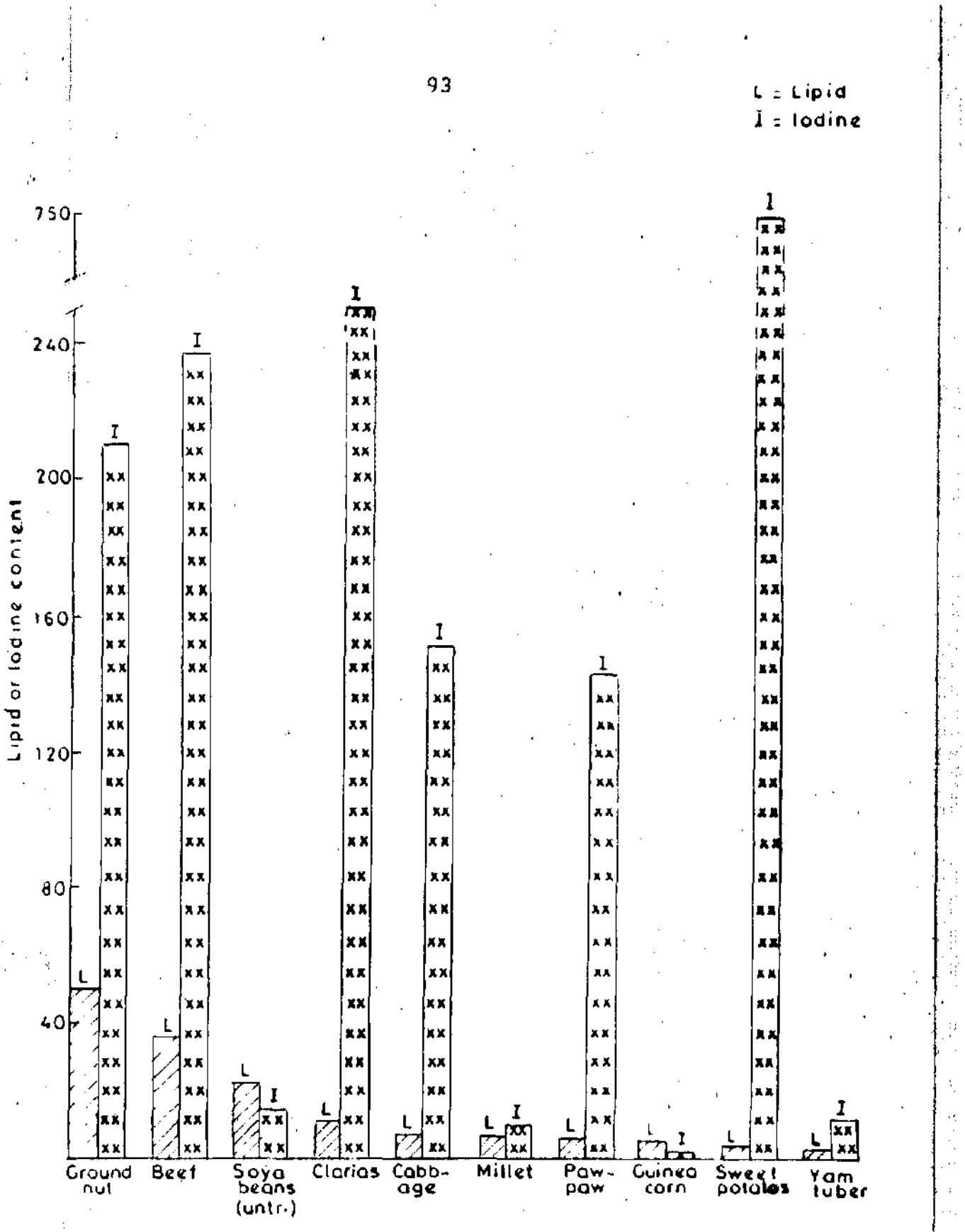


FIG. 4.9: COMPARISON OF THE LIPID (%) AND IODINE (ppm) CONTENTS OF SOME FOOD STUFFS

The grains and yam tuber on the other hand have low values for both lipid and iodine contents, and their goitrogenic effect will be determined by a consideration of other factors apart from the lipid content.

Comparison of the bar charts in figures 4.8 and 4.9 shows that foodstuffs rich in lipid (over 11%) and iodine (over 200 ug/g except soyabeans 13.3 ug/g) also have high crude protein contents (over 26%).

Conversely, it would appear that foodstuffs with relatively low lipid and iodine contents are also relatively low in crude protein. Millet and guinea corn exemplify this latter group.

4.7 Ash content of some foodstuffs

The ash contents of some foodstuffs were determined in order to use them in the calculation of the carbohydrate content (by difference) of these foodstuffs. Table 4.7 gives the values obtained. It gives an idea of the quantity of mineral elements present in each sample. However the observed contents do not show any distinct trends.

4.8 Carbohydrate content of some foodstuffs

The values of total carbohydrate (obtained by difference) for some foodstuffs are given in table 4.8.

The determination was carried out on dry matter and the values are considerably higher than some reported values (table 2.2). The beef and fish have the lowest values (less

than 4%) followed by the groundnut (17.8%). All the other samples have carbohydrate contents of over 24% in each case.

Diets high in carbohydrate and low in iodine contents are considered to have goitrogenic action (Wilson, 1954). A comparison of the carbohydrate contents of the grains and yam tuber (table 4.8) with their iodine contents would tend to implicate them as having potentials for inducing goitrogenic action.

4.9 Sodium and calcium contents of food materials

The values obtained for the sodium and calcium contents of some food samples analysed are shown in table 4.9. For most of the samples, the calcium contents are much lower than the sodium contents. A comparison of the sodium and calcium contents with some reported values (tables 2.4 and 2.5) shows that the determined values are higher. This may be due to the soil and water contents of the elements in various locations.

The removal of the testa of the brown beans leads to a significant loss in both sodium and calcium contents (24.0 and 1.4 ppm respectively for unpeeled and 4.0 and 1.0 ppm respectively for peeled beans).

As stated in section 1.1, iodine occurs in nature as sodium and calcium iodates and as iodides. As can be seen from table 4.2 and 4.9 the seeds, fish, meats and egg have relatively high values for sodium, calcium and iodine except for the low value of calcium in beef and melon seeds. Of the

grains, the corns (yellow, white, guinea), millet, and local rice gave low values of iodine (below 10 ug/g) and relatively high values for sodium and calcium (above 2000 ug/g and 100 ug/g respectively. For the fruits, vegetables, roots and tubers, no distinct trends can be seen between the three elements. These observations may imply the presence of iodine in foodstuffs in forms other than the iodides and iodates of sodium and calcium in which it is often found in nature.

