INFLUENCE OF VARIETY AND FERTILIZER TREATMENT ON THE CHEMICAL COMPOSITION OF MAIZE
(Zea mays Linn.)

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D E C L A R A T I O N

I declare that this work is wholly the author's and has not been part of any presentation for any other qualification.

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THESIS APPROVAL

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D E D I C A T I O N

To Papa, who left me while still on this course and was buried on my BIRTHDAY. May His Soul Rest in Peace.

To brother Ogbitse, who continued where Papa stopped.

To Mama, for E V E R Y T H I N G.

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ABSTRACT

Fourteen maize samples were analysed to investigate Lhe influence of variety and fertilizer treatment *on* the moisture, ash, lipid, mineral, carbohydrate and amino acid contents., Five varieties, namely TZB, 096, S 123, Bomo Local and western yellow were used for the influence of v trietal differences study while 9 groups of trv- variety used for the influence of fertilizer treatment study. The fertilizers were applied singly and in combination in the ".lowing doses: S.- (no fertilizer), S_2 (25kg N/ha), So (50kg N/ha), s_4 (30kg P/ha), s_5 (60kg P/ha), Sg 50kg K/ha), S_7 (100kg K/ha), S« (25, 30 and 50kg N, P and K/ha respectively) and Sg (50, 60 and 100kg N, P and K/ha respectively). The samples used for the influence of varietal differences study, S_{1Q} (Bomo Local), s_{la} (S 123), S_{12} (096), S_{13} (TZB) and S_{14} (Western Yellow) all received combinations (120kg N/ha, 60kg P/ha, 60kg K/ha). Both fertilizer treatment and varietal differences had significant effects on the moisture and protein contents. Fertilizer treatment had significant effect on the ash and lipid contents while varietal differences had no significant effect on these.

Analyses were done on the maize samples for the

identification of 9 minerals namely, calcium, copper, iron, manganese, zinc, sodium, phosphorus, magnesium, and potassium. Fertilizer treatment and varietal differences had significant effects on the calcium, iron, manganese, sodium zinc and phosphorus contents. In addition, while fertilizer treatment had significant effects on the copper and potassium contents, varietal differences had none. Both factors had no significant effects on the magnesium content.

The carbohydrates of the maize samples were characterised into their different fractions namely soluble carbohydrates, hemicelluloses, cellulose and lignin. Fertilzier treatment and varietal differences had significant effects on the soluble carbohydrate, hemicellulose and cellulose contents while only fertilizer treatment had significant effect on the lignin content. The soluble carbohydrates were further fractionated into fructosan, fructose, pentoses and "other sugars". Both fertilizer treatment and varietal differences had significant effects on the four fractions.

All the maize samples had low lysine and high leucine contents. These gave leucine/lysine ratios greater than 4.6. Maize protein is therefore not nutritionally safe as

a major source of protein. It is suggested that samp] S_{13} (TZB , 120, 60 and 60kg N, P and K/ha respectively) distributed to farmers for planting at the institute: for Agricultural Research, Samaru, Zaria. This suggestion is based on its leucine/lysine ratio.

CHAPTER ONE

INTRODUCTION.

Cereal grains are the principal crops which have made the continuation of mankind possible (Lachance, 1981). Maize (Zea mays) is the third most important cereal crop in the world, next to rice and wheat, with regard to cultivated area and the total production (Sundararaj and Thulasidas, 1976). It is suited to a much wider range of climatical conditions than either rice or wheat. This is because of its great adaptability from the tropical conditions to the warm temperate regions as well as the humid sub-tropical zones. Maize is cultivated on a large scale in the United States of America, China, Russia and many of the South-East Asian countries.

In Nigeria, maize is the second most important cereal crop, ranking behind only sorghum in the number of people it feeds (Ologunde, 1982a) and the third most widely cultivated, next to sorghum and millet (Oyenuga, 1968). Even in the United States of America where 85% of the maize crop is fed to livestock (Jugenheimer, 1976), people are now becoming aware of the health benefits from cereal grains

and their simple baked products (Davidson et al. 1975). These health benefits include:

1. Sources of key nutrients (especially protein, vitamins and minerals).
2. Sources of complex carbohydrates, both digestible and roughage.
3. Sources of other physiological attributes such as a role in appetite control and weight control.

It has, however, been found that the qualities of maize (and cereals in general) vary widely depending on the country of origin, the type of variety and the environmental conditions of growth including soil type, season and fertilizer treatment (Kent, 1970). Much has been done to improve the quality of maize. It was thought that with the discovery of two high-lysine mutants - Opaque 2 and floury-2 (Mertz et al, 1964; Nelson et al, 1965), maize may play an even more important role in human and animal nutrition, but this is not the case. There has been a lack of widespread adoption of Opaque-2 maize. This sprung from a set of complex and inter-related problems including comparatively low yield, non-acceptability of kernel phenotype,

and more vulnerability to ear rot organisms and insect damage during storage (CIMMYT; 1974). Due to these factors many maize producing countries are trying other avenues for obtaining improved maize.

In Nigeria, there are several (over 30) improved maize varieties but only about 18 could be regarded as most superior at the moment and which are available for production (Obilana, 1978). These varieties have been developed and improved for several traits like high grain yield, early maturity, good standability, disease and insect-pest resistance and grain quality for high protein peculiar to the different varieties.

LITERATURE REVIEW

1.1. ORIGIN OF MAIZE

The recorded history of maize is limited to the years following the discovery of America. Apparently, the first reference to the plant in recorded history occured on November 5, 1492 (Jugenheimer, 1976). On that day, two Spaniards, sent out on an expedition by Columbus to the interior of Cuba, returned with a report of "a sort of grain they call "maiz" which

was well tasted, bak'd and dry'd and made into flour" (Jugenheimer, 1976).

The origin of maize is lost in antiquity. The plant is very highly specialised and could not reproduce itself without the aid of man. This is due to its poor seed dispersal mechanism and low survival value in nature. For example, when an ear of maize drops to the ground, scores of seedlings emerge. These seedlings then complete among themselves for soil nutrients and moisture and most of them die before they reach the reproductive stage. Due to this, wild maize has never been found by modern man and therefore, no one knows when this important plant originated, but the time of origin must have been thousands of years ago - at least 5,000 years (Jugenheimer, 1976). There are many beliefs about the origin of maize. Some investigators believe it must have originated in the highlands of Peru, Bolivia and Ecuador, because of the great diversity of native forms found in the region. Others believe that it originated in Southern Mexico and Central America primarily because this appears to be the home of Euchlaena (an older species believed to have originated over 5,000 years ago), and also

because of the wide diversity of types.

There are many theories on the origin and evolution of corn. Goodman (1965) reviewed current theories on the relationship between maize and some of its relatives. An extensive review of the literature on the origin of maize was made by Galinat (1971). There are about five theories on the origin of maize (Mangelsdorf, 1974). These are

1. The Pod - corn theory.
2. The Teosinte theory.
3. The theory of a common Ancestry.
4. Minor theories:

a. The Papyrescent "Semivestidos" Theory.

b. The Corn-Grass Theory.

5. The Tripartite Theory.

Current views polarize around two hypothesis. The oldest and still persistent one is that primitive maize was selected by man either directly from its closest living relative, teosinte, or from an ancestor common to both. Podcorn has been mentioned as a wild or primitive type of corn. The kernels are enclosed in a pod which might have been characteristic of the wild plant. Pure podcorn has

many of the characteristics expected in the ancestral form of maize. However, the plant differs from ordinary maize in only one gene. Available archaeological data seem to exclude teosinte as a possible ancestor of domesticated maize. This will make maize the only cereal without a living direct ancestor. Biosystematic studies suggest that teosinte is so closely related to domesticated maize that it could be accepted as the progenitor of maize. (Jugenheimer, 1976).

How did maize get to Nigeria? In 1852, it was stated that maize was grown widely in most parts of West Africa frequented by European merchants. (Eijnatten, 1950). In several parts of Ghana and the "Bight of Benin", the crop was considered one of the main staple foods. In other parts, it was grown more "to vary the constant uniformity of the African diet, than as a commodity for sale" (Eijnatten, 1950). The introduction of maize into this part of Africa was attributed to the Spanish and Portuguese voyagers in the 16th century (Irvin, 1969). It seems that the first maize introduced into West Africa was brought from Sao Tome into Ghana. There is also evidence of later introduction of maize from

Northerly directions. Maize cultivation was recorded in Sokoto by Wallace in the later part of the 19th Century (Eijnatten, 1950). It was also known that from the early years of this century onwards, the volume of records of maize cultivation increased. A considerable quantity was grown along the Lagoons from Badagry to Epe, along the rivers up to Abeokuta and in the land of the "Ijebus". Further North and East, maize was merely grown for subsistence but from the areas mentioned, a considerable quantity was exported (Eijnatten, 1950).

1.2. STRUCTURE

The cereals of commerce and industry are harvested, transported and stored in the form of grain. The grain develops from flowers or florets. In maize, male and female flowers are contained in separate inflorescences on the same plant; the female inflorescence consists of a central rachis, the cob, bearing numerous rows of sessile spikelets, the whole being enclosed by a number of overlapping bracts which constitute the "husk" (Kent, 1975). A tuft of threadlike silks emerges from the top of the spike: these are the stigmas of the flowers. Each spikelet in the

female inflorescence comprises a pair of glumes and two florets, of which only one, the upper, is fertile. The grains of maize are naked caryopses.

The approximate range of sizes encountered among mature grains of maize and the average weight per 1000 grains are:

Length: 8-17mm

Width: 5-15mm

Weight: 324 (range 240-360)gm (Kent, 1975).

Grains of maize consist of fruit coat (pericarp) and seed. The seed is comprised of seed coat, germ and endosperm. Each of the main parts of the grain - pericarp, seed coat, germ and endosperm is futher subdivided into various layers, tissues or regions.

KERNEL (caryopsis).

1. Pericarp (fruit coat).

a. Outer

Epidermis (epicarp).
Hypoderm
Remnants of thin-walled cells
} Beeswing

} Bran

b. Inner)
)
Intermediate cells)
)
Cross cells)
)
Tube cells) Bran
)
2. Seed.)
)
a. Seed coat (testa) and pigment strand.)
)
b. Nucellar layer (hyaline layer))
)
c. Endosperm)
)
Aleurone layer)
)
Starchy endosperm)

d. Germ (embryo)

Scutellum (cotyledon)

Embryonic axis

Plumule, covered by coleoptile

Primary root, covered by coleorhiza

Secondary lateral roots

Epiblast. (Kent, 1975).

The proportion of germ (embryo) in maize is around 8-10%. The outer coverings of the grain constitute a further 16-19%, so that the starchy endosperm content is approximately 73%. (Kent-Jones and Amos, 1967).

1.3. BOTANICAL RELATIVES AND AGRICULTURAL GROUPS.

Maize is a grass and belongs to the large and important family, Gramineae. It belongs to the tribe (order) Maydeae, which includes eight genera-Coix, Schlerachne, Polytoca, Chinonachne, Trilobachne, Zea, Tripsacum and Euchlaena (teosinte). Zea is represented by the single species Zea mays, which is Indian corn or maize (Jegenheimer, 1976). Columbus gave the name "maize" to the new plant brought by the two Spaniards he sent to explore the interior of Cuba. He named it after the indigenous name "mahiz" or "marisi". In 1737, Linné gave maize the name Zea mays. The generic name of Greek origin, was used by the Greeks to describe other kinds of cereals; it derives from the word "life", while the species name originated from "mahiz". (Schrimpf, 1966).

Agricultural groups are dent, pop, flour, flint, sweet and waxy. Each group may be greatly modified by breeding, and represents an artificial classification that is not indicative of natural relationships. (Jugenheimer, 1976).

1.3.1 DENT: This is the most widely grown type. It is characterised by a depression or "dent" in the crown of the

seed. The sides of the seed have a corneous starch, while the soft starch extends to the summit of the seed. Rapid drying and shrinkage of the soft starch results in the characteristic denting.

1.3.2. FLINT: This is also a widely grown type, especially in Europe, Asia, Central America and South America. It is characterised by kernels which are hard, smooth and contain little soft starch. The relative amount of soft and corneous starch, however, vary in different varieties.

1.3.3. SWEET CORN: This is characterised by a translucent horny appearance when immature, and a wrinkled condition when dry. It is grown primarily in the U.S. Sweet corn differs from dent by only one recessive gene (su) which prevents the conversion of some of the sugar into starch.

1.3.4. FLOUR CORN: The kernels of flour corn are composed largely of soft starch and have little or no dent. It is one of the oldest types of corn.

1.3.5. POPCORN: This is an extreme form of flint with the endosperm containing only a small portion of soft starch. Popcorn is a relatively minor crop compared with dent corn. The crop is used primarily for human consumption as freshly

popped corn and is the basis of popcorn confections. The ability to pop seems to be conditioned by the relative proportion of horny endosperm where the starch grains are embedded in a tough, elastic colloidal material that confines and resists the steam pressure generated within the granule upon heating until it reaches explosive force.

1.3.6. WAXY (wx) CORN: It is so named because of the somewhat waxy appearance of the kernels. Waxy starch is composed entirely of the branched molecular form, amylopectin, whereas common corn starch is approximately 78% amylopectin and 22% amylose, the straight chain form.

1.3.7. PODCORN: It is an unusual type of corn, each kernel of which is enclosed in a pod or husks, The ear is also enclosed in husks, as are other types of corn. Homozygous podcorn usually is highly self-sterile, and the ordinary type is heterozygous. Podcorn is not being grown commercially, but is of considerable interest in studies of the origin of corn.

1.4. FERTILIZER TREATMENT.

A large number of chemical fertilizers are being manufactured and marketed. These may supply a single or

two or all the three major nutrients (N.P. or K). Fertilizer materials supplying a single nutrient are known as straight fertilizers while those supplying two or more nutrients are referred to as compound fertilizers. (Cooke, 1974; FAI, 1974).

The decision of greatest economic importance that farmers have to make in using fertilizers is the selection of the fertilizer treatment to employ (CIMMYT, 1969). If the treatment selected includes an element that is not needed, or a rate of an element higher than the economic optimum, the farmers may suffer a loss since they will not realise the maximum returns from their investment in fertilizers. On the other hand, if they apply less than the optimal amount of fertilizers, they will only be partially exploiting this source of added farm income. Therefore, when choosing a fertilizer the following factors need consideration:

i. availability

ii. cost per unit nutrient including the transportation costs.

iii. storage qualities.

iv. handling qualities.

v. crop preference.

vi. likely loss or fixation of nutrient, efficiency of utilisation.

vii. soil reaction.

It is known that Savana soils are naturally low in organic matter (Jones, 1973) and very low in native phosphorus (Bache and Rogers, 1970). Therefore, for the cultivation of maize, which requires relatively high soil fertility on these soils, the appropriate amount of fertilizers must be used for maximum yield.

1.4.1. NITROGEN FERTILIZATION.

Nitrogen is known to be the most limiting factor for production of most crops on most soils around the world. Maize has a relatively high demand for nitrogen. Due to the very low organic matter content of savana soils, 0.68%, (Jones, 1973), and the age-old practice of bush burning which destroys potential nitrogen source, frequent addition of nitrogen to soil has become necessary in order to obtain and maintain high yields. Maize response to nitrogen is very signifcant (Balasubramanian et al, 1978).

Nitrogen fertilizers can be broadly grouped into 5 classes.

1. Ammonium fertilizers.
2. Nitrate fertilizers.
3. Ammonium and nitrate fertilizers.
4. Amide fertilizers.
5. Slow release nitrogen fertilizers.

The type of nitrogen fertilizer used for the cultivation of maize at the Institute for Agricultural Research, (IAR), Samaru, Zaria is Nitrochalk (calcium ammonium nitrate 26% N or Urea 45% N (Clogunde, 1982b).

Nitrochalk is actually ammonium nitrate mixed with ground limestone and then granulated. It has excellent handling qualities but is slightly hygroscopic. It is marketed as granules of sorghum seed size (length 3-5mm, width 2.5-4.5mm, Kent, 1975) and is neutral in reaction. It has soil ameliorative properties due to high calcium content (FAO, 1980). About 400kg/ha of nitrochalk is applied during the planting of maize at IAR.

1.4.2 PHOSPHORUS FERTILIZATION

Stockinger and Heathcote (1968) have reported

phosphorus to be the most limiting factor for maize production in nearly all soils tested in Northern Nigeria, Also, Bache and Roger (1970) and Jones (1974) reported that savana soils are deficient in native phosphorus. So, the need for phosphorus fertilizers on these soils cannot be over emphasized.

The principal source of phosphorus fertilizers is rock phosphate, which occurs as a mineral deposit in many parts of the world. The two other minor sources of phosphate are animal bones and guano. Guano is the excreta of seafowls and bats and is found in large amounts alongside the coast of Peru. It is therefore also known as Peruvian guano. It contains both nitrogen (13%) and phosphorus (20% P_2O_5) (FAO, 1980). The type of phosphorus fertilizer used at IAR is the single superphosphate also known as ordinary superphosphate. It is a phosphate fertilizer containing water soluble phosphate. It is the oldest phosphate fertilizer in use and it is made by reacting rock phosphate with sulphuric acid in dug out pits or tanks. The product is hard and has to be excavated by mechanical means. It is then powdered and sold. It contains 18% P_2O_5, the

equivalent of which is 334kg superphosphate/ha (Ologunde, 1982b).

1.4.3 POTASSIUM FERTILIZATION

Maize requires large amounts of potassium. It is essential for vigorous growth, yet never becomes a part of proteins and other organic compounds. (Aldrich and Leng, 1969). Potassium enters the maize plant as the K^+ ion. Legumes like alfalfa and clover absorb more than they need for best yield. Maize does not (Aldrich and Leng, 1969).

There are two main types of potassium fertilizers, namely, Potassium chloride or Muriate of potash (KCl) containing 60% K_2O and Potassium sulphate (K_2SO_4) containing 48% K_2O. Both fertilizers have good storage and handling qualities.

Muriate of potash is used at IAR at a level of 100kg mutriate of potash/ha. This gives 60kg K_2O/ha (Ologunde, 1982b).

1.5 NUTRIENTS.

Mankind has largely failed in its effort to adequately feed millions of people now living on earth. Of these,

at least one billion are undernourished, and the diets of an additional 800 million are deficient in one or several key nutrients (Jugenheimer, 1976). From this, we ask ourselves, what do we understand by nutrition?

Nutrition has different meanings. Many people identify it with that portion of nutrition that arouses their own interest. To some nutritionists, the subject is only biochemistry. To nurses, dietitians, nutritionists and physicians, nutrition may mean meals for the sick in terms of calories, protein, carbohydrate, fat, minerals and vitamins. To the layman, it represents food or it may mean a "special diet" (Krause and Mahan, 1979). By one definition, nutrition is "the process whereby the food intake of living organisms affects growth, activity and health, including reproduction". (Kent-Jones and Amos, 1967). Some of the nutrients cannot be synthesized by the body, at least, not in amounts sufficient to satisfy the need of them; others the body can produce. Food is the source of the nutrients the body cannot synthesize. Foods provide six major classes of nutrients, water, lipids, minerals, carbohydrates, proteins and vitamins, each serving

specific functions in the body (Wilson et al, 1979).

1.5.1 WATER.

Even a brief introduction to the topic of nutrition would be incomplete without some reference to water (Osborne and Voogt, 1978). Though not strictly a nutrient, water is essential for life. It accounts for about half of total body weight and 75% of lean body weight, and without it the body cannot function and survive. Water is needed, from drink and food, to balance losses of water in urine and from the skin, lungs and feaces. Normally, losses are almost exactly balanced by gains. Normal metabolism generates some 200cm^3 per day and therefore even at rest, at least a litre of water is required daily for the maintainance of health (Bingham, 1978). About 75% of the total food intake consists of water. Cereals contain 5-15% of water (Bingham, 1978). This is little compared to a man's daily need in terms of the amount of cereals consumed. Much of the water needed by man (about 3 litres daily) is got from other foods and direct drink. However, water is normally abundant and is not withheld from even the poorest classes on the ground of cost.

1.5.2. LIPIDS.

Lipids have important and essential roles in animal nutrition. Lipid is made up of a large class of compounds. Of the various types, it appears that only certain fatty acids must be ingested preformed, the other fatty acids and lipid fractions can be synthesized from constituents of the diet in amounts sufficient to carry out their metabolic roles. These roles are for energy and as part of the structure of cell walls. The fat in food can either be used immediately for energy, or deposited in energy stores (adipose tissue). One gram of fat supplies 9 Cal, more than twice the heat obtained from the same weight of either carbohydrate or protein, and so acts as a primary source of body energy. Since fats are often comparatively expensive they are not extensively used to provide calories, but some fat in the diet is essential if the food is to be palatable.

Maize contains about 4.5% of oil (Jugenheimer, 1976). About 85% of corn lipids occur in the germ, mainly as triglycerides of fatty acids. About 10% of the energy derived from corn comes from the metabolsim of lipids.

Since the major ingredient of the corn oil of the germ is the essential fatty acid, linoleic acid (59% of the fatty acids) (Beedle et al, 1965) whole corn kernel satisfies the essential fatty acid requirement of growing animals when used as a major feed component.

1.5.3 MINERALS

The term mineral could be used for all those chemical elements found in foodstuffs which, when traced back to source, enter the plant food train from the soil, and this too, irrespective of whether they remain in the ash or not. (Nelson, 1951).

Minerals are essential nutrients which must be supplied in the diet. They may be incorporated into the body structure, or functions as cofactors for enzymes, or are required as electrolytes. Humans require at least 18 minerals (Osborne and Voogt, 1978). Eight are found in relatively large quantities in food and the body. These are referred to as macroelements. Comparatively minute amounts of the other minerals, the trace or micro elements, are required and found in the body. These elements have their recognised biological functions and these include, the control of

body fluids, building of rigid structures to support the body, involvement in chemical reactions in the body and as chemical constituents of the body (Osborne and Voogt, 1978).

Maize contains about 2.0% ash. About 80% of the minerals are in the germ. Maize is especially low in calcium (about 0.03%) with potassium and phosphorus the most abundant mineral elements (about 0.35%). Howe and Gilfillan, (1970), have shown that calcium is the first limiting nutrient when maize is the only source of food for rats. Maize contains less than 0.005ppm of sodium and no iodine. It contains (per kilogram), 0.02mg iron, 3.4mg copper, 4.1mg manganese and 10.4mg zinc (NRC, 1968). These levels are too low to safely meet the trace mineral requirements of swine when maize is fed as the only source of mineral in the diet.

1.5.4 CARBOHYDRATES.

The carbohydrates of human diet are primarily derived from plant materials, e.g. cereals and products derived from them such as flours, vegetables, sugar and preserves. Carbohydrates supply a major portion of man's energy.

Current nutritional opinion tends to favour a level of carbohydrate diet which supplies 50 - 65% of the total energy requirement. Nevertheless, ecological and economic factors produce wide variations in the percentage of total energy from carbohydrate in diet (i.e. in extreme cases 20 - 80%), which are still consistent with normal health (Osborne and Voogt, 1978).

Food carbohydrate occur in various forms. In order to gain a further knowledge of the carbohydrate portion of the grain which is useful in the diet, it is necessary to divide it into the following fractions:

a. Soluble carbohydrates (mono, di, oligo-saccharides and fructosans).
b. Hemicelluloses.
c. Cellulose.
d. Lignin.

The main function of carbohydrates is to provide metabolic energy predominantly via ATP. One of the simplest forms of carbohydrate is glucose or dextrose, and it has been described as the most important carbohydrate known to the nutritionist. Practically all carbohydrates are converted into this form in the body before being absorbed into the blood stream (Kent-Jones and Amos, 1967). While some carbohydrates provide the calories needed by the body, others act as roughage. For example, fibre, although neither digested nor absorbed, must be regarded as an important constituent of the diet. A lack of fibre in the diet of some people may be an important factor in the causation of several non-infectious diseases of the large bowel, including malignant tumours (Burkitt, 1973).

It has been suggested (Trowell, 1973) that a deficiency of fibre may increase the incidence of heart disease, diabetes and obesity. Another group of carbohydrates, pentoses, the five-carbon sugars, are not utilized by the body in the sense that the hexoses are. When ingested as such, the pentoses are largely excreted in the urine. However, the pentose ribose is a constituent of a number of important molecules in the body, such as the nucleic acids, riboflavin and certain coenzymes. Also, inulin a polysaccharide composed of D-fructose units is not hydrolysed by any of the enzymes of the gastrointestinal tract and is not utilised as food just as cellulose is not. Cellulose is the most abundant carbohydrate in nature, a component of dietary fibre found in all plants (Bingham, 1978). Plant cellulose fibres are cemented together by hemicelluloses which are polymers of D-xylose that contain side chains of other sugars. (Stenesh, 1975).

Maize contains about 80% carbohydrates (Jugenheimer, 1976). Starch is the major constituent and principal carbohydrate of the maize kernel (about 72% of the dry weight). Free sugars range between 1.0 and 3.0% with

sucrose as the major component . The kernels contain 2.1-2.3% crude fibre, mainly in the pericarp or hull (Inglett, 1970).

1.5.5. PROTEINS AND AMINO ACIDS

Proteins provide the body with the amino acids necessary for the production of body protein. Proteins result from the linking-up of numerous amino acids. The biological value of a particular food protein is determined by the extent to which it can furnish those amino acids that the body requires but cannot synthesize. Lack of certain amino acids may lead to forms of malnutrition. Proteins, being too expensive are not used as fuel foods. Their main function in the diet is the making and maintenance of muscle and the repair of worn tissues. (Kent-Jones and Amos, 1967).

For a diet to be nutritionally satisfactory, it must contain protein that is sufficient not only in quantity, but also in quality. The quality of a protein is determined by the variety of the amino acids which it contains and which, in consequence, it can give to the body. It is known that the body cannot synthesize about 8 amino acids.

(Wilson et. al, 1979) These are known as the "essential amino acids" and they have to be present in the food consumed. They are valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine and tryptophan.

The amino acid balance in all cereals is below the optimal for efficient human nutrition and growth. Lysine is the first limiting amino acid in all the cereals. The second is tryptophan in maize and threonine in most other cereals. Traditional maize, whether grown in the tropics or in the temperature climates, contains 9 - 11% protein in the whole grain. However, the balance of amino acid in the protein is poor: ordinary maize contains 1.8 - 2.3% lysine and about 0.3 - 0.4% tryptophan in protein (CIMMYT), 1974). Due to this poor amino acid balance, there has been a wide search for high quality protein maize.

In 1963, however, a Purdue University team discovered the importance of opaque-2 gene in enhancing the levels of two limiting amino acids in the maize endosperm protein (Mertz et. al, 1964). The two amino acids register a two fold increase in opaques as compared to the normal maize types. The opaque-2 mutant opened the way for

improving the quality of protein in maize through genetic manipulation. A number of other mutants such as floury-2 and opaque-7 also have been identified and have resulted in an increase in the levels of lysine and tryptophan. The mechanism for biochemical enhancement of lysine and tryptophan in all these mutants is the same: the boost in lysine and tryptophan levels is a result of a reduction in zein fraction lacking in lysine, while increasing other fractions, such as the albumins, globulins and glutens that are higher in lysine content.

Opaque-2 has been used much more extensively than the other mutants and its use in maize improvement process continues in the same pattern. Use of other mutants have been restricted to the experimental stages and have shown no advantage over the opaque-2. Recently, however, a double mutant combination involving opaque-2 and sugary-2 has shown some promise and may be considred as another major research achievement of the Purdue group .

Maize protein comes mainly from the endosperm, but the contribution of the germ (embryo) cannot be

ignored. The amino acid composition of the normal whole kernel is dictated by the amino acid pattern in the endosperm, and lies closer to that of endorsperm protein than to embryo protein (Inglett, 1970).

TABLE 1.1. AMINO ACID COMPOSITION OF NORMAL AND OPAQUE-2 CORN KERNELS. (Inglett, 1970).

CONSTITUENT	%	
	NORMAL CORN	OPAQUE-2 CORN
Protein	8.9	11.9
Dry matter	91.9	87.1
Amino Acids		
Aspartic acid	0.6	1.2
Threonine	0.3	0.4
Serine	0.4	0.5
Glutamine acid	1.9	2.2
Proline	0.9	1.0
Glycine	0.4	0.6
Alanine	0.7	0.8
Cystine	0.1	0.2
Methionine	0.2	0.2

CONSTITUENT	% NORMAL CORN	% OPAQUE-2 CORN
Isoleucine	0.3	0.4
Leucine	1.1	1.0
Tyrosine	0.4	0.4
Phenylalanine	0.4	0.5
Lysine	0.2	0.5
Histidine	0.3	0.4
Arginine	0.5	0.8
Tryptophan	0.1	0.2

1.6 USES OF MAIZE

The adage, "using everything but the squeal", applies so well to maize. This is because, the corn kernel is one of the most mysterious chemical entities in nature and can be used for almost everything. The composition of the dry substance of the kernel, though it varies somewhat according to types of seed and soil, use of fertilizer, and weather conditions, is about as follows:

Carbohydrates 80%

Protein	10%
Oil	4.5%
Fibre	3.5%
Minerals	2.0% (Jugenheimer, 1976).

Each component finds one use or the other in the industry.

The starch portion of the corn is used in foods, paper making, adhesives, textile weaving, charcoal briquettes, pharmaceutical tablets and lots of other things. The starch can be converted to sweeteners like conventional corn syrups, 42%, 55% and 90% high fructose corn syrup. These are used in food products such as baby foods, bakery products, soft drinks, tobacco, chewing gum, cordials, fruit drinks, ice cream, jams, peanut butter, vinegar, instant tea, marsh mallows, breakfast foods, gelatin, wine, non-carbonated beverages, processed cheese, olives, vitamin preparations and medicines. The liquid dextrose converted from starch can be fermented to anhydrous power alcohol, which is used for gasohol. During the fermentation, the carbon dioxide produced can be used for carbonated drinks and for refrigeration. The protein and hull of the corn kernel produce corn gluten feed and corn gluten meal. These are premium protein ingredients in animal and poultry feeds.

The oil from the germ portion is used in cooking oils, margarine, mayonnaise, salad dressings and shortening (ADM, 1981).

Due to the numerous uses of maize, breeders are able to select plants and ears suitable for many uses. In Nigeria, maize is grown chiefly for human consumption. It is utilised in many different ways for the preparation of various dishes. Some dishes are light and serve as breakfast early in the morning. Others are solid and frequently made more palatable by the addition of palm oil, vegetables or fruits and various sauces and meats. These serve as the main meal and are eaten later in the day. Some dishes are reserved and used only for special occasions. Such a dish is the MASA, (maize cake) of the Yorubas which is served only during the funeral ceremonies of a noble or a chief (Eijnattern, 1965). A list of other dishes is shown in Table 1.2. In the industries, maize is used for brewing. It is also used for the much favoured breakfast cereal, "cornflakes". With the rapid expansion of poultry and livestock industries, maize is being used as a highly suitable and palatable feeding-stuff for all classes of livestock.

TABLE 1.2. SOME MAIZE DISHES IN NIGERIA WITH AREAS OF ORIGIN (Eijnatten, 1965).

DISH	LOCAL NAME	TRIBE	STATE
1. FRESH GRAIN PREPARATIONS			
a. Fresh or green ears harvested from 3 to 5 weeks after seedset and boiled until the seeds are soft. Salt may or may not be added.	Masara ndafa	Hausa	Bauchi
	Imiyo-sise	Itsekiri	Bendel
	Okanale	Edo	Bendel
	Ikureke i jiir	Tiv	Benue
	Langbe	Yoruba	Ogun, Oyo, Ondo.
	Oka esiriesi	Ibo	Imo, Anambra
b. Green ears harvested from 3 to 5 weeks after seed set and roasted above or beside a fire or cooked in hot ashes	Epupo	Nupe	Niger Plateau
	Iwana	Yergam	
	Imiyo sisun	Itsekiri	Bendel
	Agbado sisun	Yoruba	Ogun, Oyo, Ondo.
	Oka arunaru	Ibo	Imo, Anambra.

TABLE 1.2. Contd.

DISH	LOCAL NAME	TRIBE	STATE
c. Immature grains pounded or ground, sieved in water, dried and mixed with pepper and salt. The mixture is wrapped in guinea corn or cocoa leaves and then boiled or steamed.	Agiriga Ogidigbo Sapale Utara oka	1 Ankwe Igala Yoruba Urhobo	Plateau Benue Ogun, Oyo, Ondo. Bendel.
d. Similar preparations to the above but mixed after steaming with pepper, salt, palm-oil, and raw, cut vegetables.	Igbala akpa Akpapa Adalu	Igala Tiv Yoruba	Benue. Benue. Ogun, Oyo, Ondo.

TABLE 1.2. Contd.

DISH	LOCAL NAME	TRIBE	STATE
e. Served separately with beans	Egbo	Yoruba	Ogun, Oyo, Ondo.
	Ikpa-oka	Urhobo	Bendel.
	Ukpo-oka	Bendel Ibo	Bendel
f. Similar preparations to the above but taken on their own after frying or as a stift porridge	Okereke	Ibo	Imo, Anambra
	Uka'appa	Igbirra	Kwara
	Gusasa	Hausa	Bauchi
	Zinkun	Yergam	Plateau
	Akara agbado	Yoruba	Ogun, Oyo, Ondo.
	Ikpakene	Edo	Bendel.
g. Fresh grains; boiled, crayfish fish oil and native potash added. The mixture is reboiled diluted with water and eaten as soup	Owo-imiyo	Itsekiri	Bendel

TABLE 1.2. Contd.

DISH	LOCAL NAME	TRIBE	STATE
h. Fresh grain boiled and mixed with coco-nut juice.	Imiyo ikokodie	Itsekiri	Bendel
2. MAIN DRY GRAIN PREPARATIONS.			
Grain, from which the seed coat have been removed by pounding, soaked for two or three days then ground on a stone or pounded, seived and boiled. It can be taken as a warm or cold thin	Agidi	Ibo	Imo, Anambra
	Ekamu	Igala	Benue.
	Ijobu	Igala	Benue.
	Ekwa	Nupe	Niger.
	Kunu	Hausa	Bauchi.
	Eko (mimu)	Yoruba	Ogun, Oyo,
	Ogi	Yoruba	Ondo.
	Akamu	Edo	Bendel.

TABLE 1.2. Contd.

DISH	LOCAL NAME	TRIBE	STATE
gruel, as a thick poridge, or as a thick paste wrapped in leaves.			
3. MIXED PREPARATIONS.			
a. Grain after removal of seed coat is pounded to a flour and mixed with groundnuts and sugar, then fried in oil.	Emu Unoka	Itsekiri Itsekiri	Bendel Bendel
b. As above but taken with coconut instead of groundnuts and sugar.	Okanaranmwen Ekaka Okanalami	Edo Urhobo Itsekiri	Bendel Bendel Bendel
c. As above but mixed with yam and beans and fried in oil as pancakes	Guruguru Tayitayi	Itsekiri "	Bendel "

TABLE 1.2. contd.

DISH	LOCAL NAME	TRIBE	STATE
4. MAIZE DRINKS.			
Dry grains roasted	Kun^{u}	Mada	Benue
and ground. After	Burukutu	Hausa	Bauchi
sieving the flour	Burukutu	Igala	Benue
is mixed with	Eche	Igbirra	Kwara
water and served	Ma	Mada	Benue
cool. Grains	Men	Gombe	Bauchi
soaked in water	Nwon	Tula	Bauchi
for one or two	Ntebe	Yergam	Plateau
days, allowed to	Pito	Ibo	Imo, Anambra
germinate for	Pito	Yoruba	Ogun, Ondo, Oyo
three days, ground	Oti agbado	Yoruba	Ogun, Ondo, Oyo
and sieved. The			
ground particles			
are boiled in			
water and left to			
ferment for twelve			
hours. The liquid			
is boiled again and			

TABLE 1.2. Contd.

DISH	LOCAL NAME	TRIBE	STATE
left to ferment for a period varying from one to several days. This might be called "maize beer" and is an alcoholic drink, often drunk in areas where palm wine is not readily available.			

1.7. RESEARCH OBJECTIVES

Much efforts have been directed at the cultivation of n new maize varieties by the agronomists. Apart from the yield, what are the nutrient contents of these varieties? How well do the nutrients compare with those of the old or common varieties? Does the application of fertilizer, either singly or in combination have any significant effect on the nutrient composition of the kernels?

Most studies on maize are concentrated at investigating its protein content and amino acid composition as the only

nutritional qualities. Studies on other nutrients have largely been ignored. There is an urgent need to look into maize composition with respect to other essential nutrients such as sugars, minerals and lipids.

Recently, there has been an extension of maize cultivation from the south into the northern parts of Nigeria where sorghum and millet used to be the stable crops. What could be the cause of this? Could it be due to the expected deficit of 50,000 to 94,000 tonnes of maize projected in the period 1975 to 1980 in the country? (Ologunde, 1982a). As a result of this expected deficit, the Institute for Agricultural Research (IAR), Samaru, Zaria is doing a lot of research to obtain high yielding varieties of maize whose seeds would then be distributed to farmers. It is therefore necessary to know the varieties and fertilizer treatment which could be used to obtain maximum yield and grains of high nutritional quality.

The objectives of this study therefore are:

1. To determine the (a) protein, (b) amino acid, (c) carbohydrate, (d) lipid and (e) mineral composition of some maize varieties.

2. To investigate the effect of the application of fertilizers e.g. nitrogenous, phosphatic and potassic, singly and in combination on the nutrients in I above.

It is hoped that this work will compliment the breeding and selection work of IAR.

CHAPTER TWO

MATERIALS AND METHODS

2.1. SAMPLE COLLECTION.

Samples of maize labelled S_1 - S_{10} were collected from the Agronomy Department of the Institute for Agricultural Research, (IAR), Ahmadu Bello University, Samaru, Zaria, while samples labelled S_{11} - S_{14} were obtained from the National Seeds Service, Zaria. Samples S_1 - S_9 were planted on the 2nd of June, 1981 and harvested on the 28th of September, 1981. Sample S_{10} was planted early June, 1981 and harvested in mid-September, 1981. The samples collected from the National Seeds Service were planted in June, 1980 and harvested in September, 1980.

2.2. SAMPLE DESCRIPTION

Five varieties of maize were used for the experiments. These varieties were TZB, $S_1$23, 096, Bamo Local and Western Yellow.TZB variety was used for the effect of fertilizer treatment study. Nine groups of the maize plants were used. Eight groups were subjected to different doses of the fertilizer while one group was used as a control, being subjected to no fertilizer treatment. For the study

on the effect of varietal differences, five maize varieties were used (Table 1.1).

Nitrogen was applied in the form of nitrochalk, phosphorus as Single Superphosphate and potassium as Muriate of Potash. The plant population was 53,000 plants per hectare while the spacing was 75 x 25cm.

TABLE 1.1 MAIZE SAMPLES AND AMOUNT OF FERTILIZER APPLIED.

SAMPLE	VARIETY	AMOUNT OF FERTILIZER APPLIED (Kg/ha)		
		NITROGEN (N)	PHOSPHORUS (P_2O_5)	POTASSIUM (K_2O)
S_1	TZB	-	-	-
S_2	TZB	25	-	-
S_3	TZB	50	-	-
S_4	TZB	-	30	-
S_5	TZB	-	60	-
S_6	TZB	-	-	50
S_7	TZB	-	-	100
S_8	TZB	25	30	50
S_9	TZB	50	60	100
S_{10}	Bomo Local	120	60	60
S_{11}	S,123	120	60	60

SAMPLE	VARIETY	AMOUNT OF FERTILIZER APPLIED (Kg/ha)		
		NITROGEN (N)	PHOSPHORUS (P_2O_5)	POTASSIUM (K_2O)
S_{12}	096	120	60	60
S_{13}	TZB	120	60	60
S_{14}	Western Yellow	120	60	60

2.2.1. TZB (FARZ 34)

This maize variety evolved from the International Institute for Tropical Agriculture (IITA), Ibadan. It has bold white grains, single large ears per plant, medium plant size and late maturity. Its maturity period is 120 days. TZB is open pollinated and one of the highest yielding varieties.