Table 4.7 Ash content of some foodstuffs

Foodstuff	Ash content (%)
Grains	
Millet	2.5 ± 0.2
Guinea corn	1.9 ± 0.3
Soyabeans (untreated)	6.4 ± 0.4
Seed	
Groundnut (salted and roasted)	4.0 ± 0.0
Root and tuber	
Yam tuber	3.9 ± 0.0
Sweet potato	5.7 ± 0.3
Meat and Fish	
Beef	0.01 ± 0.1
Clarias	23.0 ± 0.9

Table 4.8 Carbohydrate content of some foodstuffs

Foodstuff	Carbohydrate content (%)
Grains	
Millet	79.6
Guinea corn	82.1
Soyabeans (untreated)	24.9
Seed	
Groundnut (salted and roasted)	17.8
Root and tuber	
Yam tuber	85.1
Sweet potato	86.7
Meat and fish	
Beef	2.4
Clarias	3.9

Table 4.9 Sodium and calcium contents of some food substances

Food substance	Na content (ppm) (10^4)	Calcium content (ppm) ($x10^4$)
Grains		
Yellow corn	90.0	0.2
White corn	23.0	1.4
Guinea corn	174.0	1.8
Millet	23.5	0.8
Local rice	37.6	1.2
Polished rice	0.6	0.8
White beans (unpeeled)	2.8	2.0
White beans (peeled)	2.9	14.4
Brown beans (unpeeled)	24.0	1.4
Brown beans (peeled)	4.0	1.0
Soya beans (untreated)	0.5	9.2
Soya beans (treated)	9.8	1.8
Dried fermented locust beans	97.5	2.2
Seeds		
Melon seeds	59.0	0.05
Ogbono	10.5	1.9
Salted roasted groundnut	24.0	7.6
Fruits		
Garden egg	1.1	0.4
Pawpaw	54.4	1.1
Mango	18.0	3.6
Water melon	6.5	0.2
Tomato	0.5	0.6
Fresh pepper	0.5	2.9
Dried pepper	105.0	2.0
Vegetables		
Spinach	28.5	4.1
Water leaf	20.0	1.9
Cabbage	32.6	0.4
Fluted pumpkin leaf	36.0	10.2
Onion	0.5	1.4
Garlic	56.5	1.3

Table 4.9 cont.

Food substance	Na content (ppm) ($x10^2$)	Calcium content (ppm) ($x10^4$)
<u>Roots and tubers</u>		
Sweet potato	112.5	3.2
Garri (dried fermented cassava)	1.1	1.0
Yam tuber	1.0	1.2
Yam flour	15.0	1.3
<u>Fish</u>		
Clarias (dried)	28.0	17.2
Crayfish "	12.0	4.4
<u>Meat and Egg</u>		
Beef	10.0	0.5
Goat meat	14.0	3.6
Egg (whole)	44.0	12.4
Egg (Yolk)	29.6	2.2
Egg (albumen)	31.0	0.5
<u>Miscellaneous</u>		
Salt	4000.0	2.4
Whole milk powder	5.0	0.2
White sugar	48.8	1.0
Bread	51.0	1.5

4.10 Iron and copper contents of food materials

A determination of the iron and copper contents of some of the food substances analysed gave the values shown in table 4.10. A comparison with some reported values (tables 2.6 and 2.7) shows that for copper, most of the foodstuffs are rich sources of the element (over 8ppm) while those with contents below this value (water leaf, cabbage, and water melon) have values in the range reported in the literature for some leafy vegetables and fruits (2-8 ppm). The iron contents of the foodstuffs determined are significantly higher than the literature values. This may be due to differences in the soil and water contents of this element in the various locations at which the analyses were carried out. The ability of the parent materials from which the samples were derived to accumulate the element may also determine the value obtained.

The fruits, vegetables, fish, and seed generally show a relatively low copper content (below 20ppm) along with a relatively low iron content (below 50ppm) except for the seed and crayfish which have iron contents of over 100ppm. The grains, roots and tubers are relatively rich in both copper and iron (over 17ppm and 50ppm respectively) except for garri with iron content of 29ppm. The beef and egg yolk samples are rich in copper (over 20ppm) but low in iron (below 10ppm).

It would appear that in the plant materials studied the iron and copper contents are both relatively high, but in the

animal materials a high copper content is accompanied by low iron content and vice-versa.

Iron, copper, and iodine are thought to be involved in the formation of red blood cells in the bone marrow (sections 1.3, 2.10.3,2.10.4). A copper to iodine ratio greater than 1 has been linked with the occurrence of goitre (Kolomiitseva, 1962). Accordingly the Fe : I and Cu: I ratios have been computed and are displayed in table 4.11.

The Fe: I ratio for the vegetables, the fruits (except garden egg), crayfish, garri, sweet potato, beef, and egg yolk is less than 1 showing that for these food substances, the iodine content is higher than the iron content. For other food items, the Fe: I ratio is greater than 1. The consequence of an Fe: I ratio with respect to goitre formation has not yet been deduced.

The Cu: I ratio is generally less than 1 with the exceptions of guinea corn, millet, soyabeans, yam tuber, and garden egg. Accordingly guinea corn, millet, soyabeans, yam tuber, and garden egg all with a Cu: I ratio above 1 would appear to be goitrogenous. While millet has been mentioned by Thilly et al (1992) as being one of the goitrogenous foodstuffs, other factors as observed earlier may mitigate the goitrogenic activity of the other foodstuffs with a Cu: I ratio greater than 1.

Table 4.10 Copper and iron contents of some foodstuffs

Food substance	Copper content (ppm)	Iron content (ppm)
Grains		
Guinea corn	60.0	174.0
Millet	23.0	60.0
Soyabeans (untreated)	17.0	89.0
Dried fermented locust beans	59.0	137.0
Seed		
Ogbono	26.0	120.0
Fruits		
Garden egg	10.8	21.5
Pawpaw	10.0	47.0
Water melon	4.1	2.7
Vegetables		
Spinach	10.3	4.0
Waterleaf	7.1	3.0
Cabbage	7.9	23.4
Roots and Tubers		
Sweet potato	29.0	52.0
Garri (dried fermented cassava)	19.0	29.0
Yam tuber	37.0	200.0
Fish		
Crayfish	14.0	78.0
Meat and Egg		
Beef	27.0	6.9
Egg (Yolk)	64.0	8.0

Table 4.11 Fe: I and Cu: I ratios for some foodstuffs

Food substance	Fe: I ratio	Cu: I ratio
Guinea corn	87.0	30.0
Millet	7.2	2.8
Soyabeans (untreated)	6.7	1.3
Dried fermented locust beans	1.0	0.4
Ogbono	2.0	0.4
Garden egg	2.5	1.3
Pawpaw	0.3	0.1
Water melon	0.6	0.9
Spinach	0.1	0.1
Waterleaf	0.1	0.1
Cabbage	0.2	0.1
Sweet potato	0.1	0.1
Garri (Dried fermented cassava)	0.4	0.3
Yam tuber	15.6	2.9
Crayfish	0.5	0.1
Beef	0.1	0.1
Egg (Yolk)	0.1	0.1

4.11 Contents of some known goitrogens in the foodstuffs

In the foodstuffs in which the goitrogens, thiocyanate and phenol were determined, none showed the presence of thiocyanate but onion, garri, fluted pumpkin leaf, and millet showed the presence of phenol. This result is shown in table 4.12.