2.2.2 SAMARU, 123 (

S, 123 is a composite, with mixed colouration yellow being dominant. The seeds are dented and the cobs small-sized. The ear is placed low on the stalk which are small-sized. This variety is susceptible to foliar diseases of maize. S, 123 is high yielding and confined to the savannas.

It is open pollinated and has a maturity period of 120 days which is late maturity. S,123 used for this work was planted under irrigation at Kadawa in Kano State.

2.2.3. (096 (FARZ 23)

This is a high yielding variety with bold yellow, dent seeds. It is open pollinated. The ears are large and long and are usually two ears per plant. This is the only true ear-prolic (i.e. bearing more than one ear per plant) variety at present (Obilana, 1978). The stalks are tall and thick and together with the roots, are very strong. This gives the variety good standability in the field. It does not lodge. The plants are robust and bushy. 096 is late maturing having a maturity period of 120 days.

2.2.4. Bomo Local.

This variety is white seeded and open pollinated. It is confined to the savannas. Bomo Local is derived from local variety from Bomo village near Samaru. It is medium maturing having a maturity period of 110 days..

2.2.5. WESTERN YELLOW (FARZ 7)

This is a yellow synthetic maize cultivar evolved at the Institute of Agricultural Research and Training,

University of Ife, Ile-Ife. (Wiggin and Obajimi, 1973). The grains are dented. The ears are large and usually single and placed very high on the plant. The plants are very tall with average size stalks which fairly lodge. It is open pollinated and has a maturity period of 120 days. It is therefore late maturing.

2.3. SAMPLE PREPARATION.

The samples were picked to ensure that no foreign particles were included in the grinding. They were then ground in a Pifco 7-speed blender by Waring. At different stages of the grinding the samples were sieved to pass through sieve No. 30 (USA standard) or 600um (International Standard). Larger particles were taken back to the blender for further grinding. This process was repeated until a very fine powder was obtained. Samples were taken from this for the determination of moisture content. The remainder of each sample was then dried in an air oven at about 60^{o}C as recommeded by Oyenuga (1968). Drying was completed within 6 days. The dried samples were then stored in air tight screw-capped bottles (500.0cm^{3}) and kept in the desiccator. These were the samples used for the other analyses.

2.4. CHEMICALS

Analytical grade reagents were used for most of the determinations with a few "laboratory" and "all purpose" reagents. Chemicals used were products of BDH chemicals (Poole, England) or Hopkins and Williams (Chadwellheath, Essex, England) or May and Baker (Dageham, England) or Sigma Chemical Company (St. Louis, U.S.A.)

2.5. DETERMINATION OF MOISTURE CONTENT

The method of the Association of Official Analytical Chemists (A.O.A.C. 1975) was adopted. This method is an indirect distillation method i.e. evaporation of moisture. In this case, an air oven was used. The loss in weight after drying in the oven gives the amount of moisture present in the sample.

Apparatus.

1. Petri-dishes.
2. Oven - Laboratory Thermal Equipments, A Searl Company, England.
3. Desiccator, with activated silica gel as the drying agent.
4. Weighing balance - Mettler, Type H6T.

Method

The petri-dishes and lids were dried in the oven at 98-100°C for two hours after which they were cooled in a desiccator. Weights of the dishes with the lids on were taken at room temperature. 2.0g of the sample was accurately weighed into the dish and placed in the oven with lid separately at 98-100°C for 5 hours, after which they were cooled in a desiccator. Weights were taken with the lids on at room temperature. This procedure was repeated until a constant weight was obtained. Each treatment was replicated 3 times.

Calculation

$$\text{Moisture (\%)} = \frac{\text{loss in wt (g)}}{\text{wt of sample(g)}} \times 100$$

2.6. DETERMINATION OF TOAL ASH CONTENT

The ash content was determined by the method of A.O.A.C. (1975). The total minerals (ash) are estimated by burning away the organic material.

Apparatus

1. Porcelain crucibles.
2. Furnace- carbolite, Shefield, England.
3. Air tight desiccator with activated silica gel as the

drying agent.

4. Weighing balance - Mettler, Type H6T.

Method.

The crucible was ignited in the furnace at 550°C for 2 hours. This was cooled in a desiccator and weighed at room temperature. 2.0g of sample was weighed into the crucible and ignited in the furnace at 550°C for 12 hours. It was then cooled in a desiccator and weighed at room temperature. Each treatment was carried out in triplicate.

Calculation

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

2.7. DETERMINATION OF CRUDE PROTEIN CONTENT.

The protein content was determined using the semi-micro Kjeldahl distillation method as described by Pearson (1976). The determination of the amount of nitrogen in a cereal can be made with considerate accuracy and the figure so obtained for the nitrogen can be converted to the amount of crude protein by multiplication by known and accepted factors. The empirical factor of 6.25 is used for feeding stuffs (Kent-Jones and Amos, 1967, A.O.A.C., 1975). These factors are based upon the percentage of nitrogen in the

proteins. The determination of nitrogen is made in two stages; first, the cereal is digested with concentrated sulphuric acid and other additions that convert all the nitrogen into ammonium sulphate, and after making the mixture alkaline, the ammonia thus obtained is determined by distillation and titration of the distillate.

Apparatus

1. Heating mantle - Electrothermal, England.
2. Semi-micro Kjeldahl digestion apparatus.
3. Digestion flasks (50.0cm^3).
4. Burette (10.0cm^3).
5. Conical flasks (50.0cm^3).
6. Volumetric flasks (100.0cm^3).
7. Measuring cylinders (10.0cm^3, 20.0cm^3).
8. Glass beads.

Reagents.

1. Concentrated sulphuric acid (sp. gr. 1.84).
2. Boric acid solution (2%): prepared by dissolving 20.0g of boric acid (H_3BO_3) in water and diluting to 1,000.0cm^3.
3. Sodium hydroxide solution (40%): prepared by dissolving

400.0g of sodium hydroxide pellets in water and diluting to 1,000.0cm^3.

4. Hydrochloric acid, 0.01M, standardized.
5. Kjeldahl mixed catalyst tablets - each tablet contains 1.0g of sodium sulphate and the equivalent of 0.05g of selenuim.
6. Mixed indicator: prepared by dissolving 0.2g of methyl red and 0.1g of methylene blue in absolute ethanol and diluting to 100.0cm^3 with absolute ethanol.

Method.

a. Digestion: 0.2g of the ground sample was accurately weighed into a clean, dry 50.0cm^3 Kjeldahl flask. Half a tablet of the mixed catalyst was added. 5.0cm^3 of concentrated sulphuric acid and a glass bead were then added. The mixture was heated slowly until a clear colourless solution was obtained. This was allowed to cool and the solution quantitatively transferred to a 100.0cm^3 volumetric flask and made up to mark with distilled water.

b. Distillation: 10.0cm^3 of the above solution was transferred to the semi-micro Kjeldahl distillation flask. 20.0cm^3 of 40% sodium hydroxide solution was added to the

digest and the ammonia liberated was steam distilled into $10.0cm^3$ boric acid containing 2 drops of the mixed indicator. The process took about 10mins.

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

The blank was run using the same amount of reagents as for the samples. Each determination was carried out in triplicate.

Calculation. (Shirlaw, 1967)

$$HCl + NH_4OH \longrightarrow NH_4Cl + H_2O.$$

$1000.0cm^3$ normal HCl $\equiv$ 14.0g of Nitrogen

$1.0cm^3$ $^N/_{100}$ HCl $\equiv$ 0.00014g of Nitrogen.

The digest was made up to $100.0cm^3$. Of this, $10.0cm^3$ was used for the Nitrogen determination. Since $100.0cm^3$ of digest contains 0.2g of sample $\therefore$ $10.0cm^3$ of digest would contain 0.02g of sample.

If x cm^3 is the titre and y gm is the weight of sample taken, then

No. of gm of nitrogen in $10.0cm^3$ of digest = 0.00014x

No. of gm of nitrogen in $100.0cm^3$ of digest = 0.0014x

This is equivalent to y gm of sample.

$\therefore$ Total Nitrogen (% dry wt) = $\frac{0.14x}{y}$

Crude protein (% dry wt) = $\frac{0.14x}{y} \times 6.25$.

2.8. THE DETERMINATION OF LIPIDS - ETHER EXTRACT.

The method employed in this determination was the Soxhlet extraction as described by Shirlaw (1967). The procedure is based on the extraction of the fats by petroleum ether. The weight of the fat obtained after distilling off the solvent from the extract gives the amount of fat present.

Apparatus.

1. The Soxhlet apparatus which consists of
   a heating mantle
   a Soxhlet flask (250.0cm^3)
   a Soxhlet extractor
   a Spiral condenser
2. Fat-free thimbles.
3. Desiccator with activated silice gel as the drying agent.
4. Oven - Laboratory Thermal Equipment, A Searle Company, England.

5. Rotary vacuum evaporator - Flash evaporator, Buchler Instruments, NJ., U.S.A.

Reagent

1. Petroleum ether (b.p. 40-60^{o}C).
2. Fat-free cotton wool.

Method.

Two grammes of the sample were weighed and put in an extraction thimble which was then plugged with cotton wool, to avoid loss of sample. The thimble was placed in the extractor. An already weighed, clean and dry Soxhlet flask was attached to the bottom of the extractor. 200.0cm^{3} of petroleum ether was poured into the extractor to start a siphon (and hence empty into the flask) and then three-quarters fill the extractor again. The top was then turned on and the heating mantle switched on so that the petroleum ether just boiled. Heating was continued for at least 8 hours after which the solvent was siphoned completely into the flask and taken to near dryness using a vacuum rotary evaporator. The flask was then removed and dried to a constant weight in an air oven at 60^{o}C. The flask was cooled in a desiccator, weighed and the amount

of fat extracted was calculated from the difference between the weight of the flask before and after extraction. Each treatment was replicated 3 times.

Calculation.

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

2.9. WET DIGESTION OF MAIZE SAMPLES FOR ELEMENT ANALYSES.

The wet decomposition of organic matter by the action of various acids is of almost universal application and the conditions can be adjusted so as to prevent loss of the more volatile elements e.g. arsenic, antimony and mercury. The method employed here is that of the Analytical Methods Committee, (1960). Decomposition was done with nitric and perchloric acids which have the advatange of not giving insoluble sulphates, since the insoluble sulphates absorb a considerable proportion of trace metals.

Apparatus

1. Kjeldahl flasks (100.0cm^3)
2. Heating mantle - Electrothermal, England.
3. Volumetric flasks (50.0cm^3).
4. Measuring cylinders (10.0cm^3, 20.0cm^3).

Reagents

1. Concentrated nitric acid (sp. gr. 1.42).
2. Perchloric acid (71.0-73.0% w/w).
3. Acid washing liquid for glassware (concentrated nitric acid was diluted 100 times with water).
4. Distilled, deionised water.

Method.

One gramme of the dried sample was accurately weighed into a 100.0cm^3 Kjeldahl flask. To this was added 13.0cm^3 of concentrated nitric acid (HNO_3) and a glass bead. The mixture was boiled slowly for 30mins in a fume chamber. It was allowed to cool and 7.5cm^3 of perchloric acid ($HClO_4$) added. The mixture was boiled very gently for about 1hr until it was colourless and dense white fumes were evolved. The mixture was allowed to cool and quantitatively transferred to a 50.0cm^3 volumetric flask and diluted to volume with distilled, deionised water. A blank was prepared using the same amount of reagents. The digests and blank were then transferred to plastic reagent bottles (60.0cm^3) and stored in the refrigerator until ready for use. Each treatment was carried out in triplicate.

ELEMENT ANALYSES.

2.9.1 ATOMIC ABSORPTION SPECTROPHOTOMETRY

The method employed is as described by Osborne and Voogt (1978). It was used for the determination of calcium, copper, iron, magnesium, manganese and zinc in the digested maize samples. The solution obtained from the wet digestion was sprayed into the flame of an atomic absorption spectrophotometer and the absorption of the metals to be analysed were measured at specific wavelengths.

2.9.1.1. Calcium.

Calcium was determined with the Pye Unicam Atomic Absorption Spectrophotometer at 422.7nm. The air-acetylene flame was used and the maximum current was 6mAmp. There was no interferance by other metals.

Apparatus.

1. Atomic Absorption Spectrophotometer-Unicam SP 1900 with digital readout.
2. Micro-pipettes (0.1, 0.2, 0.5cm^3).
3. Volumetric flasks (100.0cm^3, 1000.0cm^3.)

Reagents.

1. Hydrochloric acid, 3M: prepared by diluting 260.0cm^3

of concentrated hydrochloric acid (sp. gr. 1.18) to 1,000.0cm^3 with distilled, deionised water.

2. Stock calcium solution: This was prepared by dissolving 2.5g of calcium carbonate ($CaCO_3$) analytical grade in 100.0cm^3 3M hydrochloric acid and diluted to 1000.0cm^3 with distilled, deionised water. This solution contained 1000ppm of calcium.

3. Working standard solution: 10.0cm^3 of the stock solution was diluted to 100.0cm^3 with distilled, deionised water to give a solution containing 100.0ppm of calcium ions. 0.1, 0.2, 0.3, 0.4, 0.5 cm^3 of this solution were pipetted into separate 100.0cm^3 volumetric flasks and made up to volume with distilled, deionised water. These gave solutions containing 0.1, 0.2, 0.3, 0.4, 0.5 ppm of calcium respectively. A blank solution was also prepared.

Method.

A calibration curve was plotted from the absorbances of the standard solutions. The absorbances of the sample solutions were also measured and the concentrations read off from the calibration curve.

2.9.1.2. Copper.

Apparatus. As for calcium.

Reagents.

1. Hydrochloric acid, 3M. Prepared as for calcium.
2. Stock copper solution: This was prepared by dissolving 3.8g of cupric nitrate ($Cu(NO_3)_2 \cdot 3H_2O$), analytical grade, in 100.0cm^3 3M hydrochloric acid and diluted to 1,000.0cm^3 with distilled, deionised water.
3. Working standard solution: 10.0cm^3 of the stock solution was diluted to 100.0cm^3 with distilled, deionised water. 0.1, 0.2, 0.3, 0.4, 0.5cm^3 of this solution were pipetted into separate 100.0cm^3 volumetric flasks and made up to volume with distilled, deionised water. These gave solutions containing 0.1, 0.2, 0.3, 0.4, and 0.5ppm of copper respectively. A blank solution was also prepared.

Method.

Copper was determined at 324.8nm, with a maximum current of 5m Amp. The concentrations of copper in the sample solutions were read off from the calibration curve.

2.9.1.3. Iron

Apparatus: As for calcium.

Reagents.

1. Hydrochloric acid, 3M. Prepared as for calcium .
2. Stock iron solution: This was prepared by dissolving 4.98g of ferrous sulphate ($FeSO_4.7H_2O$), analytical grade, in 100.0cm^3 3M hydrochloric acid and diluted to 1,000.0cm^3 with distilled, deionised water.
3. Working standard solution. 10.0cm^3 of the stock solution was diluted to 100.0cm^3 with distilled, deionised water. 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5 and 2.0cm^3 of this solution were pipetted into separate 100.0cm^3 volumetric flasks and made up to volume with distilled, deionised water. These gave solutions containing 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, and 2.0 ppm of iron respectively. A blank solution was also prepared.

Method.

Iron was determined at 248.3nm with a maximum current of 15m Amp. The concnetration of iron in the sample solutions were read off from the calibration curve.

2.9.1.4. Manganese.

Apparatus: As for calcium

Reagents.

1. Hydrochloric acid, 3M. Prepared as for calcium.
2. Stock manganese solution: This was prepared by dissolving 4.06g of manganous sulphate ($MnSo_4.4H_2O$), analytical grade in 100.0cm^3 3M hydrochloric acid and diluted to 1,000.0cm^3 with distilled, deionised water.
3. Working standard solution: 10.0cm^3 of the stock solution was diluted to 100.0cm^3 with distilled, deionised water. 0.1, 0.2, 0.3 and 0.4cm^3 of this solution were piptetted into separate 100.0cm^3 volumetric flasks and made up to volume with distilled, deionised water. These gave solutions containing 0.1, 0.2, 0.3 and 0.4 ppm of managense respectively. A blank solution was also prepared.

Method.

Manganese was determined at 279.5nm, with a maximum current of 12mAmp. The concentration of manganese in the sample solutions were read off from the calibration curve.

2.0.1.5. Magnesium.

Apparatus: As for calcium.

Reagents.

1. Hydrochloric acid, 3M. Prepared as for calcium.
2. Stock magnesium solution: This was prepared by dissolving 10.54g of magnesium nitrate ($Mg(NO_3)_2 \cdot 6H_2O$) analytical grade in 100.0cm^3 3M hydrochloric acid and diluted to 1000.0cm^3 with distilled, deionised water.
3. Working standard solution: 10.0cm^3 of the stock solution was diluted to 100.0cm^3 with distilled, deionised water. 0.1, 0.2, 0.3, 0.4 and 0.5 cm^3 of this solution were pipetted into separate 100.0cm^3 volumetric flasks and made up to volume with distilled, deionised water. These gave solutions containing 0.1, 0.2, 0.3, 0.4 and 0.5 ppm of magnesium respectively. A blank solution was also prepared.

Method.

Magnesium was determined at 285.2nm, with a maximum current of 6mAmp. The sample solutions were diluted a hundred times so as to give absorbance readings that would fall within the limit of detection of magnesium at

285.2nm. The concentration of magnesium in the sample solutions were read off from the calibration curve.

2.9.1.6. Zinc.

Apparatus: As for calcium.

Reagents.

1. Hydrochloric acid, 3M. Prepared as for calcium.
2. Stock zinc solution: This was prepared by dissolving 4.55g of zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$), analytical grade, in 100.0cm^3 3M hydrochloric acid and diluted to 1000.0cm^3 with distilled, deionised water.
3. Working standard solution: 10.0cm^3 of the stock solution was diluted to 100.0cm^3 with distilled, deionised water. 0.1, 0.2, 0.3, 0.4 and 0.5cm^3 of this solution were pipetted into separate 100.0cm^3 volumetric flasks and made up to volume with distilled, deionised water. These gave solutions containing 0.1, 0.2, 0.3, 0.4, and 0.5ppm of zinc respectively. A blank solution was also prepared.

Method.

Zinc was determined at 213.9nm, with a maximum current of 10mAmp. The concentration of zinc in the sample

solutions were read off from the calibration curve.

2.9.1.7. Calculation: For calcium, copper, iron, manganese, magnesium and zinc. (Osborne and Voogt, 1978).

Let: weight (g) of sample = W.

Volume (cm^3) of extract = V.

Concentration (ppm or ug/cm^3) of sample solution = a.

Concentration (ppm or ug/cm^3) of blank solution = b.

Then: metal content (ug/100g) $= \frac{(a-b) \times V}{W} \times 100$

Metal content (mg/100g) $= \frac{(a-b) \times V}{W} \times \frac{100}{1000}$

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

2.9.2. FLAME PHOTOMETRIC METHOD.

The method described by Osborne and Voogt (1978) was used for the determination of potassium and sodium. Flame photometry is an emission photometry and it is the recommended technique for the determination of these two metals.

2.9.2.1. Potassium.

Potassium was determined with a flame photometer using an air pressure of $10 lb/in^2$.

Apparatus

1. Flame photometer - Corning Eel, Scientific Instruments,

England.

2. Pipettes: 5.0cm^3, 10.0cm^3.

3. Volumetric flasks: 100.0cm^3, 1,000.0cm^3.

Reagents.

1. Hydrochloric acid, 3M. Prepared as for calcium.

2. Stock potassium solution: This was prepared by dissolving 1.91g of potassium chloride (KCl) analytical grade, which had previously been dried in an air oven at 105^{o}C for 2hours, in 100.0cm^3 3M hydrochloric acid and diluted to 1,000.0cm^3 with distilled, deionised water.

3. Working standard solution: 10.0cm^3 of the stock solution was diluted to 100.0cm^3 with distilled, deionised water. 0.1, 0.2, 0.3, 0.4 and 0.5 cm^3 of this solution were pipetted into separate 100.0cm^3 volumetric flasks and made up to volume with distilled, deionised water. These gave solutions containing 0.1, 0.2, 0.3, 0.4 and 0.5ppm of magnesium respectively. A blank solution was also prepared.

Method.

The flame photometer was calibrated using the blank for 0% transmittance and the highest working standard solution (10.0ppm) for 100% transmittance. A calibration curve was plotted from the transmittance of the standard solutions.

The transmittances of the sample solutions were also measured and the concentrations read off from the calibration curve. The sample solutions were diluted ten times so as to give transmittance readings that would fall within the calibrated instrument.

2.9.2.2. Sodium

Apparatus: As for potassium

Reagents.

1. Hydrochloric acid, 3M. Prepared as for calcium.
2. Stock sodium solution. This was prepared by dissolving 2.45g of sodium chloride (NaCl), analytical grade, which had been previously dried in an air oven at 105°C for 2 hours, in 100.0cm^3 3M hydrochloric acid and diluted to 1000cm^3 with distilled, deionised water.
3. Working standard solution: 10.0cm^3 of the stock solution was diluted to 100.0cm^3 with distilled, deionised water. 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0cm^3 of this solution were pipetted into separate 100.0cm^3 volumetric flasks and made up to volume with distilled, deionised water. These gave solutions containing 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5. 4.0, and 5.0 ppm of sodium respectively. A blank solution was

prepared.

Method.

The flame photometer was calibrated using the blank for 0% transmittance and the highest standard solution (5ppm) for 100% transmittance. A calibration curve was plotted from the transmittances of the standard solutions. The transmittance of the sample solutions were also measured and the concentrations read off from the calibration curve. The sample solutions were diluted ten times so as to give transmittance readings that would fall within the calibrated instrument.

2.9.2.3. Calculation: For potassium and sodium.

Same as in section 2.9.1.7.

2.9.3. SPECTROPHOTOMETRIC METHOD.

Phosphorus was determined as phosphate by the Vanado-Molybdate spectrophotometric method as described by Pearson (1976). This method was first proposed by Hanson (1950). It is based on Misson's reaction. The acid solution containing orthophosphate is treated with an acid reagent containing molybdic acid and vanadic acid. A stable orange-yellow coloured complex of vanadi-molybdi phosphoric

acid ($H_3PO_4.VO_3$, $11MoO_3.nH_2O$) is formed. The maximum absorption is at 330nm, but Donald et. al (1956) have reported that satisfactory results can be obtained in the region 420-480nm, provided the light is monochromatic.

2.9.3.1. Phosphorus.

Apparatus.

1. Spectrophotometer - Beckman DB-GT grating spectrophotometer.
2. Volumetric flasks ($100.0cm^3$, $1000cm^3$).
3. Pipettes ($1.0cm^3$, $2.0cm^3$, $5.0cm^3$, $10.0cm^3$).
4. Glass rods for stirring.

Reagents.

1. Vanadate-molybdate composite reagent: This was prepared as follows: 20.0g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) analytical grade) was dissolved in $400.0cm^3$ warm, distilled, deionised water and then cooled. 1.0g of ammonium metavanadate (NH_4VO_3, analytical grade) was dissolved in $300.0cm^3$ boiling distilled, deionised water. This was cooled and $140.0cm^3$ of concentrated nitric acid (sp. gr. 1.42) was slowly added to it with stirring. The molybdate

solution was then added gradually to the acid vanadate solution with stirring and diluted to 1,000.0cm^3 with distilled, deionised water.

2. Ammonia solution (sp. gr. 0.88).

3. Dilute nitric acid - two times dilution with water.

4. Hydrochloric acid, 3M. Prepared as for calcium.

5. Stock phosphate solution: This was prepared by dissolving 4.58g of disodium hydrogen orthophosphate (Na_2HPO_4) analytical grade, in 100.0cm^3 3M hydrochloric acid and diluted to 1000cm^3 with distilled, deionised water.

6. Working standard solution: 10.0cm^3 of the stock solution was diluted to 100.0cm^3 with distilled, deionised water. 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0cm^3 of this solution were pipetted into separate 100.0cm^3 volumetric flasks. These were then diluted to about 50.0cm^3 with distilled, deionised water. Four drops of ammonia solution were added and then 10.0cm^3 of dilute nitric acid. 25.0cm^3 of the vanadate-molybdate reagent was added and the mixture made up to 100.0cm^3 with distilled, deionised water.

These gave solutions containing 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 and 10.0 ppm of phosphate respectively.

Method.

$10.0cm^3$ of the sample solution was transferred to a $100.0cm^3$ volumetric flask. This was neutralised by the dropwise addition of ammonia solution and then made up to about $50.0cm^3$ with distilled, deionised water. The solution was then made acid by adding $10.0cm^3$ of dilute nitric acid. $25.0cm^3$ of vandate-molybdate reagent was added and diluted to mark with distilled, deionised water. By this preparation, the sample had a ten times dilution. The solution was mixed thoroughly and the optical density measured with the spectrophotometer at 470nm after allowing to stand for 10 minutes. A calibration curve was plotted from the absorbances of the standard solutions. The absorbances of the sample solutions were also measured and the concentrations read off from the calibration curve.

2.9.3.2. Calculation: See Appendix I.

2.10. DETERMINATION OF CARBOHYDRATES

The method described by Shirlaw (1967) was adopted. The carbohydrates were characterised into their different fractions namely soluble carbohydrates, hemicelluloses, cellulose and lignin. Different methods of extraction were used and the amounts of carbohydrates in each fraction were estimated.

2.10.1. Extraction of soluble carbohydrates.

The soluble carbohydrates are taken as those that are soluble in boiling water.

Apparatus.

1. Cold-finger condenser.
2. Funnels.
3. Conical flasks (500.0cm^3).
4. Volumetric flasks (50.0cm^3).
5. Pipettes (5.0cm^3.
6. Screw capped plastic sample bottles (60.0cm^3).
7. Heating mantle, Electrothermal, England.

Method.

0.2g of the ground sample was accurately weighed and placed in a 500.0cm^3 conical flask. 20.0cm^3 of distilled

water was then added. A cold-finger condenser was placed in the mouth of the flask and the suspension boiled for 30 minutes after which the contents of the flask were filtered. 5.0cm^3 of the filtrate was pipetted into a 50-cm^3 volumetric flask and made up to mark with distilled water. This solution was labelled A and stored in the refrigerator. Each treatment was replicated 3 times.

2.10.2. Hydrolysis and extraction of Hemicelluloses

The hemicelluloses are taken as those that are hydrolysed by boiling with 5 percent sulphuric acid.

Apparatus.

As for soluble carbohydrates, including

1. Vacuum pump.
2. Buchner funnel.
3. Filter paper.

Reagent.

Five percent sulphuric acid: This was prepared by diluting 27.0cm^3 of concentrated sulphuric acid (sp. gr. 1.84) to 1,000.0cm^3 with distilled water.

Method.

The residue from section 2.10.1 was washed with distilled water in a Buchner funnel, and transferred back to the 500.0cm^3 conical flask. 20.0cm^3 of 5 per cent sulphuric acid was added. The cold-finger condenser was placed back in the flask and the suspension boiled for 3 hours. At the end of this time, the suspension was filtered and 5.0cm^3 of the filtrate pipetted into a 50.0cm^3 volumetric flask and made up to mark with distilled water. This was labelled solution B and left in the refrigerator until it was ready for use.

2.10.3. Hydrolysis and extraction of cellulose.

Cellulose is the most abundant organic compound in the biosphere, containing more than half of all the organic carbon (Stryer, 1981). Total hydrolysis of cellulose yields only D-glucose, while partial hydrolysis yields cellobiose (Mahler and Cordes, 1968). The method of hydrolysis employed is that given by Shirlaw (1967) where 72 per cent sulphuric acid was used.

Apparatus.

As for soluble carbohydrates including

1. Sintered glass crucible (porosity 1).

Reagent.

72 per cent sulphuric acid. This was prepared by diluting 651.0cm^3 of concentrated sulphuric acid (sp. gr. 1.84) to 1,000.0cm^3 with distilled water.

Method.

The residue from section 2.10.2 was transferred to a 500.0cm^3 conical flask and 10.0cm^3 of 72 per cent sulphuric acid added. This was allowed to stand for 4 hours at room temperature after which 235.0cm^3 of distilled water was added. The contents of the flask were then boiled for 4 hours with a cold-finger condenser in the neck of the flask. At the end of that time, the content of the flask were filtered through a sintered glass crucible and carefully washed with distilled water. The filtrate was made up to 500.0cm^3 with distilled water. This solution was labelled C and kept in the refrigerator.

2.10.4. Determination of Lignin.

The method of Shirlaw (1967) was adopted.

Apparatus.

1. Sintered glass crucible (porosity 1).
2. Oven - Laboratory Thermal Equipment, A Searle Company, England.

3. Furnace - Carbolite, Shefield, England.

4. Weighing balance - Mettler, Type H6T.

Reagent.

Methanol (CH_3OH).

Method.

The residue from section 2.10.3. was carefully washed with about 20.0cm^3 of methanol. The crucible was then dried to constant weight in an air oven at 100°C for about 12 hours. The crucible and contents were weighed, then ignited in a furnace at 450°C for 4 hours, cooled and reweighed. The loss in weight was attributed to the crude lignin.

2.10.4.1. Calculation

$$\text{Crude lignin (\% dry wt)} = \frac{\text{loss in wt (g)}}{\text{wt of sample (g)}} \times 100$$

2.10.5. SPECTROPHOTOMETRIC ESTIMATION OF CARBOHYDRATES IN SOLUTIONS.

Each of the solutions will contain carbohydrate derived either by solution (A) or by hydrolysis and solution (B and C). The carbohydrate was estimated spectrophotometrically with anthrone-sulphuric acid reagent, each extract being

treated identically.

Apparatus.

1. Burrette (50.0cm^3).
2. Measuring cylinder (1000.0cm^3).
3. Pipettes (5.0cm^3, 10.0cm^3).
4. Volumetric flasks (100.0cm^3, 1000.0cm^3).
5. Test-tubes.
6. Rubber bungs.
7. Water bath-B & T, A Searle Company, England.
8. Spectrophotometer - Beckman DB-GT Grating spectrophotometer.

Reagents.

1 Anthrone - sulphuric acid: This was prepared by adding 760.0cm^3 of concentrated sulphuric acid (sp. gr. 1.84) to 330.0cm^3 of distilled water. 1.0g of thiourea ($CS(NH_2)_2$) and 1.0g of anthrone ($C_{14}H_{10}O$) were then dissolved in the solution. The solution was allowed to cool and then made up to 1000.0cm^3 with concentrated sulphuric acid.

2. Stock carbohydrate solution: This was prepared by dissolving 0.1g of glucose in 1000.0cm^3 of distilled

water. This solution contained 100.0 ppm of glucose

3. Working standard solutions: 10.0, 20.0, 40.0, 60.0 and 80.0cm^3 of the stock solution were pipetted into separate 100.0cm^3 volumetric flasks and made up to volume with distilled water. These gave solutions containing 10.0, 20.0, 40.0, 60.0 and 80.0 ppm of glucose respectively.

Method.

2.0cm^3 of the solutions A, B and C were pipetted into separate test-tubes and 10.0cm^3 of anthrone-sulphuric acid reagent added to each from a burrette. Duplciate blanks were prepared using 2.0cm^3 of distilled water and 10.0cm^3 of the reagent. Duplicate standards were also prepared using 2.0cm^3 of each working standard solution and 10.0cm^3 of the reagent. The tubes were then shaken, stoppered lightly with rubber bungs and placed in a water bath at 100^{o}C for 20 minutes, after which they were removed and cooled in running water. The colour developed was measured with a spectrophotometer at 625nm using the blank to set zero. A calibration curve was plotted from the absorbances of the standard solutions. The absorbances of the sample solutions

were also measured after making an eleven times dilution for solutions A and B and no dilution for solution C. The concentrations of carbohydrate in solutions A and B were read off from the calibration curve (Appendix II) while that for solution C was obtained from the calculation below. Each determination was carried out in duplicate.

2.10.5.1. Calculation. For solution C. (Shirlaw, 1967).

Let reading on the spectrophotometer for solution C be X_c, and for 100ppm X_s.

Concentration of cellulose in solution C

$$= \frac{X_c \times 100}{\text{reading for standard}} \text{ ppm or mg per litre.}$$

Since there were $500.0cm^3$ of this solution there were

$$\frac{X_c \times 100 \times 500}{1000 \times \text{reading for standard}} \text{ mg of cellulose.}$$

$$= \frac{X_c \times 50}{\text{reading for standard}} \text{ mg of cellulose.}$$

and this was from 0.2g of original sample. Therefore in 100.0g of sample there would be

$$\frac{X_c \times 50}{\text{reading for standard}} \times \frac{100,000}{200} \text{mg of cellulose.}$$

Thus, percentage cellulose in dry matter

$$= \frac{X_c \times 50}{\text{reading for standard}} \times \frac{100,000}{200 \times 1000}$$

$$= \frac{25\ X_c}{\text{reading for standard}}$$

However, to allow for the molecule of water loss in the condensation of any two molecules of monosaccharides, this figure is multiplied by 0.9. Thus:

$$\text{Cellulose (\% dry matter)} = \frac{22.5\ X_c}{\text{reading for standard.}}$$

$$= \frac{22.5\ X_c}{X_s}$$

2.10.6. Determination of Fructosan.

The method adopted was that of Shirlaw (1967).

Apparatus.

1. Volumetric flasks ($100.0cm^3$, $1000.0cm^3$).
2. Test-tubes.
3. Rubber bungs.
4. Water bath - B & T, A Searle Company, England.
5. Spectrophotometer - Beckman DB-GT Grating spectrophotometer.
6. Pipette ($2.0cm^3$).
7. Burette ($50.0cm^3$).

Reagent.

1. Resorcinol (0.1% w/v). This was prepared by dissolving 0.1g resorcinol ($C_6H_4(OH)_2$) in 100.0cm^3 of ethanol. (C_2H_5OH).
2. Ferric chloride solution. Prepared by dissolving 7.5mg of ferric chloride ($FeCl_3$) in 100.0cm^3 of concentrated hydrochloric acid.
3. Standard fructosan solution. Prepared by dissolving 0.1g of inulin in 100.0cm^3 of warm water and making up to 1000.0cm^3 in a volumetric flask. This gave a solution containing 100.0 ppm of inulin.

Method.

2.0cm^3 of the diluted extract from section 2.10.1. (Solution A) were pipetted into a test-tube, followed by 2.0cm^3 of resorcinol reagent and 5.0cm^3 of hydrochloric acid - ferric chloride reagent. Standards and blanks were prepared in the same way using 2.0cm^3 of standard fructosan and 2.0cm^3 of distilled water respectively. The tubes were then shaken, lightly stoppered, and placed in a water bath at 80^{o}C for 8 minutes, after which they were removed, cooled and the colour developed read on

a spectrophotometer at a wavelength of 540nm. Zero was set with the blank. Each determination was carried out in duplicate.

2.10.6.1. Calculation.

The steps of the calculation are practically the same as those for cellulose in section 2.10.5.1.

Let the readings on the spectrophotometer be X_a for solution A and X_s for the standard.

Concentration of fructosan in solution A

$$= \frac{X_a \times 100}{X_s} \text{ ppm or mg per litre.}$$

Since there were $50.0cm^3$ of this solution there were

$$\frac{X_a \times 100 \times 50}{X_s \times 1000} \text{ mg of fructosan.}$$

$$= \frac{X_a \times 5}{X_s} \text{ mg of fructosan}$$

This was in $5.0cm^3$ of original extract.

Therefore in $20.0cm^3$ of original extract there would be

$$\frac{X_a \times 5}{X_s} \times \frac{20}{5} \text{ mg fructosan.}$$

This was from 0.2g of original sample.

Therefore in 100.0g of sample there would be

$$\frac{X_a \times 20}{X_s} \times \frac{100,000}{200} \text{ mg fructosan.}$$

$$= \frac{X_a \times 10,000}{X_s} \text{ mg fructosan.}$$

Thus percentage fructosan in dry matter

$$\frac{X_a \times 10,000}{X_s} = \frac{10X_a}{X_s}$$

2.10.7. Determination of fructose, Pentoses and other sugars.

The soluble carbohydrates obtained in section 2.10.1 (solution A) were further fractioned into fructose, pentoses other sugars using the method described by Shirlaw (1967).

Apparatus.

1. Volumetric flasks (100.0cm^3).
2. Pipettes (1.0cm^3, 5.0cm^3, 10.0cm^3).
3. Burette (50.0cm^3).
4. Water bath - B & T, A Searle Company, England.
5. Test-tubes.
6. Spectrophotometer-Beckman DB-GT Grating spectrophotometer.

Reagents.

1. Phenol (80% w/v). This was prepared by dissolving 80.0g of phenol (C_6H_5OH) in 20.0cm^3 of distilled water.
2. Concentrated sulphuric acid (sp. gr. 1.84).
3. B-methylumbelliferon (7-Hydroxy-4-methylcoumarin) a 2 per cent solution prepared by dissolving 2.0g of B-methylumbelliferon ($C_{10}H_8O_3$) in 95 per cent ethanol and diluted to 100.0cm^3 with ethanol.
4. Standard solutions: a concentrated standard containing 1000.0ppm of each of the three sugars - glucose, fructose and xylose was prepared by dissolving 1.0g of each in 1000.0cm^3 of distilled water. Serial dilutions were then made to give a range of 10.0-80.0ppm by diluting 1.0cm^3 of the concentrate to 100.0cm^3 with distilled water for each 10.0ppm required.

Method.

1.0cm^3 of the diluted extract from section 2.10.1 (solution A) was pipetted into a test tube followed by 1.0cm^3 of distilled water, 2 drops of phenol reagent and 2 drops of B-methylumbelliferone (fluorescent indicator,

pH range 0.2 - 6.0). 5.0cm^3 of concentrated sulphuric acid was added rapidly from the burette and the tube shaken. The tube was allowed to stand for 20 minutes at room temperature and then for 20 minutes in a water bath at 24^{o}C. A blank was prepared the same way using 2.0cm^3 of distilled water. The optical density was read at 470nm, 485nm and 490nm for fructose, pentoses and other sugars respectively. Each determination was carried out in duplicate. A calibration curve was prepared by plotting optical density readings against concentration for the standards at each wavelength. The concentration of each of the fractions was then read off the graph in terms of parts per million.

2.10.7.1. Calculation (Shirlaw, 1967).

The calculation of each fraction was similar to that depicted for fructosans in section 2.10.6.1 and it could be shown that

$$\left.\begin{matrix}\text{Fructose}\\ \text{Pentoses}\\ \text{Other sugars}\end{matrix}\right\}\text{fraction (\% dry wt)} = \frac{\text{concentration of fraction in ppm dilution}}{10}$$

2.11. AMINO ACID ANALYSIS.

The amino acid analyzer is used for the separation and quantitative analysis of amino acids in a variety of mixtures such mixtures include proteins, polypeptides and physiological fluids among others. Determination of amino acids are required in investigations of the distribution of amino acids and their derivatives in physiological fluids (Stein, 1953; Stein et. al, 1954) and mammaliam tissue (Tallan et. al, 1954) as well as in analysis of foods (Schran et. al. 1953).

Amino acids fall into broad chromatography classifications.

i. Basic: Lysine, Histidine, (Ammonia) and Arginine.

ii. Acidic with Neutral: Aspartic acid, Threonine, Serine, Glutamic acid, Proline, Glycine, Alanine, ½-Cystine, Valine, Methionine, Isoleucine, Leucine, Tyrosine and Phenylalanine. Tryptophan should be listed under the acidic with neutral amino acids but is measured using the short column (basic). The acidic and neutral amino acids migrate more quickly and may be separated in a 60.0cm long x 0.9 cm diameter column while the slow-moving basic group cells make use of a 10.0cm long x 0.9 cm diameter column.