The fluted pumpkin leaf showed the highest content of phenol (324.5 ug/g) followed by garri (183.2 ug/g) millet (118.5 ug/g), and onion (100.4 ug/g). The presence of phenol in these foodstuffs indicate that they may be goitre causing (Bondy and Rosenberg, 1974).

Phenols are known to be present in polluted water from chemical plants (Allen et al, 1974). Diphenyl or 0-phenylphenol and its sodium salt are often used as a food preservatives. D-phenyl and 0-phenylphenol are solids which suppress mould growth and are used for preventing mould growth on fruits. The sodium salt of 0-phenylphenol is used as a spray or dip for citrus fruits (Fox and Cameron, 1972).

Vegetables are grown in gardens which are close to water sources such as for irrigation. The presence of phenol in some of these foodstuffs may be due to contamination from chemicals used for preservation, or a contamination of the water sources by phenol containing substances from chemical plants. Zaria for example has a tobacco industry and a number of other factories that could contaminate irrigation waters.

Table 4.12 Thiocyanate and phenol content of some foodstuffs.

Foodstuff	Thiocyanate content (Ug/g)	Phenol content (Ug/g)
Cabbage	N.D	N.D
Onion	N.D	100.4 ± 0.0
Fluted pumpkin leaf	N.D	324.5 ± 29.5
Garri (dried fermented Cassava)	N.D	183.2 ± 10.8
Millet	N.D	118.5 ± 23.7

N.D. - Not detectable (by the method of analysis).

Table 4.13 Summary of parameters determined in some foodstuffs

Food Substance	Moisture (%)	I(Ug/g)	Starch (%)	Crude protein (%)	Lipid (%)	Ash(%)	Carbohydrate(%)	Na(ppm) (10^2)	Ca(ppm) (10^2)	Cu(ppm)	Fe(ppm)	Phenol (Ug/g)
Grain								90.0	0.2			
Yellow corn	8.2	4.8	56.0					23.0	1.4			
White "	7.2	9.0	60.0					174.0	1.8	60.0	174.0	
Guinea corn	11.0	2.0	40.0	11.4	4.6	1.9	82.1	23.5	0.8	23.0	60.0	118.5
Millet	8.3	8.3	50.0	11.4	6.5	2.5	79.6	37.6	1.2			
Local Rice	2.0	8.3	70.0					0.6	0.8			
Polished Rice	8.7	15.3	68.0					2.8	2.0			
White bean (Unpeeled)	9.9	22.3	42.0					2.9	14.4			
White bean (Peeled)	11.2	15.5	28.0					24.0	1.4			
Brown bean (Unpeeld)	10.1	24.5	44.0					4.0	1.0			
Brown bean (Peeled)	10.3	7.8	44.0					0.5	9.2	17.0	89.0	
Soya beans (Untreated)	6.9	13.3	0.03	47.2	21.5	6.4	24.9	9.8	1.8			
Soya bean (treated)	7.0	14.0	0.03					97.5	2.2	59.0	137.0	
Dried fermented locust beans	12.4	132.5	1.0					59.0	0.05			
								10.5	1.9	26.0	120.0	
Seeds			1.6									
Melon seeds	3.9	11.8	10.0									
Ogbono	5.2	58.8										
Unsalted roasted groundnut		46.0						24.0	7.6			
Salted roasted groundnut	3.0	210.0	1.7	26.8	51.4	4.0	17.8					
								1.1	0.4	10.8	21.5	
Fruits		0.2						54.4	1.1	10.0	47.0	
Carrots	91.6	8.5	0.04					18.0	3.6			
Garden egg	90.8	145.0	0.03	4.3	6.1			6.5	0.2	4.1	2.7	
Pawpaw	87.8	35.0	1.0					0.5	0.6			
Mango	96.8	4.6	0.01									
Water Melon	93.8	6.4	0.0									
Tomato												

Table 4.13 continued.

Fresh pepper	89.9	8.3	0.1					0.5	2.9			
Dried pepper	3.7	26.5	7.2					105.0	2.0			
Vegetables												
Spinach	88.7	160.0	0.06	-	-			28.5	4.1	10.3	4.0	-
Water leaf	91.5	70.1	0.03					20.0	1.9	7.1	3.0	
Cabbage	90.8	152.2	0.04	21.4	6.6			32.6	0.4	7.9	23.4	324.5
Fluted pumpkin leaf	88.5	60.0	0.05					36.0	10.2			100.4
Onion	88.7	7.0	0.03					0.5	1.4			
Garlic	70.9	6.3	0.0					56.5	1.3			
Roots and Tubers												
Sweet potato	71.9	750.0	9.0	4.1	3.5	5.7	86.7	112.5	3.2	29.0	52.0	
Garri	6.4	66.0	58.0					1.1	1.0	19.0	29.0	183.2
Yam tuber	63.4	12.8	22.0	8.1	2.9	3.9	85.1	1.0	1.2	37.0	200.0	
Yam flour	6.7	6.8	62.0					15.0	1.3			
Irish potato		3.5										
Fish												
Alestes (dried)	5.7	210.0										
Clarias "	7.3	280.0	0.3	69.7	11.2	23.0	3.9	28.0	17.2			
Tilapia "		148.4										
Crayfish "	8.0	145.0	0.4					12.0	4.4	14.0	78.0	
Meat and Egg												
Beef	78.0	237.5	0.06	66.8	35.6	0.01	2.4	10.0	0.5	27.0	6.9	
Goat meat	78.2	112.5	0.1					14.0	3.6			
Egg (Whole)	79.6	280.0	0.05					44.0	12.4			
Egg (albumen)	84.4	530.0	0.0					31.0	0.5			
Egg (Yolk)	51.9	625.0	0.07					29.6	2.2	64.0	8.0	
Miscellaneous												
Salt	0.0	95.0	0.0					4000.0	2.4			
Whole milk powder	3.6	5.0	0.6					5.0	0.2			
Cocoa beverage		4.0										
White sugar	0.0	11.5	4.0					48.8	1.0			
Bread	27.9	95.0	44.0					51.0	1.5			
Biscuit		13.3										
Jam		18.0										

CHAPTER 5

5.0 SUMMARY AND CONCLUSION

From the investigation of the iodine contents of certain foodstuffs, it can be seen that there are some readily available foodstuffs that provide good and available sources of iodine. Examples are the leafy vegetables, soyabeans and cowpeas, locust beans, and the seeds (melon, groundnut, ogbono).

It has also been shown that starchy foods are usually low in iodine content while the proteinous ones are richer in iodine. Although animal proteins are expensive, plant proteins are relatively cheap and affordable. More use of these proteins can be encouraged as one of the ways to prevent iodine deficiency disorders (IDD). The study has also shown that foods rich in iodine and protein are usually also rich in fat, all of which are important nutrients. The consumption of such food substances like groundnut, beef, soyabean, fish, and cabbage should hence be encouraged. A diet high in starch should be accompanied by some of these food items in order to increase the iodine content of the diet. Bread is a food item rich in both starch and iodine (probably due to the addition of salt or potassium halide).

The methods by which some of the foods are prepared and processed have also been shown to determine to what extent the iodine and other nutrients are retained. For example, the

dried fish used in this study showed a high value for iodine with probably no loss due to drying. Brown beans on the other hand showed a significant loss of iodine on peeling the testa. White beans also showed a significant reduction in starch content on peeling the testa. A significant reduction of the sodium and calcium contents was also observed for brown beans on peeling. It would thus appear that grains are more nutritious when eaten with the testa than when peeled.

Millet has been shown in many ways to be a likely goitrogenous food item. It has low iodine and protein contents. It also has a Cu: I ratio greater than 1 and showed a significant presence of phenol (a goitrogen). Its carbohydrate content is also high. Millet based diets would therefore require appropriate iodine supplementation.

Other food items with a Cu: 1 ratio greater than 1 (implying likely goitrogenous effect) are guinea corn, soyabeans, yam, and garden egg. Of these, the carbohydrate content of guinea corn and yam are also high. Yam is also known to contain substances like sapogenins, alkaloids, tannins, calcium oxalate crystals, and some may contain enough free oxalic acid to be poisonous (Inglett and Charalambous, 1979). Yam will therefore seem a food item to be taken with caution.

The food items apart from millet also shown to contain phenol are onion, fluted pumpkin leaf, and garri. Onion and garri (dried cassava with varying degree of fermentation) have

been cited in literature as containing goitrogens (Sapieka, 1969; Thilly et al, 1992). Apart from the presence of phenol in onion, it also has a low iodine content.

Results from the study of the sodium, calcium, and iodine contents of some foodstuffs indicate the presence of iodine in other forms apart from the compounds of sodium and calcium.

In conclusion, foods high in fats and carbohydrate but low in protein and iodine should be supplemented with those rich in iodine to prevent likely goitrogenic effects. Foods should also be prepared and processed in ways which will minimise nutrient loss especially that of iodine.

5.1 Suggestion for further work

The full extent of iodine deficiency in Nigeria may not be known until further investigations are carried out into the iodine contents of the foods, waters, and soils of the different parts of the country. This will thus provide comprehensive data for health workers and others in combatting IDD in this country. Further work in this area is thus advocated.