2.11.1. Principle.

The operation princple of the Technicon TSM Sequential Multisample Analyser for Amino Acids is based on elution chromatography from buffered columns of ion-exchange resin. The separated amino acids are reacted with ninhydrin and the absorbance of the resulting fractions measured with a colorimeter. The resultant abosrbances are plotted on a strip of chart recorder. The area under each peak gives an estimate of the quantity of each amino acid present. (Fig A).

Method.

Samples for amino acid analysis were hydrolysed with constant boiling hydrochloric acid in sealed ampoules under nitrogen at 110 $\pm$ 1^{o}C for 24 hours. Hydrolysis was followed by ion-exchange chromatography in the TSM Technicon Autoanalyser (Technicon, Basingstoke, England. Spackman et. al, 1958).

2.12. STATISTICAL ANALYSIS

Analysis of variance was performed on the data for mositure, ash, protein, lipid, mineral and carbohydrate contents of the maize samples on a dry weight basis. (Dixton and Massey, 1957; Fisher and Yates, 1974) (Appendix III).

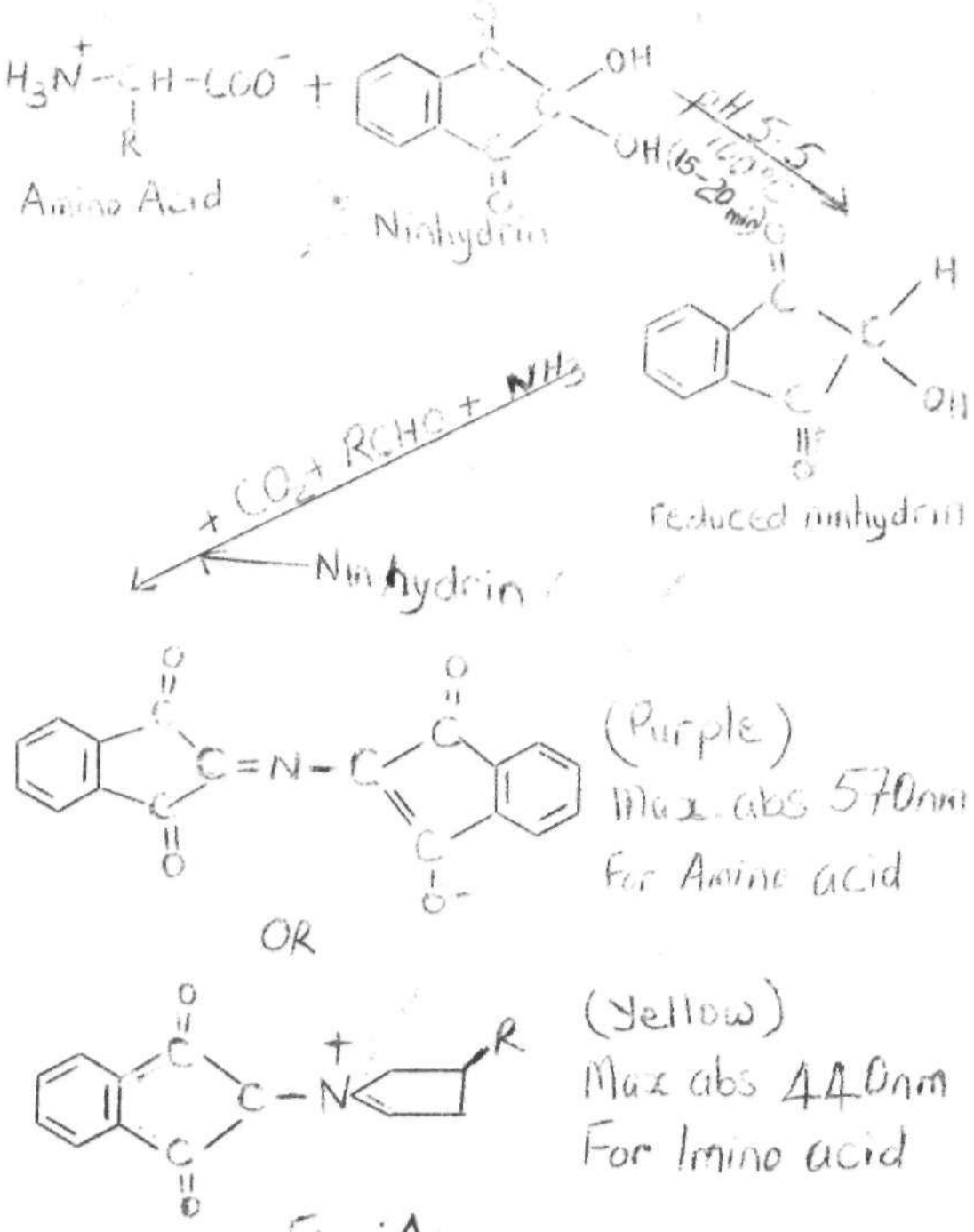


Fig A:
Principle of amino acid determination using the Ninhydrin reagent.

CHAPTER THREE

RESULTS

3.1. MOISTURE CONTENT

The results of the moisture content of the maize samples are shown in Tables 3.1 and 3.2 for the effect of fertilizer treatment and varietal differences respectively. The values range from 6.1 - 7.2% and 6.4 - 8.0% for the effects of fertilizer treatment and varietal differences respectively. Both fertilizer treatment and varietal differences had significant effects on the moisture content of the maize samples.

3.2. ASH CONTENT

The values of the ash content of the maize samples analysed are shown in Tables 3.1 and 3.2 respectively for the effect of fertilizer treatment and varietal differences. The values range from 1.1 - 1.4% and 1.1 - 1.6% for the effects of fertilizer treatment and varietal differences respectively. Fertilizer treatment had a significant effect on the ash content of the maize samples whereas varietal differences had no significant effect.

Table 3.1: MOISTURE, ASH AND LIPID CONTENTS OF MAIZE SAMPLES WITH RESPECT TO FERTILIZER TREATMENT.

SAMPLE	(%) MOISTURE	(%) DRY WEIGHT	
		ASH	LIPID
S_1	6.99 ± 0.02*	1.20 ± 0.01*	6.52 ± 0.63*
S_2	7.16 ± 0.04	1.10 ± 0.01	6.19 ± 0.25
S_3	6.91 ± 0.03	1.12 ± 0.01	6.73 ± 0.61
S_4	7.07 ± 0.08	1.28 ± 0.04	5.98 ± 0.02
S_5	6.93 ± 0.05	1.42 ± 0.04	5.17 ± 0.44
S_6	6.40 ± 0.06	1.19 ± 0.04	4.73 ± 0.11
S_7	6.13 ± 0.03	1.26 ± 0.06	5.22 ± 0.07
S_8	6.69 ± 0.01	1.12 ± 0.03	6.09 ± 0.07
S_9	6.65 ± 0.01	1.34 ± 0.05	7.15 ± 0.30
Mean	6.77	1.22	5.98

*Each result represents the mean of 3 determinations.

± Standard deviation.

TABLE 3.2: MOISTURE, ASH AND LIPID CONTENTS OF MAIZE SAMPLES WITH RESPECT TO VARIETAL DIFFERENCES.

SAMPLE	(%) MOISTURE	(%) DRY WEIGHT	
		ASH	LIPID
S_{10}	6.43 ± 0.04+	1.29 ± 0.03+	5.17 ± 0.78+
S_{11}	7.88 ± 0.07	1.43 ± 0.18	4.62 ± 0.49
S_{12}	7.78 ± 0.09	1.34 ± 0.00	5.10 ± 0.14
S_{13}	7.20 ± 0.19	1.24 ± 0.02	5.18 ± 0.70
S_{14}	6.81 ± 0.05	1.15 ± 0.05	5.28 ± 0.33
Mean	7.22	1.29	5.07

+ Each result represents the mean of 3 determinations.

± Standard deviation.

3.3. LIPID CONTENT

The results of the lipid content of the maize samples are shown in Tables 3.1 and 3.2 for the effects of fertilizer treatment and varietal differences respectively. The values range from 4.6 - 7.4% and 4.1 - 5.6% respectively for the effects of fertilizer treatment and varietal differences. Statistical analyses have shown that fertilizer treatment had a significant effect on the lipid content of the maize

samples whereas varietal differences had no significant effect.

3.4. CRUDE PROTEIN CONTENT

The values of the crude protein content of the maize samples are shown in Tables 3.3 and 3.4 for the effects of fertilizer treatment and varietal differences respectively. The values range from 6.0 - 12.1% for the effect of fertilizer treatment and 8.6 - 10.6% for the effect of varietal differences. Both fertilizer treatment and varietal differences had significant effects on the protein content of the maize samples.

3.5. MINERALS

3.5.1. CALCIUM

The results of the calcium content of the samples analysed are given in Tables 3.5 and 3.6 for the effects of fertilizer treatment and varietal differences respectively. The values range from 1.4 - 2.5mg/100gm for the effect of fertilizer treatment and 1.4 - 2.2mg/100gm for the effect of varietal differences. Both fertilizer treatment and varietal differences had significant effects on the calcium content of the maize samples.

TABLE 3.3. CRUDE PROTEIN CONTENT OF MAIZE SAMPLES WITH RESPECT TO FERTILIZER TREATMENT.

SAMPLE	N P K	(% DRY WEIGHT) PROTEIN
S_1	- - -	7.52 ± 0.20+
S_2	25 - -	6.95 ± 0.34
S_3	50 - -	10.98 ± 0.18
S_4	- 30 -	7.55 ± 0.08
S_5	- 60 -	6.52 ± 0.33
S_6	- - 50	6.82 ± 0.12
S_7	- - 100	6.30 ± 0.34
S_8	25 30 50	10.19 ± 0.22
S_9	50 60 100	11.77 ± 0.37
Mean		8.29

TABLE 3.4. CRUDE PROTEIN CONTENT OF MAIZE SAMPLES WITH RESPECT TO VARIETAL DIFFERENCES

SAMPLE		N P K	(% DRY WEIGHT) PROTEIN
BL	S_{10})	120 60 60	10.52 ± 0.09+
S123	S_{11})		9.30 ± 0.19
096	S_{12})		9.74 ± 0.20
TZB	S_{13})		8.83 ± 0.20
WY	S_{14})		10.00 ± 0.24
Mean			9.68

\+ Each result represents the mean of 6 determinations.

± Standard deviation.

TABLE 3.5: CALCIUM, COPPER, IRON, MANGANESE AND ZINC CONTENTS OF MAIZE SAMPLES WITH RESPECT TO FERTILIZER TREATMENT.

SAMPLE	(mg/100gm) of dry weight.				
	Ca	Cu	Fe	Mn	Zn
S_1	2.25 ± 0.20+	0.17 ± 0.05+	1.50 ± 0.00+	0.41 ± 0.03+	1.64 ± 0.15+
S_2	1.73 ± 0.09	0.10 ± 0.00	1.70 ± 0.14	0.42 ± 0.00	1.63 ± 0.06
S_3	1.60 ± 0.00	0.10 ± 0.00	2.50 ± 0.28	0.58 ± 0.00	1.72 ± 0.12
S_4	1.90 ± 0.08	0.20 ± 0.00	2.85 ± 0.28	0.42 ± 0.00	1.91 ± 0.01
S_5	2.50 ± 0.00	0.20 ± 0.00	1.98 ± 0.14	0.42 ± 0.00	1.93 ± 0.01
S_6	2.00 ± 0.00	0.20 ± 0.00	1.80 ± 0.00	0.36 ± 0.00	2.03 ± 0.05
S_7	2.08 ± 0.12	0.20 ± 0.00	1.80 ± 0.00	0.30 ± 0.05	1.83 ± 0.01
S_8	1.52 ± 0.12	0.10 ± 0.00	2.15 ± 0.00	0.42 ± 0.00	1.42 ± 0.00
S_9	2.00 ± 0.00	0.20 ± 0.00	2.15 ± 0.28	0.70 ± 0.00	1.52 ± 0.08
Mean	1.95	0.16	2.05	0.45	1.74

\+ Each result represents the mean of 3 determinations.

± Standard deviation.

TABLE 3.6: CALCIUM, COPPER, IRON, MANGANESE AND ZINC CONTENTS OF MAIZE SAMPLES WITH RESPECT TO VARIETAL DIFFERENCES

SAMPLE	(mg/100gm) of dry weight.				
	Ca	Cu	Fe	Mn	Zn
S_{10}	1.35 ± 0.00+	0.19 ± 0.07+	2.05 ± 0.41+	0.45 ± 0.04+	1.76 ± 0.20+
S_{11}	2.25 ± 0.00	0.28 ± 0.00	3.43 ± 0.16	0.36 ± 0.16	1.35 ± 0.00
S_{12}	2.00 ± 0.00	0.22 ± 0.04	3.78 ± 0.16	0.36 ± 0.00	1.39 ± 0.01
S_{13}	1.70 ± 0.08	0.13 ± 0.05	2.15 ± 0.00	0.58 ± 0.00	2.12 ± 0.00
S_{14}	1.60 ± 0.00	0.22 ± 0.04	2.50 ± 0.00	0.42 ± 0.00	1.62 ± 0.00
Mean	1.78	0.21	2.78	0.43	1.41

+ Each result represents the mean of 3 determinations.

± Standard deviation.

3.5.2. COPPER

The values of the copper content of the maize samples are given in Tables 3.5 and 3.6 for the effects of fertilizer treatment and varietal differences respectively. The values range from 0.1 - 0.2mg/100gm and 0.09 - 0.30mg/100gm respectively for the effects of fertilizer treatment and varietal differences. Fertilizer treatment had a significant effect on the copper content while varietal differences had none.

3.5.3. IRON

The results of the iron content of the maize samples analysed are given in Tables 3.5 and 3.6 respectively for the effects of fertilizer treatment and varietal differences. The values range from 1.5 - 3.1mg/100gm for the effect of fertilizer treatment and 2.1 - 3.9mg/100gm for the effect of varietal differences. Both fertilizer treatment and varietal differences had significant effects on the iron content of the maize samples.

3.5.4. MANGANESE

The values of the manganese content of the maize samples are given in Tables 3.5 and 3.6 for the effects of fertilizer treatment and varietal differences respectively. The values range from 0.25 - 0.70mg/100gm for the effect of fertilizer treatment and 0.36 - 0.58mg/100gm for the effect of varietal differences. Both fertilizer treatment and varietal differences had significant effects on the manganese content of the maize samples.

3.5.5. ZINC

The results of the zinc content of the analysed maize samples are shown in Tables 3.5 and 3.6 for the effects of fertilizer treatment and varietal differences respectively.

The values range from 1.4 - 2.1mg/100gm for both the effects on fertilizer treatment and varietal differences. Both fertilizer treatment and varietal differences had significant effects on the zinc content of the maize samples.

3.5.6. SODIUM

The values of the sodium content of the analysed samples are shown in Tables 3.7 and 3.8 for the effects of fertilizer treatment and varietal differences respectively. The values range from 10.0 - 59.5mg/100gm and 10.0 - 19.7mg/100gm respectively for the effects of fertilizer treatment and varietal differences. Both fertilizer treatment and varietal differences had significant effects on the sodium content of the maize samples.

3.5.7. PHOSPHORUS

The values of the phosphorus content of the maize samples are given in Tables 3.7 and 3.8 respectively for the effects of fertilizer treatment and varietal differences. The values range from 159.7 - 315.3mg/100gm for the former and 154.6 - 290.3mg/100gm for the later. Both fertilizer treatment and varietal differences had significant effects on the phosphorus content of the maize samples.

TABLE 3.7. SODIUM, PHOSPHORUS, MAGNESIUM AND POTASSIUM CONTENTS OF MAIZE SAMPLE WITH RESPECT TO FERTILIZER TREATMENT.

SAMPLE	(mg/100gm) of dry weight.			
	Na	P	Mg	K
S_1	57.50±2.00*	241.70±31.20*	92.50±12.42	325.00±0.00*
S_2	38.30±2.40	216.70±23.60	95.00±0.00	307.33±6.13
S_3	42.50±3.50	225.00±20.40	104.17±5.89	293.33±11.78
S_4	36.20±1.00	200.00±0.00	98.33±2.36	346.67±6.24
S_5	24.20±2.40	291.70±11.80	108.33±5.89	403.33±2.36
S_6	21.20±1.00	216.70±23.60	95.00±0.00	340.00±0.00
S_7	23.30±1.20	291.70±23.60	107.00±8.48	365.00±0.00
S_8	10.00±0.00	183.30±23.60	95.00±0.00	310.00±4.08
S_9	12.50±0.00	233.30±23.60	108.33±5.89	278.33±4.71
Mean	29.52	225.34	100.41	329.89

*Each result represents the mean of 3 determinations.

± Standard deviation.

TABLE 3.8. SODIUM, PHOSPHORUS, MAGNESIUM AND POTASSIUM CONTENTS OF MAIZE SAMPLES WITH RESPECT TO VARIETAL DIFFERENCES.

SAMPLE	(mg/100gm) of dry weight			
	Na	P	Mg	K
S_{10}	10.00±0.00*	175.00±20.40*	102.50±7.36*	276.67±4.71*
S_{11}	10.80±1.20	233.30±23.60	104.17±5.89	311.67±2.36
S_{12}	18.70±1.00	250.00±0.00	100.00±0.00	288.33±4.71
S_{13}	13.30±3.10	266.70±23.60	112.50±0.00	311.67±2.36
S_{14}	10.00±0.00	216.70±11.80	95.00±0.00	286.67±2.36
Mean	12.56	228.34	102.83	295.00

* Each result represents the mean of 3 determinations.

± Standard deviation.

3.5.8. MAGNESIUM

The results of the magnesium content of the samples are given in Tables 3.7 and 3.8 respectively for the effects of fertilizer treatment and varietal differences. The values range from 80.09 - 114.22mg/100gm for the former and 95.00 - 112.50mg/100gm for the later. Fertilizer treatment had no significant effect on the magnesium content of the maize samples. Varietal differences had a significant effect at

5% level but no significant effect at 1% level.

3.5.9. POTASSIUM

The values of the potassium content of the maize samples are given in Tables 3.7 and 3.8 respectively for the effects of fertilizer treatment and varietal differences. The values range from 273.6 - 405.7mg/100gm for the former and 272.0 - 314.0mg/100gm for the later. Fetilizer treatment had a significant effect on the potassium content of the maize samples while varietal differences had no significant effect.

3.6. CARBOHYDRATES

3.6.1. SOLUBLE CARBOHYDRATES

The results of the soluble carbohydrates content of the maize samples are shown in Tables 3.9 and 3.10 for the effects of fertilizer treatment and varietal differences respectively. The values range from 22.8 - 49.4% for the former and 25.9 - 48.9% for the later. Fertilizer treatment had a significant effect on the soluble carbohydrate content of the maize samples. Varietal differences also had a significant effect on the soluble carbohydrate content of the maize samples.

TABLE 3.9. SOLUBLE CARBOHYDRATE, HEMICELLULOSE, CELLULOSE, LIGNIN AND TOTAL CARBOHYDRATE CONTENTS OF MAIZE SAMPLES WITH RESPECT TO FERTILIZER TREATMENT.

SAMPLE	(%) DRY WEIGHT				
	SOLUBLE CARBOHYDRATES	HEMICELLULOSE	CELLULOSE	LIGNIN	TOTAL CARBOHYDRATE
S_1	33.28±2.78[X]	31.76±3.47[X]	10.00±0.39[•]	1.90±0.07[•]	76.94
S_2	31.99±5.01	34.92±4.02	10.42±0.34	1.97±0.10	79.30
S_3	29.33±1.48	31.93±2.91	11.85±0.26	2.18±0.44	75.29
S_4	30.80±3.39	33.46±0.60	11.94±0.60	1.10±0.08	77.30
S_5	30.43±2.02	33.88±3.94	12.17±0.32	1.58±0.12	78.06
S_6	26.17±3.32	36.21±2.88	7.22±0.11	1.12±0.17	70.72
S_7	36.08±1.84	37.77±2.19	8.18±0.64	1.55±0.50	83.58
S_8	41.87±3.97	34.54±0.86	8.44±0.34	1.20±0.07	86.05
S_9	46.75±2.64	35.87±1.16	6.71±0.74	1.33±0.19	90.66
Mean	34.08	34.48	9.66	1.55	79.77

[X] Each result represents the mean of 6 determinations.

[•] Each result represents the mean of 3 determinations.

± Standard deviation.

TABLE 3.10. SOLUBLE CARBOHYDRATE, HEMICELLULOSE, CELLULOSE, LIGNIN AND TOTAL CARBOHYDRATE CONTENTS OF MAIZE SAMPLES WITH RESPECT TO VARIETAL DIFFERENCES.

SAMPLE	(%) DRY WEIGHT				
	SOLUBLE CARBOHYDRATE	HEMICELLULOSE	CELLULOSE	LIGNIN	TOTAL CARBOHYDRATE
S_{10}	45.38±3.53+	35.02±1.87+	7.96±0.48*	1.23±0.21*	89.59
S_{11}	32.13±4.85	38.06±2.25	5.57±0.32	1.33±0.34	77.09
S_{12}	30.80±4.92	41.80±2.20	5.53±0.32	1.38±0.02	79.51
S_{13}	31.90±3.54	41.98±2.41	6.33±0.16	1.31±0.03	81.52
S_{14}	34.88±3.90	40.27±2.74	5.52±0.10	1.27±0.12	81.89
Mean	35.01	39.43	6.18	1.30	81.92

+ Each result is the mean of 6 determinations.

* Each result is the mean of 3 determinations.

± Standard deviation.

3.6.2. HEMICELLULOSE

The values of the hemicellulose content of the analysed maize samples are given in Tables 3.9 and 3.10 for the effects of fertilizer treatment and varietal differences respectively The values range from 28.3 - 40.0% for the former and 33.1 - 44.4% for the later. Both fertilizer treatment and varietal

differences had significant effects on the hemicellulose contents of the maize samples.

3.6.3. CELLULOSE

The results of the cellulose content of the maize samples are given in Tables 3.9 and 3.10 respectively for the effects of fertilizer treatment and varietal differences. The values range from 6.0 - 12.5% for the former and 5.2 - 8.4% for the later. Both fertilizer treatment and varietal differences had significant effects on the cellulose content of the maize samples.

3.6.4. LIGNIN

The values of the lignin content of the analysed maize samples are shown in Tables 3.9 and 3.10 for the effects of fertilizer treatment and varietal differences respectively. The values range from 1.0 - 2.6% for the former and 1.0 - 1.4% for the later. Fertilizer treatment had a significant effect on the lignin content while varietal differences had no significant effect.

3.6.5. FRUCTOSAN

The results of the fructosan content of the maize samples are given in Tables 3.11 and 3.12 respectively for the effects of fertilizer treatment and varietal differences

The values range from 0.60 - 1.56% for the former and 0.76 - 1.53% for the later. Both fertilizer treatment and varietal differences had significant effects on the fructosan content on the maize samples.

TABLE 3.11. FRUCTOSAN, FRUCTOSE, PENTOSE AND "OTHER SUGARS" CONTENTS OF MAIZE SAMPLES WITH RESPECT TO FERTILIZER TREATMENT.

SAMPLE	(%) DRY WEIGHT			
	FRUCTOSAN	FRUCTOSE	PENTOSE	OTHER SUGARS
S_1	0.84 ± 0.06^X	12.80 ± 0.56^X	7.50 ± 0.41^X	9.07 ± 0.43^X
S_2	0.61 ± 0.01	13.77 ± 1.33	6.77 ± 0.41	8.43 ± 0.77
S_3	0.97 ± 0.07	11.53 ± 0.41	6.33 ± 0.24	7.27 ± 0.09
S_4	1.18 ± 0.19	12.20 ± 0.20	7.37 ± 0.45	7.63 ± 0.29
S_5	1.23 ± 0.02	11.40 ± 0.31	6.40 ± 0.45	7.17 ± 0.03
S_6	1.54 ± 0.02	10.63 ± 0.57	10.33 ± 0.41	17.00 ± 0.43
S_7	1.00 ± 0.08	10.00 ± 0.89	7.87 ± 0.38	14.83 ± 0.14
S_8	1.07 ± 0.09	9.63 ± 0.50	9.30 ± 0.49	14.37 ± 0.50
S_9	0.84 ± 0.07	9.13 ± 0.27	8.37 ± 0.07	11.53 ± 0.74
Mean	1.03	11.23	7.80	10.81

X Each result represents the mean of 6 determinations.

$\pm$ Standard deviation.

TABLE 3.12. FRUCTOSAN, FRUCTOSE, PENTOSE AND "OTHER SUGARS" CONTENTS OF MAIZE SAMPLES WITH RESPECT TO VARIETAL DIFFERENCES.

SAMPLE	(%) DRY WEIGHT			
	FRUCTOSANS	FRUCTOSE	PENTOSES	OTHER SUGARS
S_{10}	0.84 ± 0.08^{X}	6.13 ± 0.88^{X}	5.40 ± 0.16^{X}	8.47 ± 0.39^{X}
S_{11}	1.52 ± 0.07	7.87 ± 0.48	6.40 ± 0.20	8.23 ± 0.59
S_{12}	1.44 ± 0.19	9.07 ± 0.75	8.27 ± 0.09	8.27 ± 0.36
S_{13}	1.34 ± 0.20	8.93 ± 0.44	6.23 ± 0.21	7.73 ± 0.63
S_{14}	1.44 ± 0.25	9.90 ± 0.36	6.83 ± 0.53	7.80 ± 0.45
Mean	1.32	8.38	6.63	8.1

^{X} Each result represents the mean of 6 determinations.

$\pm$ Standard deviation.

3.6.6. FRUCTOSE

The values of the fructose content of the maize samples are given in Tables 3.11 and 3.12 respectively for the effects of fertilizer treatment and varietal differences. The values range from 8.86 - 13.36% for the former and 5.25 - 10.26% for the later. Both fertilizer treatment and varietal differences had significant effects on the fructose content of the maize samples.

3.6.7. PENTOSES

The values of the pentose content of the analysed maize samples are shown in Tables 3.11 and 3.12 for the effects of fertilizer treatment and varietal differences respectively. The values range from 5.95 - 10.74% and 5.24 - 8.36% respectively for the former and the later. Both fertilizer treatment and varietal differences had significant effects on the pentose content of the maize samples.

3.6.8. OTHER SUGARS

The values obtained for "other sugars" are shown in Tables 3.11 and 3.12 for the effects of fertilizer treatment and varietal differences respectively. The values range from 6.84 - 17.43% for the former and later respectively. Both fertilizer treatment and varietal differences had significant effects on the "other sugars" of the maize samples.

3.7. AMINO ACIDS

The amino acid profiles for the maize samples are shown in Tables 3.13 and 3.14 respectively for the effects of fertilizer treatment and varietal differences.

TABLE 3.13: AMINO ACID PROFILES OF THE MAIZE SAMPLES WITH RESPECT TO FERTILIZER TREATMENT.

AMINO ACID	gm a.a/16gm Nitrogen									
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	C*
Trypto-+ phan	-	-	-	-	-	-	-	-	-	-
Lysine+	2.5	3.9	2.2	2.1	3.3	3.9	3.4	3.1	2.9	17.2
Histi-dine	15.9	11.5	7.1	-	8.2	14.3	7.9	6.4	8.3	6.2
Arginine	9.6	6.6	5.3	3.7	-	6.6	7.4	5.9	6.8	8.0
Aspartic acid	17.9	17.5	16.3	15.5	17.7	18.9	22.3	13.8	18.2	15.1
Threonine	11.2	10.3	8.7	8.0	10.1	11.5	18.5	8.8	10.0	9.9
Serine	13.6	11.1	9.2	12.5	11.2	15.7	15.2	9.5	11.5	12.8
Glutamic acid	43.0	47.1	43.5	41.0	43.0	52.6	55.0	43.3	40.6	46.8
Proline	25.9	23.1	25.6	22.1	25.9	26.7	21.9	19.2	20.4	24.6
Glycine	11.9	8.7	8.0	8.5	11.0	12.6	11.9	9.4	9.3	4.2
Alanine	20.6	16.3	15.4	17.3	16.8	19.7	18.3	16.9	16.4	6.5
Cystine ($\frac{1}{2}$)	2.5	-	-	-	-	-	-	1.1	-	-
Valine	16.9	16.0	11.7	12.4	18.2	18.4	14.6	11.7	11.6	14.3
Methio-nine+	7.8	3.2	3.4	4.1	6.5	6.4	5.8	5.2	3.8	5.9
Isoleu-cine+	14.3	10.6	10.6	8.2	6.9	9.8	12.4	10.0	14.0	11.5
Leucine+	32.7	28.8	38.0	27.5	25.0	29.4	32.5	29.4	31.9	20.2
Tyrosine	7.0	8.2	11.8	10.9	12.6	11.5	15.0	8.7	10.6	12.4
Phenyla-lanine+	3.7	4.3	2.8	3.2	2.3	4.1	4.4	6.7	4.5	11.1

\- Not determined.

C* Casein. See FAO (1970).

\+ Essential amino acids.

TABLE 3.14 AMINO ACID PROFILES OF THE MAIZE SAMPLES WITH RESPECT TO VARIETAL DIFFERENCES.

AMINO ACID	gm a.a/16gm Nitrogen					
	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	C*
Tryptophan+	-	-	-	-	-	-
Lysine+	2.7	3.9	2.6	3.7	3.8	17.2
Histidine	-	6.7	6.2	8.0	8.8	6.2
Arginine	-	5.2	6.5	8.4	9.1	8.0
Aspartic acid	19.1	14.8	11.6	15.3	18.5	15.1
Threonine+	11.7	7.4	7.8	8.8	9.3	9.9
Serine	15.1	10.2	13.3	8.8	11.3	12.8
Glutamic acid	40.3	42.3	41.7	36.7	36.4	46.8
Proline	19.9	17.8	21.4	24.4	23.5	24.6
Glycine	10.6	8.1	10.0	8.7	8.8	4.2
Alanine	17.0	16.2	19.0	12.9	17.5	6.5
Cystine (½)	-	-	-	-	-	-
Valine+	10.9	11.6	12.8	10.6	11.6	14.3
Methionine+	5.5	4.9	7.5	4.2	5.7	5.9
Isoleucine+	13.1	11.4	11.6	8.2	10.5	11.5
Leucine+	27.3	31.4	32.1	21.5	32.3	20.2
Tyrosine	9.3	8.4	13.0	7.9	9.6	12.4
Phenylalanine+	5.8	7.1	7.6	6.0	6.3	11.1

- Not determined.

C* Casein. See FAO (1970).

+ Essential amino acids.

TABLE 3.15. LEUCINE/LYSINE RATIOS OF THE MAIZE SAMPLES WITH RESPECT TO FERTILIZER TREATMENT.

SAMPLE	N	P	K	LEUCINE/LYSINE RATIO
S_1	-	-	-	13.08
S_2	25	-	-	7.38
S_3	50	-	-	17.27
S_4	-	30	-	13.10
S_5	-	60	-	7.58
S_6	-	-	50	7.54
S_7	-	-	100	9.56
S_8	25	30	50	9.48
S_9	50	60	100	11.00

TABLE 3.16. LEUCINE/LYSINE RATIOS OF THE MAIZE SAMPLES WITH RESPECT TO VARIETAL DIFFERENCES.

SAMPLE		N	P	K	LEUCINE/LYSINE RATIO
BL	S_{10})	120	60	60	10.33
S123	S_{11})				8.05
096	S_{12})				12.35
TZB	S_{13})				5.81
WY	S_{14})				8.28

CHAPTER FOUR

DISCUSSION

The use of fertilizers on crops affects the yield and composition of grains. This has been the finding of many workers (McVicker, 1963; Kent, 1970; Singh and Majhi, 1975). Apart from fertilizer treatment, varietal differences also have effect on the nutrients of plants. (Cox, 1950; McCay et. al, 1975).

Five varieties of maize were used for this study. These were, TZB, S 123, 096, Bomo Local and Western Yellow These were chosen because they are the varieties most commonly grown around Samaru, Zaria. TZB was used for the studies of the effect of fertilizer treatment on the chemical composition of maize. This variety was chosen because it is the variety being distributed to farmers presently at IAR. This variety is high yielding and responds well to fertilizer (Ologunde, personal communication).

4.1.. MOISTURE.

The values obtained for the effects of fertilizer treatment and varietal differences on the chemical composition of the maize samples are shown in Tables 3.1 and 3.2 respectively. These values, which fall within the range of

moisture content for cereals, 5 - 15% (Bingham, 1978) are lower than those reported for maize by other workers: 14.4% (Schrimpf, 1966), 10.8% (Kent-Jones and Amos, 1967), 9.6% (Oyenuga, 1968), 8.1% (Inglett, 1970), 10.0% (Jugenheimer, 1976) and 10.8% (Olatunji et. al, 1980). The low values obtained in this work could be attributed to the fact that the moisture content determination was done during the harmattan, (January) when the air is normally very dry. Duckworth (1966), reported that both climate and soil can affect the moisture content of materials. Both fertilizer treatment and varietal differences had significant effects on the moisture content of the maize samples. Sample S_2 (25 kgN/ha) had the highest moisture content while sample S_7 (100kg K/ha) had the lowest. McVickar (1963) reported that well-fertilized crops use water more efficiently than poor-fertilized crops. Why this is so is not known but it is reasoned that crops from well-fertilized soils have a larger root system and a greater root area, which no doubt contribute to the more efficient utilization of moisture. It is therefore thought that the moisture content of well-fertilized crops should be lower

since much of the moisture would be utilised in anabolic activities. The effect of varietal differences on moisture content is significant. This may be expected. Since the varieties were subjected to the same fertilizer treatment, the difference then in their moisture content could be due to their different ability to absorb water from the soil. According to Cox (1950) and McCay et. al (1975), varietal differences affect the nutrient content of plants. Sample S_{11} (S 123) had the highest moisture content while sample S_{10} (Bomo Local) had the lowest. Bomo Local will therefore be a better variety for the dry milling industries.

4.2. ASH

This gives an insight to the mineral content of the maize. The results obtained (Tables 3.1 and 3.2) are close to those obtained by some workers 1.23% (Schrimpf, 1966), 1.50% (Kent-Jones and Amos, 1967), 1.40% (Olatunji et. al, 1980). Some workers have reported higher values; 3.13% (Oyenuga, 1968) and 2.0% (Jugenheimer, 1976). Sample S_5 (60kg P/ha) had the highest value of 1.42% for the effect of fertilizer treatment on the ash content while sample S_2 (25kg N/ha) had the lowest value of 1.10%.

(Table 3.1). The reason for this could be as proposed by McVickar (1963). He reported that increase in yield that follows the use of nitrogen fertilizers may actually result in a decrease in the mineral content of the plant. For the effect of varietal differences, sample S_{11} (S 123) had the highest value (1.43%) while sample S_{14} (Western Yellow) had the lowest (1.15%). There was a significant effect of fertilizer treatment on the ash content of the maize whereas varietal differences had no significant effect. The reason for the significant effect of fertilizer treatment can be traced back to source. The ash (mineral) content are the chemical elements of the grain which enter the plant-food train from the soil (Nelson, 1951). It therefore follows that a plant from a soil deficient in chemical substances will have a decreased ash content. For the effect of varietal differences there was no significant effect. This is similar to the findings of Olatunji et. al, (1977) who worked on both the white and yellow farmers' varieties of maize.

4.3. LIPIDS

The results obtained are as shown in Tables 3.1 and 3.2.

The values obtained are slightly higher than those obtained by other workers: 4.80% (Schrimpf, 1966), 4.09% (Oyenuga, 1968), 4.50% (Jugenheimer, 1976) and 4.40% (Olatunji et. al, 1980). From Table 3.1, it is seen that sample S_9 (50,60 and 100kg N, P and K/ha respectively) had the highest lipid content while sample S_6 (50kg P/ha) had the lowest value. There is actually no particular trend in the lipid content of the different samples due to fertilizer application. Fertilizer treatment, had a significant effect on the lipid content of the maize samples. This is in agreement with results by workers who reported that the quality of crops are often improved by addition of fertilizers (Kent, 1970; Singh and Majhi, 1975). Under some conditions, the additional plant-food elements (in form of chemical fertilizers) affect the fat content of crops (McVickar, 1963). For the effect of varietal differences, sample S_{14} (Western Yellow) gave the highest value of 5.28% while sample S_{11} (S 123) gave the lowest value of 4.62% (Table 3.2). Varietal differences had no significant effect on the lipid content of the maize samples. This is in agreement with the results obtained by Olatunji et. al, (1977) for both the

white and yellow farmers' varieties of maize.

4.4. PROTEIN

The results obtained (Tables 3.3 and 3.4) are comparable to those obtained by other workers: 9.70% (Schrimpf, 1966), 10.0% (Kent-Jones and Amos, 1967; Jugenheimer, 1976), 10.65% (Oyenuga, 1968), 8.90% (Inglett, 1970) and 9.7% (N x 5.7, Olatunji, 1980). Both fertilizer treatment and varietal differences had significant effects on the protein content of maize. It has been reported, (McVickar, 1963) that an application of nitrogen generally results in an increased uptake of the element and a corresponding increase in the protein content of the crop. Duckworth, (1966) also reported that the protein content of a plant is dependent on the fertilizer applied. Also, CIMMYT, (1974) reported that traditional maize, whether grown in the tropics or in the temperature climates, contains 9 - 11% protein in the whole grain. The results obtained in this work thus confirm these reports. From Table 3.3, it is seen that, the three samples that contain nitrogen, S_3 (50kg N/ha), S_8 (25, 30 and 50kg N, P and K/ha respectively) and S_9 (50, 60 and 100kg N, P and K/ha respectively) had the

highest protein values of 10.98%, 10.19% and 11.77% respectively. Sample S_2 (25kg N/ha) though containing nitrogen, had a very low protein content (6.95%), while sample S_8 having the same amount of nitrogen had a high value. This high value may have been obtained due to the presence of the other fertilizers applied. Also, sample S_9 which had the same amount of nitrogen as sample S_3 had a high protein value. The presence of other fertilizers might have caused this increase too. These go to show that, the application of fertilizers in combination aid in the nutritive quality of crops since plants need all the primary plant food (nitrogen, phosphorus and potassium) for normal growth (McVickar, 1963). Also, in potassium deficient soils, full benefit of nitrogen and phosphorus fertilizers will be realised only when potassium is also applied (Balasubramanian et. al, 1978). Table 3.4 shows the protein content of the different varieties. Sample S_{10} (Bomo Local) had the highest value of 10.52% while sample S_{13} (TZB) had the lowest value of 8.83%. This sample S_{13} (TZB) was the same variety used for the studies of the effect of fertilizer treatment. Sample S_9 (TZB, 50, 60 and 100kg N, P and K/ha respectively) had a protein content of 11.77%

while sample S_{13} (TZB, 120, 60 and 60kg N, P and K/ha respectively) had a value of 8.83%. With the high nitrogen application on sample S_{13}, what then could account for the very low protein content? Apart from nitrogen, another important environmental factor that affects the protein content of a particular hybrid of maize is the thickness of stand (Leonard and Martin, 1963). It is therefore reasoned that the low protein content of sample S_{13} could have been due to growing a high-yielding hybrid at a thick rate of planting. Fertilizer treatment had a significant effect on the protein content of maize. This confirms the reports of workers like Hera (1971) and Mengel (1971) who said that fertilizer treatment, especially the use of nitrogen fertilizers affect the protein content of cereals. On the effect of varietal differences, the results obtained compare well to those obtained by other workers. Olatunji et. al, (1977) worked on both the white and yellow farmers' varieties, and their results show that varietal differences had significant effect on the protein content of maize. Taylor et. al, (1979) worked on the protein content of varieties 096 and S 123. The values of 9.74% and 9.3%

which were obtained for samples S_{12} (096) and S_{11} (S 123) respectively, in this work correspond closely to the mean values of 9.7% and 9.0% for 096 and S 123 respectively obtained by Taylor et. al, (1979).

4.5. MINERAL ELEMENTS

Duckworth (1966) reported that there are considerable differences in the mineral content between species and varieties of plants and also between different batches of the same variety grown under different environmental conditions. These different conditions include climate, soil and fertilizer treatment.