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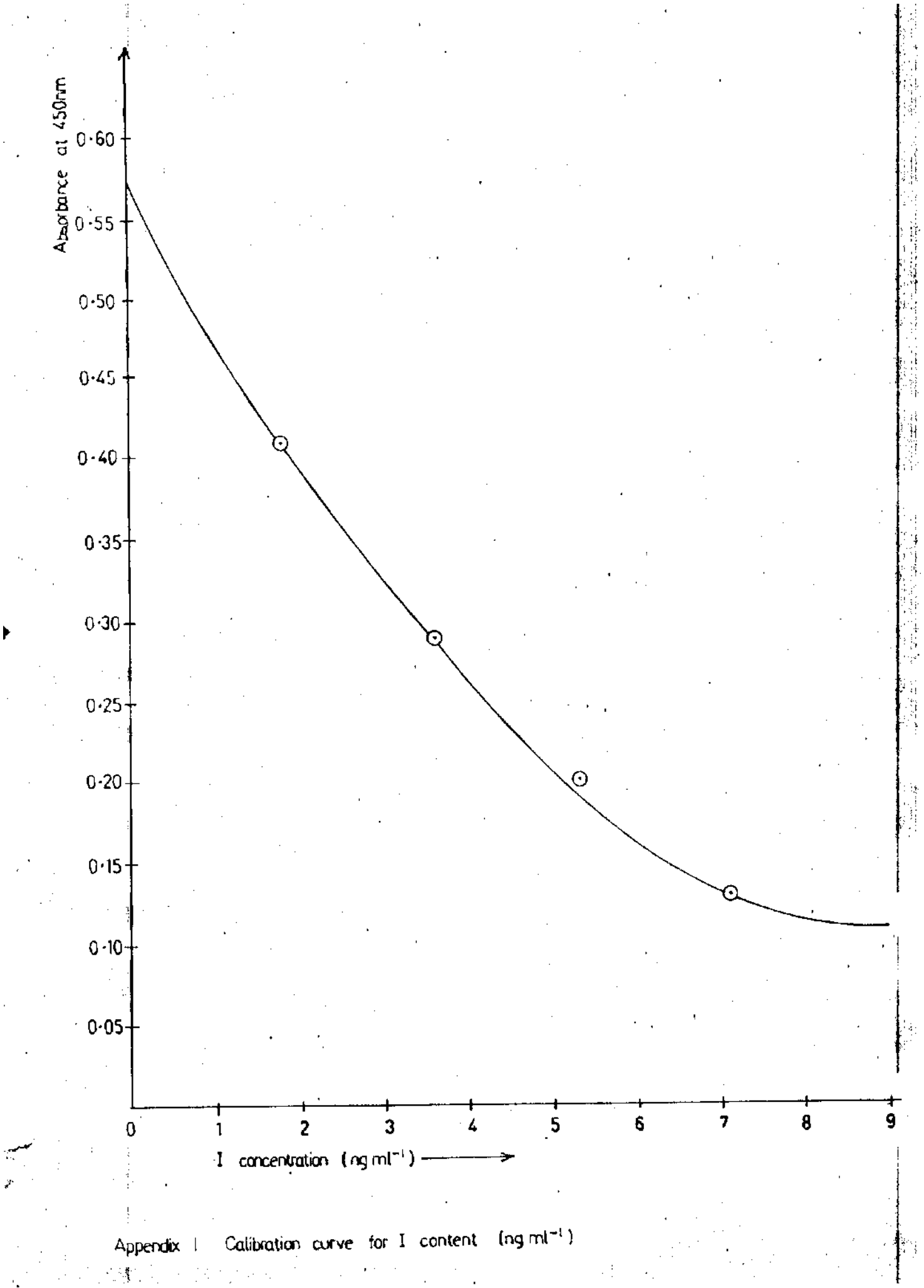
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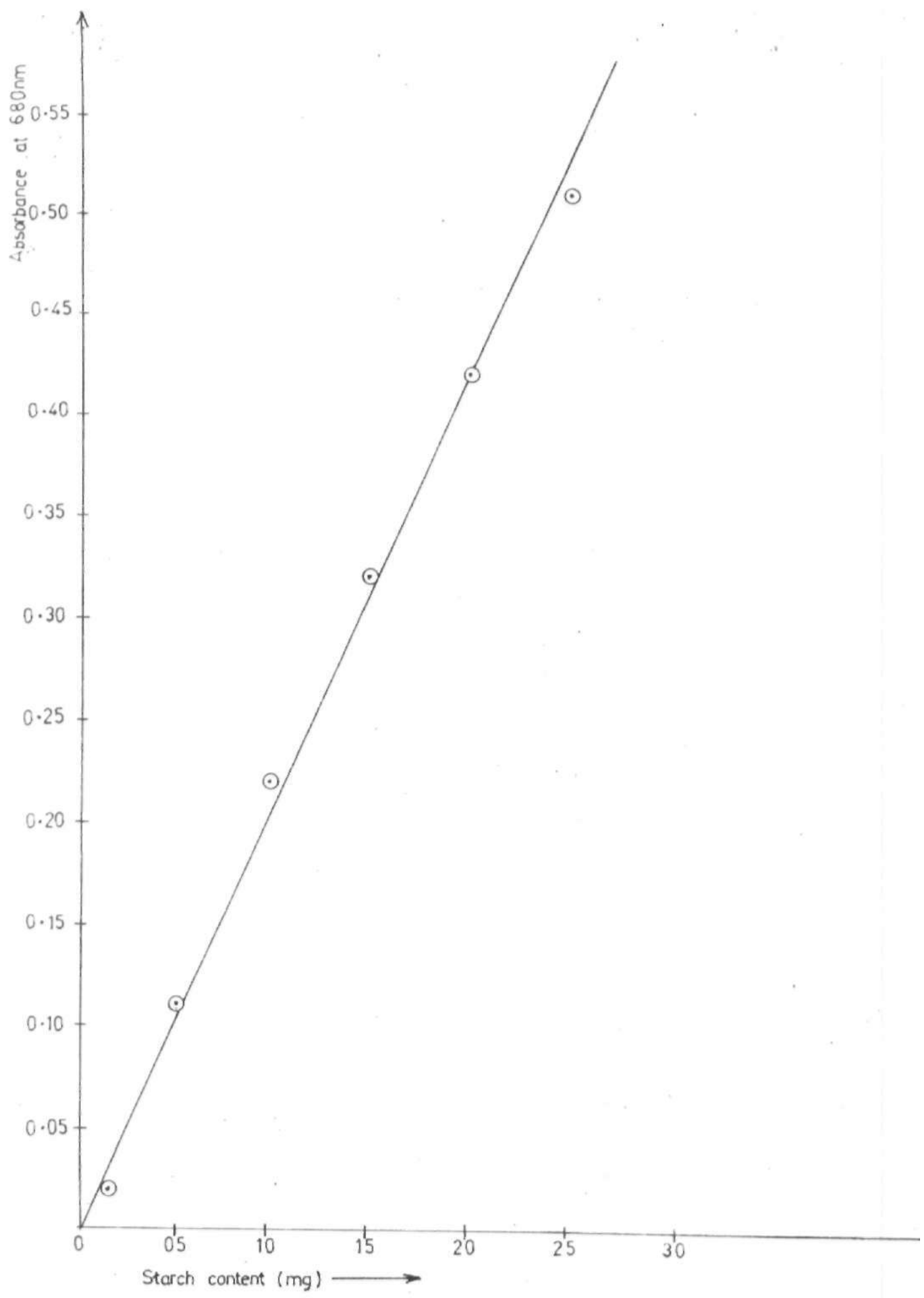
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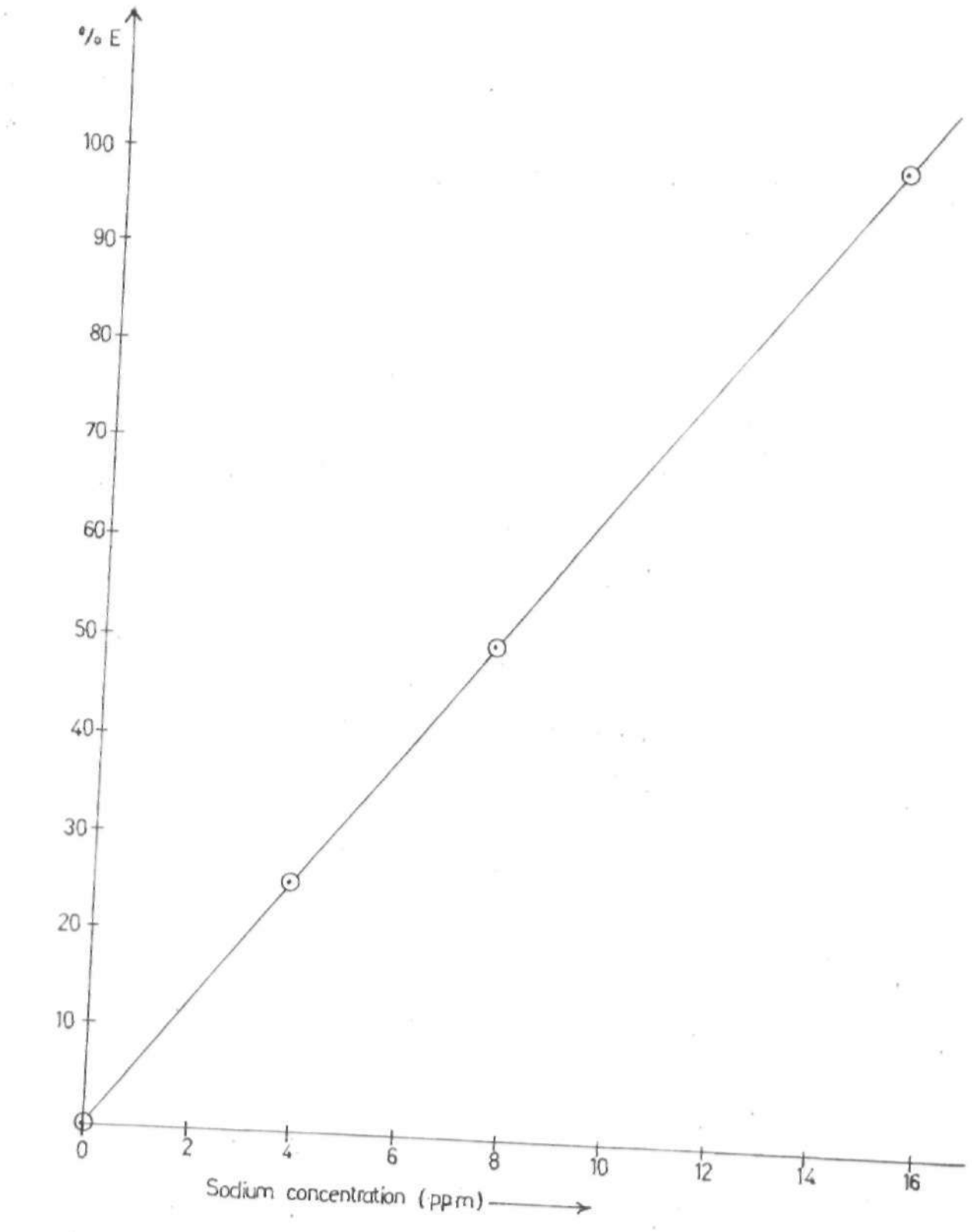
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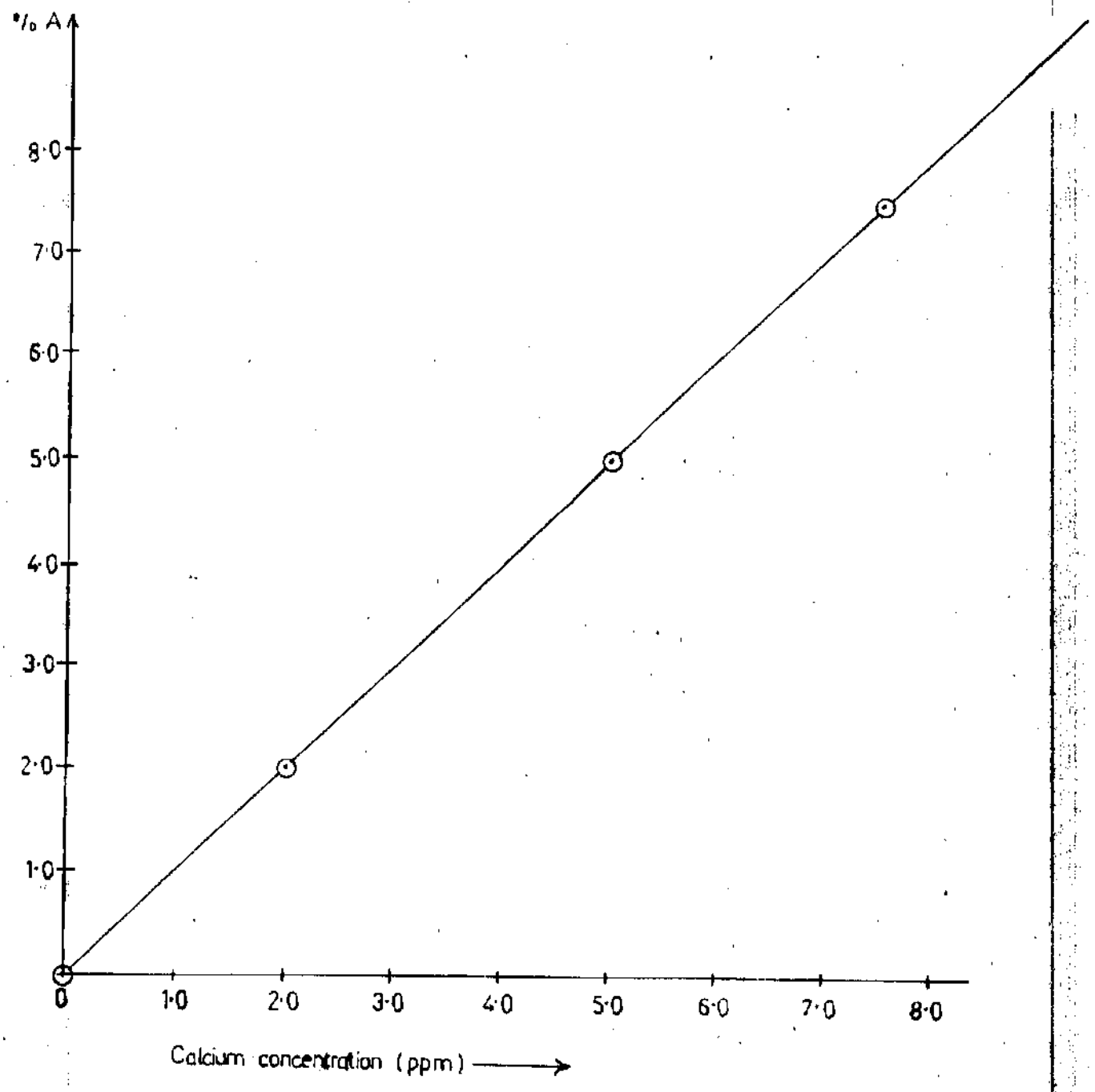
Appendix I Calibration curve for I content (ng ml^{-1})



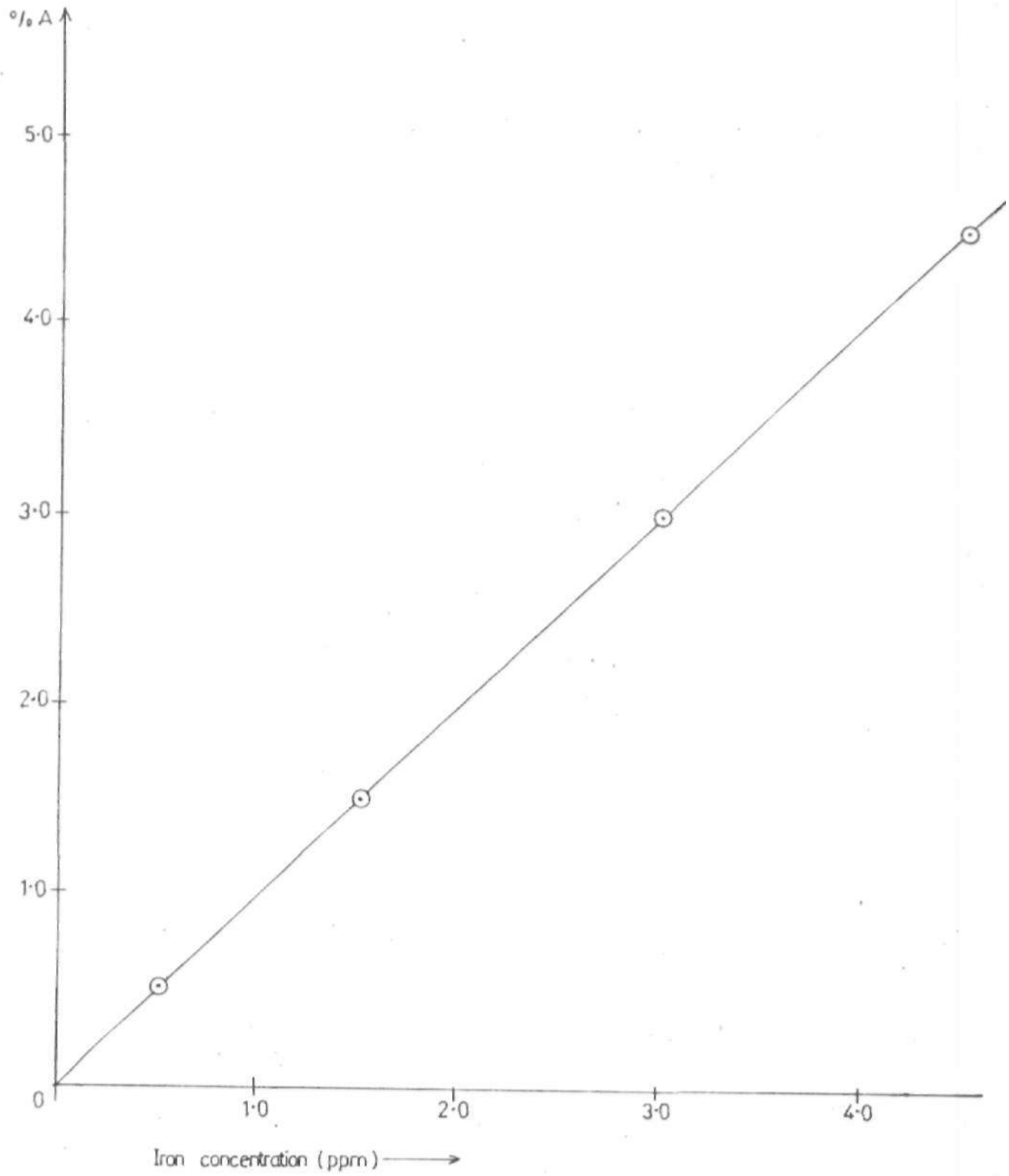
Appendix II Calibration graph for starch content