4.5.1. CALCIUM.

The values obtained, (Tables 3.5 and 3.6), fall within the range of 0.00 - 0.45% given by Miller (1958). Table 3.5 shows sample S_5 (60kg P/ha) to have the highest value of 2.5mg/100gm while sample S_8 (25, 30 and 50kg N, P and K/ha respectively) had the lowest value of 1.52mg/100gm. Sample S_8 was one of the samples with applied nitrogen fertilizer. McVickar (1963) reported that the increase in yield that follows nitrogen fertilization may result in a decrease in the mineral content of plant,

particularly calcium and phosphorus. McVickar (1963) also reported that potassium or magnesium applications may result in a reduction in the calcium content of crops. It was observed that the results obtained in this work for the effect of fertilizer treatment on the calcium content of maize confirms the reports of McVickar (1963). For the effect of varietal differences, sample S_{11} (S 123) had the highest value of 2.25mg/100gm while sample S_{10} (Bomo Local) had the lowest value of 1.35mg/100gm. Varietal differences had a significant effect on the calcium content of maize. This is in agreement with the report of Sauchelli (1965) who said that varietal differences within plant species influence the uptake of nutrients. Howe and Gilfillan (1970), reported that maize has an especially low amount of calcium.

4.5.2. COPPER.

The results obtained (Tables 3.5 and 3.6) are comparable to the values of 0.34mg/100gm and 4.49ppm given by NRC (1968) and Oyenuga (1968) respectively. Highest value of 0.2mg/100gm was obtained for samples S_4 (30kg P/ha), S_5 (60kg P/ha), S_6 (50kg K/ha), S_7 (100kg K/ha) and

S_9 (50, 60 and 100kg N, P and K/ha respectively), while the lowest value of 0.1mg/100gm was obtained for samples S_2 (25kg N/ha), S_3 50kg N/ha) and S_8 (25, 30 and 50kg N, P and K/ha respectively). From these results, it was observed that maize plants require high application of fertilizers, either singly or in combination in high doses. It was also noticed that all the samples with nitrogen application had very low copper content (even lower than when no fertilizer was applied) except sample S_9 which had nitrogen in high dose in combination with high doses of phosphorus and potash. McVickar (1963) reported that the trace element content in plant is usually lowered through excessive use of nitrogen. The high doses of phosphorus and potash in sample S_9 might have over-shadowed the effect of nitrogen on the copper content of that sample. Fertilizer treatment had a significant effect on the copper content of maize. For the effect of varietal differences (Table 3.6), the highest value of 0.28mg/100gm was obtained for sample S_{11} (S 123) while the lowest value of 0.13mg/100gm was obtained for sample S_{13} (TZB). Varietal differences had no significant effect on the copper content of maize.

4.5.3. IRON.

The values are as shown in Tables 3.5 and 3.6. For the effect of fertilizer treatment, the highest value of 2.85mg/100gm was obtained for sample S_4 (30kg P/ha) while the lowest value of 1.50mg/100gm was obtained for sample S_1 (no fertilizer). There was significant effect on the iron content of maize due to fertilizer application. This confirms the report by Duckworth (1966) that there are considerable differences in the mineral content of plants grown under different fertilizer application. For the effect of varietal differences, sample S_{12} (096) had the highest value of 3.78mg/100gm while sample S_{10} (Bomo Local) had the lowest value of 2.05mg/100gm. Varietal differences also had significant effect on the iron content of maize. Literature values obtained are a range of 0.001 - 0.010mg/100gm (Miller, 1958), 0.002mg/100gm (NRC, 1968) and 2.50mg/100gm (Oyenuga, 1968). Values obtained in this work are comparable to that obtained by Oyenuga (1968).

4.5.4. MANGANESE.

The values obtined (Tables 3.5 and 3.6) agree with the literature values of 0.41mg/100gm and 6.88ppm given by

NRC (1968) and Oyenuga (1968) respectively. Highest value of 0.7mg/100gm was obtained for sample S_9 (50, 60 and 100kg N, P and K/ha respectively), while the lowest value of 0.30mg/100gm was obtained for sample S_7 (100kg K/ha). Phosphorus fertilizers increase the supply of available manganese. This has been found to be important for soybeans, oats and field beans but probably not for maize, since manganese deficiencies have not been found on maize in the field (Aldrich and Leng, 1969). Sample S_9 which had a high phosphorus application had the highest manganese content. This result therefore confirms the findings of Aldrich and Leng (1969) on soybeans, oats and field beans. Fertilizer treatment had a significant effect on the manganese content of maize. For the effect of varietal differences, sample S_{13} (TZB) had the highest value of 0.58mg/100gm while samples S_{11} (S 123) and S_{12} (096) had the lowest value of 0.36mg/100gm (Table 3.6). There was also a significant effect for the effect of varietal differences on the manganese content of maize.

4.5.5. ZINC

The values obtained (Tables 3.5 and 3.6) are comparable

to the literature value of 1.04mg/100gm given by NRC (1968). Sample S_6 (50kg K/ha) had the highest value of 2.03mg/100gm while sample S_8 (25, 30 and 50kg N, P and K/ha respectively) had the lowest value of 1.42mg/100gm. There was a significant effect due to fertilizer treatment on the zinc content of maize. Singh and Majhi (1975) reported that the use of fertilizers on crops affect the yield and composition of grain. Aldrich and Leng (1969) also reported that phosphorus induces a shortage of zinc. So, the above results confirm the findings of Aldrich and Leng (1969). Sample S_{13} (TZB) had the highest value of 2.12mg/100gm while sample S_{11} (S 123) had the lowest value of 1.39mg/100gm for the effect of varietal differences. Varietal differences also had a significant effect on the zinc content of maize.

4.5.6. SODIUM.

The values obtained (Tables 3.7 and 3.8) correspond to literature values of 0.0 - 130mg/100gm and 50.0mg/100gm given by Miller (1958) and Oyenuga (1968) respectively. Sample S_1 (no fertilizer) had the highest value of 57.5mg/100gm. While sample S_8 (25, 30 and 50kg N, P and K/ha

respectively) had the lowest value of 10.0mg/100gm. Fertilizer treatment had a significant effect on the sodium content of maize. Results in Table 3.5 show that low fertilizer treatment is needed for high sodium yield. For the effect of varietal differences, sample S_{12} (096) had the highest value of 18.7mg/100gm while samples S_{10} (Bomo Local) and S_{14} (Western Yellow) had the lowest value of 10.0mg/100gm. There was also a significant effect on the sodium content due to varietal differences. These results confirm the reports of Duckworth (1966) and McCay et. al, (1975) who said that both fertilizer treatment and varietal differences affect the nutrient composition of plants.

4.5.7. PHOSPHORUS

The values obtained are shown in Tables 3.7 and 3.8. Samples S_5 (60kg P/ha) and S_7 (100kg K/ha) had the highest value of 291.7mg/100gm while sample S_8 (25, 30 and 50kg N, P and K/ha respectively) had the lowest value of 183.3mg/100gm. From the results on Table 3.7, it seemed that whatsoever the combination, higher phosphorus contents were obtained when the fertilizers were applied in higher doses. Fertilizer treatment had a significant effect on

the phosphorus content of maize. This could be due to the fact that the addition of phosphorus to soils low in phosphorus often result in a marked uptake of the element by the plant (McVickar, 1963). For the effect of varietal differences, the highest value of 266.7mg/100gm was obtained for S_{13} (TZB) while the lowest value of 175.0mg/100gm was obtained for sample S_{10} (Bomo Local) varietal differences had a significant effect on the phosphorus content of maize.

4.5.8. MAGNESIUM.

The values obtained (Tables 3.7 and 3.8) fall within the range of 20.0 - 920.0mg/100gm given by Miller, (1958) but lower than the value of 160mg/100gm given by Oyenuga (1968). It will be seen (Table 3.7) that when ferilizers were applied in higher doses, either singly or in combination, the results obtained were almost the same. These results are on the other hand higher than when the fertilizers were applied in lower doses, either singly or in combination. The differences obtained notwithstanding, had no significant effect when analysed statistically. This shows that fertilizer treatment had no significant effect on the magnesium content of maize. For the effect of v

varietal differences, the highest value of 112.5mg/100gm was obtained for S_{13} (TZB) while the lowest value of 95.0mg/100gm was obtained for S_{14} (Western Yellow). There was significant effect on the magnesium content due to varietal differences at 5% level while at 1% level there was no significant effect.

4.5.9. POTASSIUM.

The values obtained (Tables 3.7 and 3.8) agree with those given by Miller (1958), Oyenuga (1968) and Howe and Gilfillan (1970). The values are 30.0 - 920.0mg/100gm, 400.0mg/100gm and 350.0mg/100gm respectively. The highest value of 403.33mg/100gm was obtained from sample S_5 (60kg P/ha), while the lowest value of 278.33mg/100gm was from sample S_9 (50, 60 and 100kg N, P and K/ha respectively). Sample S_5 had no applied potassium fertilizer while sample S_9 had the greatest amount of potassium application. McVickar.(1963) reported that it is not always true that the application of fertilizer would result in an increase in the plant's content of the elements that were added in the fertilizer. Maize, it is known requires large amounts of potassium. This is essential for vigorous growth, yet

never becomes a part of proteins and other organic compounds (McVickar, 1963, Aldrich and Leng, 1969). Fertilizer treatment had a significant effect on the potassium content of maize. McVickar (1963) reported that the addition of potash on soils is necessary for economical returns. This might be in terms of yield and quality. For the effect of varietal differences, sample S_{11} (S 123) and S_{13} (TZB) had the highest value of 311.67mg/100gm while sample S_{10} (Bomo Local) had the lowest value of 276.67mg/100gm. There was nonetheless, no significant effect on the potassium content due to varietal differences.

4.6. CARBOHYDRATES.

The results obtained for total carbohydrates were got by the addition of the values obtained for soluble carbohydrates, hemicelluloses, cellulose and lignin. (Tables 3.9 and 3.10). Literature values given are 68.3% (Schrimpf, 1966), 71.7% (Kent-Jones and Amos, 1967), 83.88% (Oyenuga, 1968), 80.0% (Jugenheimer, 1976) and 70.0% (Bingham, 1978). These literature values were obtained mainly by difference. That is, substracting from 100, the values obtained for moisture, ash, lipid, protein

and vitamin contents of the samples. The values obtained by difference cannot be taken as valid in totality since the final results obtained for the carbohydrates will include the amount of nucleic acids present in the materials. All the same, less errors are encountered by this method. The method employed in this study was used so as to know the composition of the individual fractions that make up the total carbohydrates. From Table 3.9, the highest value obtained was from sample S_9 (50, 60 and 100kg N, P and K/ha respectively) while the lowest value was from Sample S_3 (50kg N/ha). The low value obtained for sample S_3 could be due to the fact that potassium plays a vital part in the process whereby sugars are made from carbon dioxide and water and plants not getting enough potassium are weak and often contain low percentage of carbohydrates (McVickar, 1963). It therefore shows that fertilizer treatment plays a vital role in the carbohydrate content of maize. This was verified by the statistical analyses done on the results obtained. For the effect of varietal differences, sample S_{10} (Bomo Local) had the highest value of 89.59% while Sample S_{11} (S 123) had the lowest value of

77.09%. There were significant effects on the individual fractions except lignin due to varietal differences.

4.6.1. SOLUBLE CARBOHYDRATES.

The highest value of 46.75% was obtained from sample S_9 (50, 60 and 100kg N, P and K/ha respectively), while the lowest value of 26.17% was obtained from sample S_6 (50kg K/ha). This result was not as expected because S_6 had some amount of applied potash, which is necessary for high percentage of carbohydrate. On the other hand, it could be reasoned that the low soluble carbohydrates value for sample S_6 would have been so because the fertilizer was applied singly, since sample S_9 which had a high amount of applied potash and also in combination with other fertilizers had the highest value. For the effect of varietal differences, sample S_{10} (Bomo Local) had the highest value of 45.38% while sample S_{12} (096) had the lowest value of 30.8%. Both fertilizer treatment and varietal differences had significant effects on the soluble carbohydrate contents of maize.

4.6.2. HEMICELLULOSES.

Sample S_7 (100kg K/ha) had the highest value of 37.77%

while sample S_1 (no fertilizer) had the lowest 31.76%. Potassium increases the percentage of carbohydrates and also helps plants to resist diseases, cold weather and other adverse conditions (McVickar, 1963). Since hemicelluloses serve to cement plant cellulose fibres together, a high percentage of this carbohydrate would be expected in crops from soils with high potassium application. Fertilizer treatment had significant effect on the hemicellulose content of maize. For the effect of varietal differences, sample S_{10} (Bomo Local) had the lowest value of 35.02% while sample S_{13} (TZB) had the highest value of 41.98%. Varietal differences also had a significant effect on the hemicellulose content of maize.

4.6.3. CELLULOSE

Sample S_5 (60kg P/ha) had the highest value of 12.17% while sample S_9 (50, 60 and 100kg N, P and K/ha respectively) had the lowest value of 6.71%. Fertilizer treatment had a significant effect on the cellulose content of maize. For the effect of varietal differences, sample S_{10} (Bomo Local) had the highest value of 7.96% while sample S_{14} (Western Yellow) had the lowest value of 5.52%. Varietal differences

also had a significant effect on the cellulose content of maize.

4.6.4. LIGNIN.

This is a complex phenylpropanoid polymer that strengthens the cellulose framework of wood fibers and vascular plants. The values obtained (Tables 3.9 and 3.10) are close to the values of 2.8% (Schrimpf, 1966) and 1.7% (Kent-Jones and Amos, 1967) given for crude fiber. The highest value of 2.18% was obtained from sample S_3 (50kg N/ha) while the lowest value of 1.10% was obtained from sample S_4 (30kg P/ha). Fertilizer treatment had a significant effect on the lignin content of maize. For the effect of varietal differences sample S_{12} (096) had the highest value while sample S_{10} (Bomo Local) had the lowest. There was no significant effect on the lignin content due to varietal differences.

4.6.5. FRUCTOSAN

This is also known as fructan. It is a homopolysaccharide of fructose that occurs in plants. Values obtained are shown in Tables 3.11 and 3.12. Preece, (1957) gave values of zero for maize and 0.089% for oats, another

cereal grain. The highest value of 1.54% was obtained for sample S_6 (50kg K/ha) while the lowest value of 0.61% was obtained for sample S_2 (25kg N/ha). Preece (1957) reported that in oats, the fructosan content is low and the amount present is normally a function of the degree of ripeness so that with a fully ripe sample, no fructosan would be detected. This might also be true in the case of maize. For the effect of varietal differences, sample S_{11} (S 123) had the highest value of 1.52% while sample S_{10} (Bomo Local) had the lowest value of 0.84%. Both fertilizer treatment and varietal differences had significant effects on the fructosan content of maize.

4.6.6. FRUCTOSE

Literature value of 0.055% was given by Preece (1957). This value is low compared to those obtained in this work. (Tables 3.11 and 3.12). For the effect of fertilizer treatment, sample S_2 (25kg N/ha) had the highest value of 12.80% while sample S_9 (50, 60 and 100kg N, P and K/ha respectively) had the lowest, 9.13%. Fertilizer treatment had a significant effect on the fructose content of maize. For the effect of varietal differences, sample S_{10} (Bomo Local)

had the lowest value of 6.13% while sample S_{14} (Western Yellow) had the highest value of 9.90%. Varietal differences also had a significant effect on the fructose content of maize.

4.6.7. PENTOSES.

The highest value of 10.33% was obtained for sample S_6 (50kg K/ha) while the lowest value of 6.33% was obtained for sample S_3 (50kg N/ha). For the effect of varietal differences, highest value of 8.22% was obtained for sample S_{12} (096) while the lowest value of 5.4% was obtained for sample S_{10} (Bomo Local). Fertilizer treatment and varietal differences had significant effects on the pentose content of maize.

4.6.8. OTHER SUGARS.

Highest value of 17.0% was obtained for sample S_6 (50kg K/ha) while the lowest value of 7.17% was obtained for sample S_5 (60kg P/ha). For the effect of varietal differences sample S_{10} (Bomo Local) had the highest value of 8.47% while sample S_{13} (TZB) had the lowest value of 7.73%. Both fertilizer treatment and varietal differences had significant effects on the "other sugars" content of maize.

4.7. AMINO ACIDS.

The amino acid contents of crops are affected by both fertilizer treatment and varietal differences. These are the findings of many workers, McElory et. al (1949); Price (1950); Gunthardt et. al (1957); Pleskov and Powden (1959); Waggle et. al (1967); Hisclike et. al (1968); Keeny (1970); Hera (1971); Robbins et. al (1971); Deosthale (1972); Deosthale et. al (1972); Frey (1973); Warsi and Wright (1973); Eppendorfer (1975, 1977, 1978); Gleiter and Parker (1975); Rhodes and Jenkins (1975); Breteler (1976); Mitchell et. al (1976); Neucere and Sumrell (1979); Ajakaiye (1980) and Elmore and Polles (1980). The nutritional value of a protein is generally considered directly dependent on its amino acid composition (Kakade, 1974; Eggum, 1970). It has however been found that maize endosperm protein is limiting in two amino acids namely lysine and tryptophan (CIMMYT, 1974). Efforts are therefore being made to produce maize hybrids which have high lysine and tryptophan contents. The effects of both fertilizer treatment and varietal differences on 17 amino acids of maize samples were determined and the results

obtained are shown in Tables 3.13 and 3.14 for the effects of fertilizer treatment and varietal differences respectively. From the tables, it is seen that no values were obtained for tryptophan. This is because acid hydrolysis was used to breakdown the protein of the maize samples into their different amino acid components and acid hydrolysis destroys tryptophan (West et. al, 1966).

The results of the amino acid contents of the maize samples with respect to fertilizer treatment would be discussed in relation to the essential and non-essential amino acid contents. From Table 3.13, it is seen that with the exception of lysine and phenylalanine, it can be said that fertilizer treatment decreases the essential amino acid contents of maize. Though not essential for man, histidine and arginine are essential for some animals e.g. rats (West et. al, 1966). It was also observed that fertilizer treatment decreased the histidine and arginine content of maize. The results obtained for the essential amino acids agree with the report given by Ajakaiye, (1980) who worked on sorghum. Eppendorfer (1975), on the other hand reported an increase in the phenylalanine content

of grains of rye, wheat and barley with increasing nitrogen content. The results obtained in this work are contrary to this report. It was observed that, the phenylalanine content of sample S_3 (50kg N/ha) was lower than that of sample S_2 (25kg N/ha) and sample S_9 (50, 60 and 100kg N, P and K/ha respectively) ; had a lower value than sample S_8 (25, 30 and 50kg N, P and K/ha respectively), Table 3.13. The effect of nitrogen fertilization on the phenylalanine content of maize corresponds to the findings of Ajakaiye (1980), who reported that applied nitrogen tend to decrease the essential amino acid content of sorghum.

The lysine content of the maize samples ranged from 2.1 - 3.9g/16g N (Table 3.13). Sample S_4 (30kg P/ha) had the lowest value of 2.1g/16g N while samples S_2 (25kg N/ha) and S_6 (50kg K/ha) had the highest value of 3.9g/16g N. Both S_2 and S_6 had very low crude protein values (Table 3.3). What then could be responsible for their high lysine content? Infact, several investigators have reported in rice and sorghum, an inverse relationship between the lysine content of protein and the protein content itself (Juliano et. al, 1964); Cogampang et. al., 1966; Houston et. al, 1969; Srikantia, 1979; Ajakaiye, 1980).