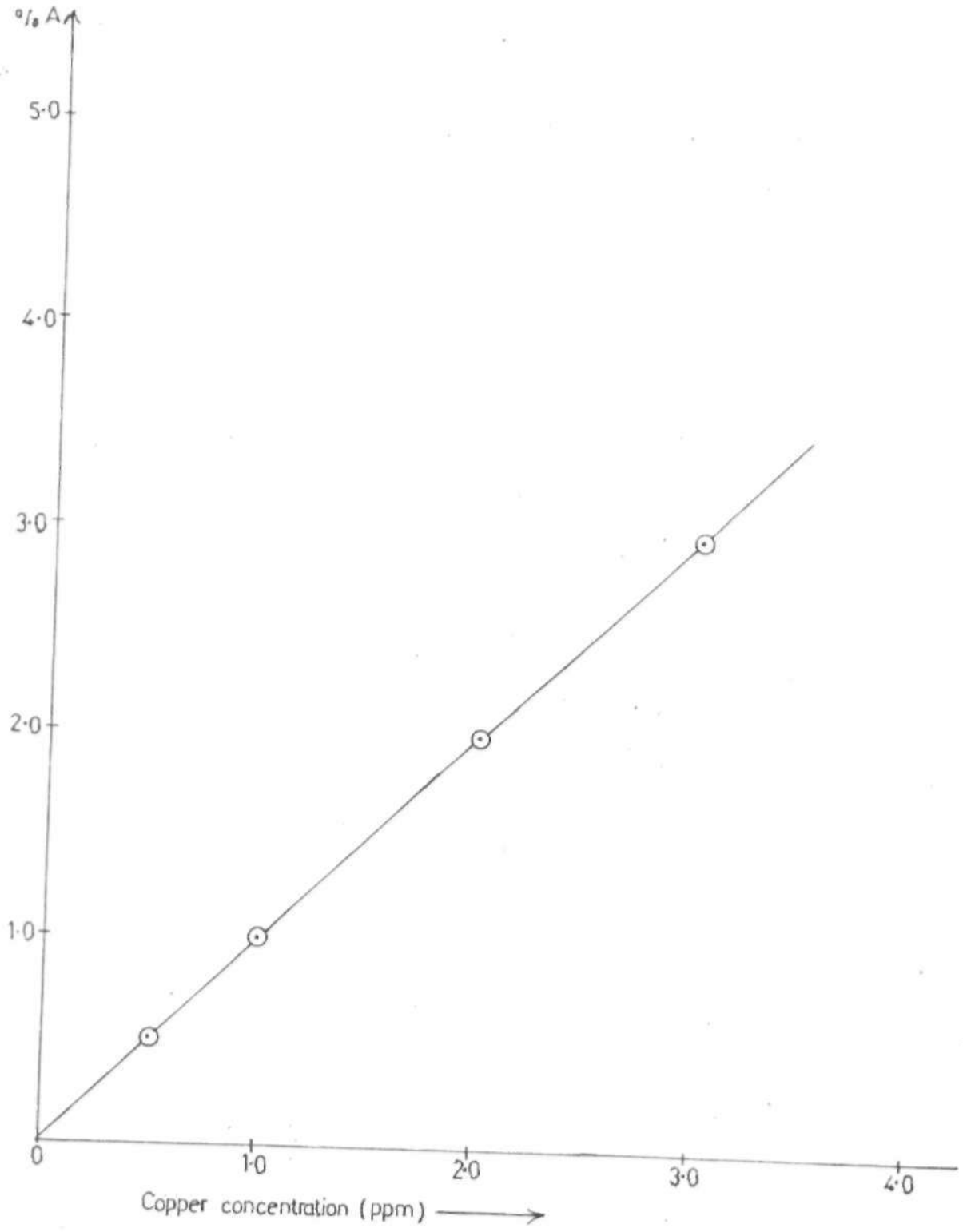
Appendix III Calibration graph for sodium (ppm)



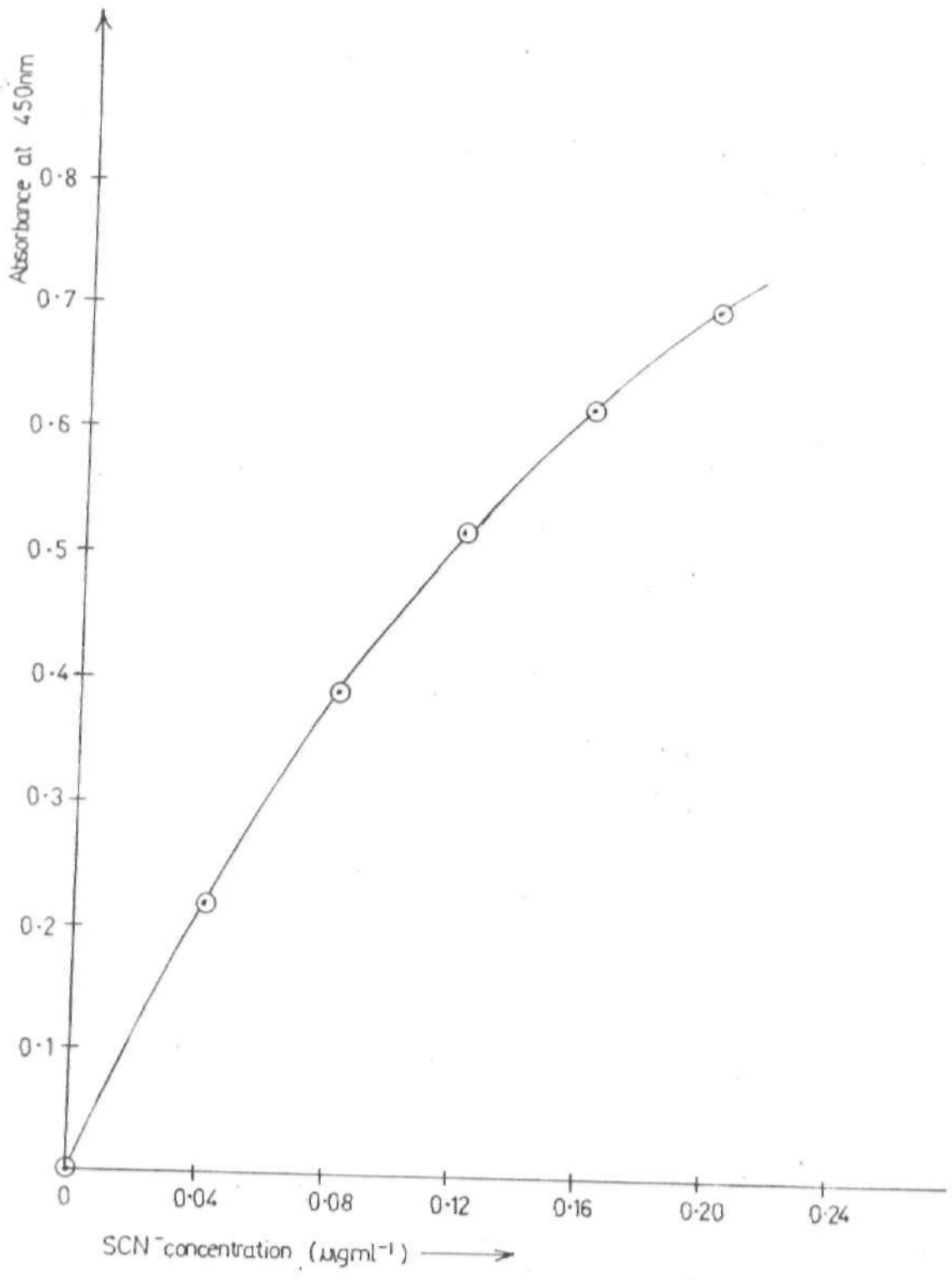
Appendix IV Calibration graph for calcium



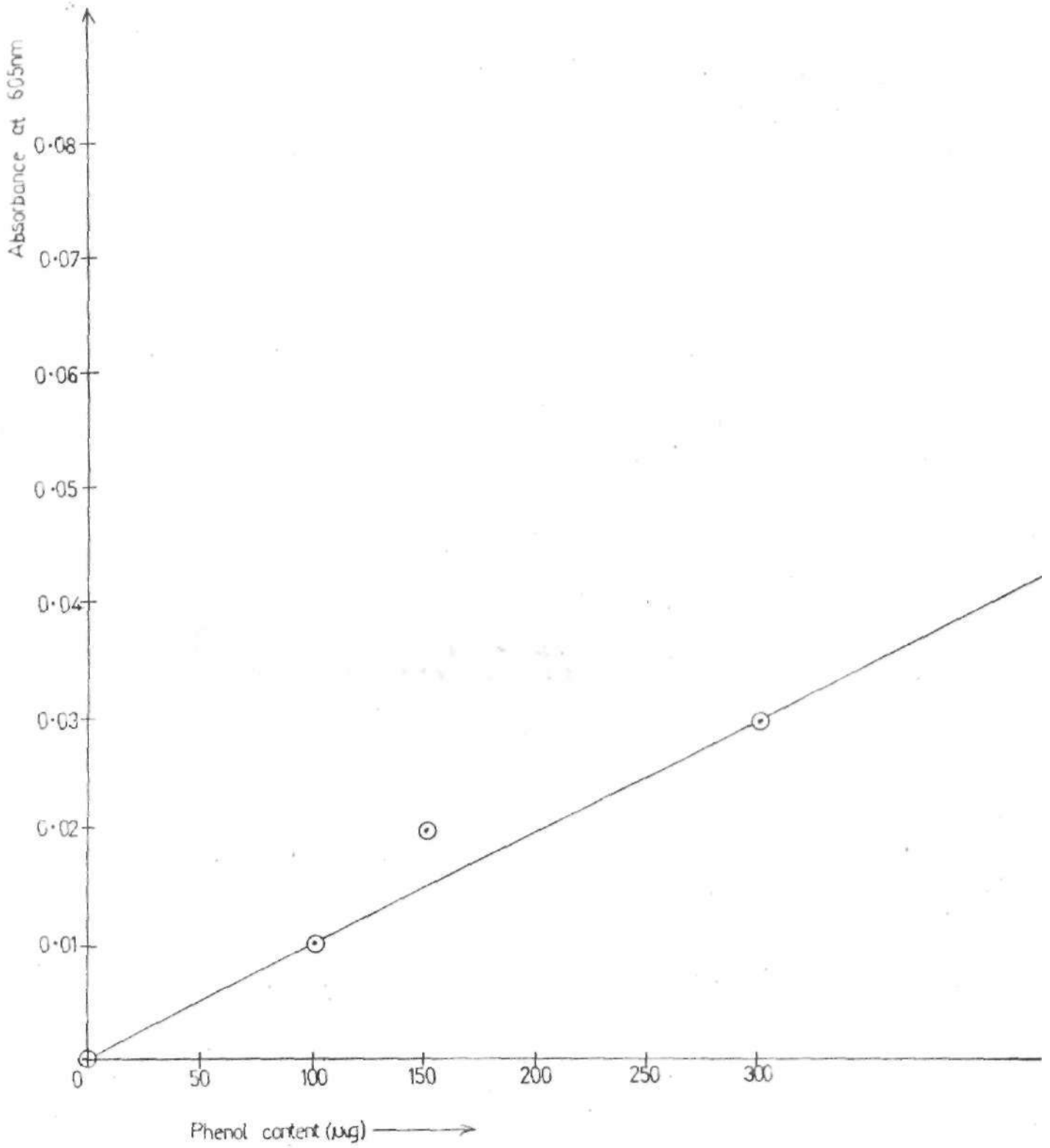
Appendix V calibration graph for iron



Appendix VI Calibration graph for copper



Appendix VII Calibration graph for SCN^-



Appendix VIII Calibration graph for phenol