Comparing the results obtained for the protein content in Table 3.3 to the results for lysine content, Table 3.13, it could be seen that the results are in agreement with the findings of these authors. It was also observed that sample S_6 had the lowest carbohydrate content (Table 3.9). This result corresponds to the findings of Doll and Koie (1978) who reported reduced carbohydrate accumulation in the grains of high lysine mutants and also the production of reduced seed protein. Koie and Doll (1979) also suggested that there could be a possible common mechanism responsible for the reduction in starch synthesis in all high lysine mutants. The lysine content of all the maize samples in Table 3.13 are low when compared to values of 5.0 and 4.07g/16g N (Pridilla et. al, 1975) for two hybrids of Opaque-2 modified maize endorsperm. When compared to the lysine content of casein, (17.2g/16g N, FAO, 1970), a milk protein of high biological value, it was seen that the lysine content of maize is still very low. It is therefore advised that maize meals be supplemented with other high lysine foods such as milk and eggs. Arginine values range from 3.9 - 9.6g/16g N for the effect of

fertilizer treatment. Nelson et. al, (1965) reported that mutants with high lysine and tryptophan contents are normally associated with high arginine and lower quantities of leucine and glutamate. Though samples S_2 and S_6 had the highest lysine content, they did not have the highest arginine value. Nonetheless, the value of 6.6g/16g N obtained for both samples was relatively high. Low values were obtained for leucine but not for glutamic acid. From Table 3.13, it is seen that the leucine values of the maize samples range from 25.0 - 38.0g/16g N. These values are higher than the values of 19.5 and 16.1g/16g N given by Pradilla et. al, (1975) for two opaque-2 modified maize endosperm. The values of leucine obtained in this work are also higher than the value of 20.2g/16g N given for casein (FAO, 1970). It is known that the leucine content is the single most important factor in the development of pellagra associated with maize, as in the case of sorghum (Srikantia, 1979). It is therefore important to note that just as much efforts are being made for high lysine maize, efforts should be made too to obtain low leucine maize.

For the non-essential amino acids, Ajakaiye (1980),

reported an increase in their contents in sorghum with respect to nitrogen fertilization. From the results obtained in this work (Table 3.13), there was actually no particular trend in the effect of fertilizer treatment on the non-essential amino acid content of the maize samples. Some non-essential amino acids increased with applied fertilizer treatment while some decreased, or just didn't have a particular trend. Aspartic acid content increased with increase potassium application 18.9 and 22.3g/16g N respectively for samples S_6 (50kg K/ha) and S_7 (100kg K/ha). When the potassium fertilizer was applied in combination with nitrogen and phosphorus fertilizers, the aspartic acid contents decreased. Sample S_7 (100kg K/ha) had the highest aspartic acid content while sample S_4 (30kg P/ha) had the lowest. It could be said that the high aspartic acid value of sample S_7 is in agreement with the findings of Gleiter and Parker (1975) who worked with alfalfa and reported that phosphorus deficiency in alfalfa resulted in large increases in free aspartic acid. Glutamic acid plays a central role in the synthesis of other amino acids. Values obtained for the effect for the effect of fertilizer

treatment range from 40.6 - 55.0g/16g N (Table 3.13). The lowest value of 40.6g/16g N was obtained for sample S_9 (50, 60 and 100kg N, P and K/ha respectively) and the highest value of 55.0g/16g N was obtained for sample S_7 (100kg K/ha). These results are contrary to the findings of Hera (1971), who reported that the simultaneous presence of ammonium and nitrate could stimulate the formation of glutamic acid. Mengel (1971) also reported that glutamic acid responds most distinctly to the nitrogen supply giving a good indication of the nitrogen nutritional status of the plant. It would be realised that nitrogen was applied in the form of calcium ammonium nitrate (nitrochalk), so all the maize samples with nitrogen application should have given higher glutamic acid values if Hera and Mengel's findings were to apply in this case. Eppendorfer (1977) reported slight reduction in the concentration of glycine in oats as a result of increasing nitrogen. The results obtained for the samples with nitrogen fertilization corresponded to this report. The value of 8.7g/16g N obtained for sample S_2 (25kg N/ha) is higher than that of 8.0g/16g N obtained for sample S_3 (50kg N/ha). Also, the value of 9.4g/16g N obtained for

sample S_8 (25, 30 and 50kg N, P and K/ha respectively) is higher than the value of 9.3g/16g N obtained for sample S_9 (50, 60 and 100kg N, P and K/ha respectively).

For the effect of varietal differences on the amino acid content of maize samples, the values obtained were compared with those of a reference protein, casein (FAO, 1970) and those of two opaque-2 modified maize endosperm (Pradilla et. al, 1975). For the essential amino acids, lysine, leucine and phenylalanine values of the maize samples differed remarkedly from the values for casein. The values of the lysine content of the samples ranged from 2.6 - 3.9g/16g N. These values are much lower than the value of 17.2g/16g N for casein. (Table 3.14). The leucine value of the samples range from 21.5 - 32.3g/16g N as compared to the value of 20.2g/16g N given for casein. The leucine of the samples are higher than that for casein. These high leucine values obtained for the samples are not favourable nutritionally. This is because high leucine is pellagragenic. (Srikantia, 1979). From the results in Table 3.14, it was observed that for the samples studied, sample S_{13} (TZB) had the lowest leucine value of 21.5g/16g N.

This value is close to the value of 20.2g/16g N for the leucine content of casein. The phenylalanine value for casein is higher than the values of 5.8 - 7.6g/16g N obtained for the maize varieties. The other essential amino acids did not differ much from the values given for the essential amino acids of casein. When the values were compared with those of the two opaque-2 modified maize endosperms, it was found that the lysine contents of 5.0 and 4.07g/16g N obtained for the opaque-two hybrids (Pradilla et. al, 1975) were higher than the values obtained for the maize varieties. Also, the leucine contents of 12.7 and 11.6g/16g N obtained for the opaque-two hybrids were less than those obtained for the maize varieties. They are also lower than the value of 11.1g/16g N obtained for the leucine content of casein. This low leucine value of the opaque-2 hybrids is nutritionally favourable and it has been reported that opaque-2 maize is nonpellagragenic because of its low leucine content (Srikantia, 1979).

For the non-essential amino acids of the maize varieties, apart from the values for glycine and alanine

which were higher than the glycine and alanine values of casein, the other amino acids compare favourably. Table 3.14.

4.7.1. LEUCINE/LYSINE RATIO.

An excess or deficiency of certain amino acids in protein can have deleterious effects on nutrition. It has been suggested that the disproportionately high amounts of leucine in some cereals is a possible cause of pellagra. This observation has been made in some parts of India, where sorghum is a staple food (Deosthale et. al, 1970; Kakade, 1974; Srikantia, 1979). It has also been reported that maize varieties with low level of leucine is in fact non-pellagragenic (Srikantia, 1979). Since a low level of leucine and a high level of lysine have been reported to be nutritionally favourable, it was therefore necessary to know what ratio of these amino acids could be considered nutritionally safe as a major source of protein. Deosthale et. al, (1970) reported that any protein fraction with a leucine/lysine ratio below 4.6 could be considered nutritionally safe as a major source of protein. Thus, the leucine/lysine ratios of the maize samples were considered

for both the effects of fertilizer treatment and varietal differences. From Tables 3.15 and 3.16, it was seen that none of the samples had a leucine/lysine ratio less than 4.6. The values of 7.38 - 17.27 and 5.81 - 12.35 for the effects of fertilizer treatment and varietal differences respectively, were much higher than the value of 1.17 for casein, a milk protein with very high biological value. The leucine/lysine ratio of casein was obtained from the values of leucine (20.2gm/16g N) and lysine (17.2gm/16g N) as given by FAO, (1970).

4.8. GENERAL DISCUSSION.

It has been seen that both fertilizer treatment and varietal differences influence the chemical composition of maize. Fertilizer treatment, especially with nitrogen has the effect of increasing the protein content of maize. This has been found to be nutritionally disadvantageous since there is an inverse relationship between the lysine content of the protein and the protein content itself. It was reported by Breteler (1976) in his experiment supplying nitrogen (ranging from 35 - 276kg N/ha) to two genotypes of maize, that lysine and tryptophan content of

both genotypes fell sharply between 35 and 69kg N/ha but there was a slower reduction at higher nitrogen levels. This shows an adverse effect of fertilizer treatment at some levels of application on the nutrients of maize. Also, it was reported that leucine, which is pellagragenic was high in the maize samples especially at fertilizer rates 50, 60 and 100kg N, P and K/ha respectively; 100kg K/ha; and 50kg K/ha for samples S_9, S_7 and S_3 respectively (Table 3.13). How then can the quality of maize nutrients be improved?

Methods suggested include induced mutations and selection from crosses. Balint et. al (1970), proposed that the protein content of different inbred strains can be increased more rapidly by means of induced mutations than by selection from crosses. Nelson Jr (1970) had shown that there is evidence that by breeding and selection from plants initially identified as producing seeds of high protein content, one can approach in many instances, and exceed in a few, the desired lysine values. He proposed genetic manupulation in achieving this. According to him, cereal grain protein is non-functional storage protein.

It is therefore possible to have wider variation in its amino acid composition without loss of seed viability. Thus, by changing genetically the relatively proportion of nutritionally-rich and nutritionally-poor protein bodies, dramatic increases in protein quality may be achieved as in the opaque-2 and floury-2 mutants of maize.

Many of the mineral elements needed by the body are contained in maize. Maize could therefore be said to be a good source of mineral elements for both man and animals.

All the maize samples analysed had high lipid contents especially sample S_9 (50, 60 and 100kg N, P and K/ha respectively). It is known that maize has a high amount of unsaturated fatty acids (West et. al, 1966). Though the lipid was not characterised into individual fractions, it is hoped that, if the lipid content of maize is anything to go by, more of the unsaturated fatty acid fraction should contain the essential fatty acids, linoleic and linolenic acids.

From the characterization of the carbohydrates, it was observed that the hemicellulose content of the maize was higher than the soluble carbohydrate content in most of the samples. Tables 3.9 and 3.10. Since the function

of the hemicellulose is to cement plant cellulose fibers together, one would wonder why there was high amount in the maize. It should have been expected that the soluble carbohydrate content be higher since it contains fractions like glucose and fructose which serve more in nutrition. On further fractionation of the soluble carbohydrates, it was observed that the "other sugars" fraction had the highest amount of carbohydrates. This result would be said to be favourable since this fraction contains glucose which is the most useful of the carbohydrates in terms of nutritional quality.

Lastly, it is important to realise that even where high nutrient contents are obtained either by the influence of fertilizer treatment or genetic manipulations, assessment of bioavailability of nutrients is as important, if not more so than actual concentrations.

4.9. CONCLUSION.

The use of fertilizers influences the chemical composition of maize. When viewing seed improvement in relation to protein quantity and quality, the use of fertilizers is not the best method to be employed in achieving these. Instead, genetic manipulations should be used.

None of the maize varieties analysed are nutritionally favourable to be taken as an only source of protein. This is because all the varieties had low lysine and high leucine values just as the other samples used for the influence of fertilizer treatment on the chemical composition of maize. High leucine is pellagragenic (Srikantia, 1979) and a leucine/lysine ratio greater than 4.6 is considered nutritionally unsafe. (Deosthale et. al, 1970).

The chemical composition of the maize plant or of the grain, reflects its value for feed, food and industrial uses (Leonard and Martin, 1963). Due to this, maize breeders could choose hybrids which favour these uses for planting. For example, maize with low moisture content favour the dry milling industries, high lipid content maize for the oil industries, high carbohydrate maize for

wet mill industries, high protein and high lysine hybrids for feeds and foods. One of the aims of this work is to compliment the breeding and selection work of the Institute for Agricultural Research, Samaru, Zaria (IAR). From the results obtained in this work, it is advised that IAR should continue the distribution of the TZB variety of maize to farmers for planting. Though sample S_{13} (TZB) had low protein content (8.83 $\pm$ 0.20%), Table 3.4, its lysine and leucine contents were relatively high and low respectively when compared to the other varieties, Table 3.14. This was also reflected in its leucine/lysine ratio which was lower than those for the other varieties, Table 3.15. Its leucine/lysine ratio of 5.81 is still closest to the value of 4.6 which could be considered nutritionally safe as a major source of protein. The TZB variety was used for the study of the influence of fertilizer treatment on the chemical composition of maize. The lowest leucine/lysine value for this study was obtained for sample S_2 (25kg N/ha). This value of 7.38 was higher than the value of 5.81 obtained for sample S_{13} (TZB , 120, 60 and 60kg N, P and K/ha respectively). It is

therefore advised that a fertilizer combination of 120, 60 and 60kg N, P and K/ha respectively be used for the planting of TZB variety of maize in IAR, Samaru, Zaria.

It was observed that though chances for further and rapid progress for improved protein crops are readily given, selection for improved protein will only be successful if the efforts of breeders are supported by the advice and competence of nutritionists. Nelson and Mertz (1973) advised that attempts to improve plant protein quality should be monitored biologically as well as by chemical analysis. This is evident in the result obtained for sample S_3 (50kg N/ha) which had high protein content, 10.98 $\pm$ 0.18% (Table 3.3) but is least nutritionally favourable in terms of its leucine/lysine ratio, 17.27, (Table 3.15).

Though fertilizer treatment and varietal differences influenced the moisture, lipid, mineral and carbohydrate contents of the maize samples, the values obtained for all the factors could still be considered to be nutritionally favourable if maize is to be taken as a main source of food or feed.

4.10 SUGGESTIONS FOR FURTHER STUDIES.

The main nutritional deficiences affecting populations in developing countries include the controversial but real protein-calorie deficiency, anaemia, goitre and vitamin-A deficiency (Bressani and Elias, 1979). Thus various nutrients are simultaneously responsible for the malnutrition problem. It is therefore suggested that further analyses be carried out on the maize samples such as the vitamin contents especially vitamin A and some toxic substances.

Many of the essential minerals in the maize grain are located in the pericarp-aleurone area of the seed. It is suggested that perhaps further analyses of component parts such as flour fractions will give more exact information on comparative mineral contents.

Protein Efficiency Ratios (PER's) and Net Protein Ratios (NPR's) measured in growing rats provide more direct measurements of nutritional quality. It is therefore suggested that these methods be employed for the maize samples especially sample S_{13}, which in this work has been found more nutritionally favourable than the other maize samples, due to its leucine/lysine ratio.

Tryptophan, the second limiting amino acid in maize,

was not determined in this work. This was due to the fact that acid hydrolysis, which destroys tryptophan, was used as the method of hydrolysis of the maize samples. Tryptophan could therefore be determined using techniques and equipment needed for colorimetric tryptophan methods available from the Protein Quality Laboratory, Centro International De Mejoramiento De Maiz Y Trigo (CIMMYT), El Batan (CIMMYT, 1971).

Leucine is the single most important factor in the development of pellagra associated with maize. It is important, therefore, that just as much efforts are being made for high lysine maize, efforts should be made too to obtain low leucine maize.

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APPENDICES.

APPENDIX I.

Calculation of Phosphorus content (as phosphate) of maize samples.

Wt (g) of digested sample	=	1.0
Volume (cm^3) of extract	=	50.0
Volume (cm^3) of extract used for determination	=	10.0
$\therefore$ Wt (g) of sample used for determination	=	0.2
Dilution factor	=	10.0

Conc. (ppm or ug/cm^3) of sample from the calibration curve = a

Conc. (ppm or $ug.cm^3$) of blank solution = b

Then: Metal content (ug/100g) $= \left[\frac{(a-b) \times 10}{0.2} \times 100\right] 10$

metal content (mg/100g) $= \left[\frac{(a-b) \times 10}{0.2} \times \frac{100}{1000}\right] 10$

$= 50\ (a-b).$

APPENDIX II.

Calculation of Soluble Carbohydrates and Hemiccellulose contents of maize samples.

Wt (g) of sample = 0.2

This was made up to $20.0cm^3$

$5.0cm^3$ of this extract was then diluted to $50.0cm^3$.

Of this, $2.0cm^3$ was used for the determinations.

$\therefore$ Wt (g) of maize used for determination = 0.002

Conc (ppm or ug/cm^3) of soluble carbohydrates or hemicelluloses from the calibration curve = a

Dilution factor = 11

$\therefore$ conc (ug/100g) $= \left[\frac{a \times 100}{0.002}\right] 11$

$\therefore$ conc (mg/100g) $= \left[\frac{a \times 100}{0.002} \times \frac{1}{1000}\right] 11$

$= 550a$

$\therefore$ % soluble carbohydrate or hemicellulose $= \frac{550a}{1000}$

$= 0.55a.$

APPENDIX III.

Analysis of Variance

The data for ash content is used as example.

TABLE AI. Raw data for the Ash content of the maize samples with respect to fertilizer treatment.

	ASH (% dry weight)								
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9
a	1.1389	1.0932	1.1323	1.3356	1.3554	1.2040	1.2621	1.1394	1.4070
b	1.2046	1.0945	1.0995	1.2585	1.4485	1.2232	1.3273	1.1419	1.3128
c	1.2052	1.1075	1.1153	1.2404	1.4414	1.1338	1.1845	1.0825	1.2866
Total	3.5987	3.2952	3.3471	3.8345	4.2453	3.5610	3.7739	3.3638	4.0064
T^2	12.950	10.858	11.203	14.703	18.023	12.681	14.242	11.315	16.051
$\Sigma(\text{Total})^2$	4.3171	3.6196	3.7349	4.9062	6.0129	4.2313	4.7577	3.7740	5.3504
mean	1.1996	1.0984	1.1157	1.2782	1.4151	1.1870	1.2580	1.1213	1.3355

$$CF = \frac{(\text{Total})^2}{27} = \frac{(33.0259)^2}{27}$$

$$= 40.3967.$$

$$\text{Between ss} = \sum \frac{T^2}{n} - CF$$

$$= \frac{122.0275}{3} - 40.3967$$

= 40.6758 - 40.3967

= 0.2791.

Total ss = $(\text{Total})^2$ - CF

= 40.7118 - 403967

= 0.3156

Within ss = Total ss - Between ss

= 0.3156 - 0.2791

= 0.0365

Degrees of freedom

Between = 9 - 1 = 8

Within = 26 - 8 = 18

Total = 27 - 1 = 26.

ANOVA TABLE.

SOURCE	SS	DF	MS	COMPUTED F
Between	0.2791	8	0.0349	$\frac{0.0349}{0.0020} = 17.45$
Within	0.0365	18	0.0020	
Total	0.3156	26		

For (8, 18) df.

α = 0.05 Tabled F = 3.01

α = 0.01 Tabled F = 4.28

Since the computed F is greater than either of the tabled F,

there is then significant effect on the ash content of maize due to fertilizer treatment.

KEY.

CF = Correction Factor.

SS = Sum of Squares.

n = Number of determinations.

DF = Degree of freedom.

MS = Mean square.

F = F statistics.

α=0.01 Type I error for rejecting a hypothesis

α=0.05 where it is true, at 1% and 5% levels respectively.

TABLE A2. Raw data for the Ash content of the maize samples with respect to varietal differences.

	ASH (% dry weight)				
	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}
a	1.2706	1.6903	1.1347	1.2431	1.1912
b	1.3360	1.2712	1.1418	1.2728	1.1818
c	1.2754	1.3420	1.1325	1.2190	1.0708
Total	3.8820	4.3035	3.4090	3.7349	3.4438
T^2	15.0700	18.5201	11.6213	13.9495	11.8598
$(Total)^2$	5.0260	6.2740	3.8738	4.6513	3.9622
mean	1.2940	1.4345	1.1363	1.2450	1.1479

$$CF = \frac{(Total)^2}{15} = \frac{(18.7732)^2}{15}$$

$$= 23.4955$$

$$\text{Between ss} = \frac{T^2}{n} - CF$$

$$= 23.6736 - 23.4955$$

$$= 0.1781.$$

$$\text{Total ss} = (Total)^2 - CF$$

$$= 23.7872 - 23.4955$$

$$= 0.2917.$$

Within ss = Total ss - Between ss

= 0.2917 - 0.1781

= 0.1136.

Degrees of freedom.

Between = 5 - 1 = 4

Within = 14 - 4 = 10

Total = 15 - 1 = 14

ANOVA TABLE

SOURCE	SS	DF	MS	COMPUTED F
Between	0.1781	4	0.0445	$\frac{0.0445}{0.0114} = 3.9035$
Within	0.1136	10	0.0114	
Total	0.2917	14		

For (4,10) df

α = 0.05 Tabled F = 4.47

α = 0.01 Tabled F = 7.34

Since the computed F is less than either of the tabled F, there is then no significant effect on the ash content of maize due to varietal differences.

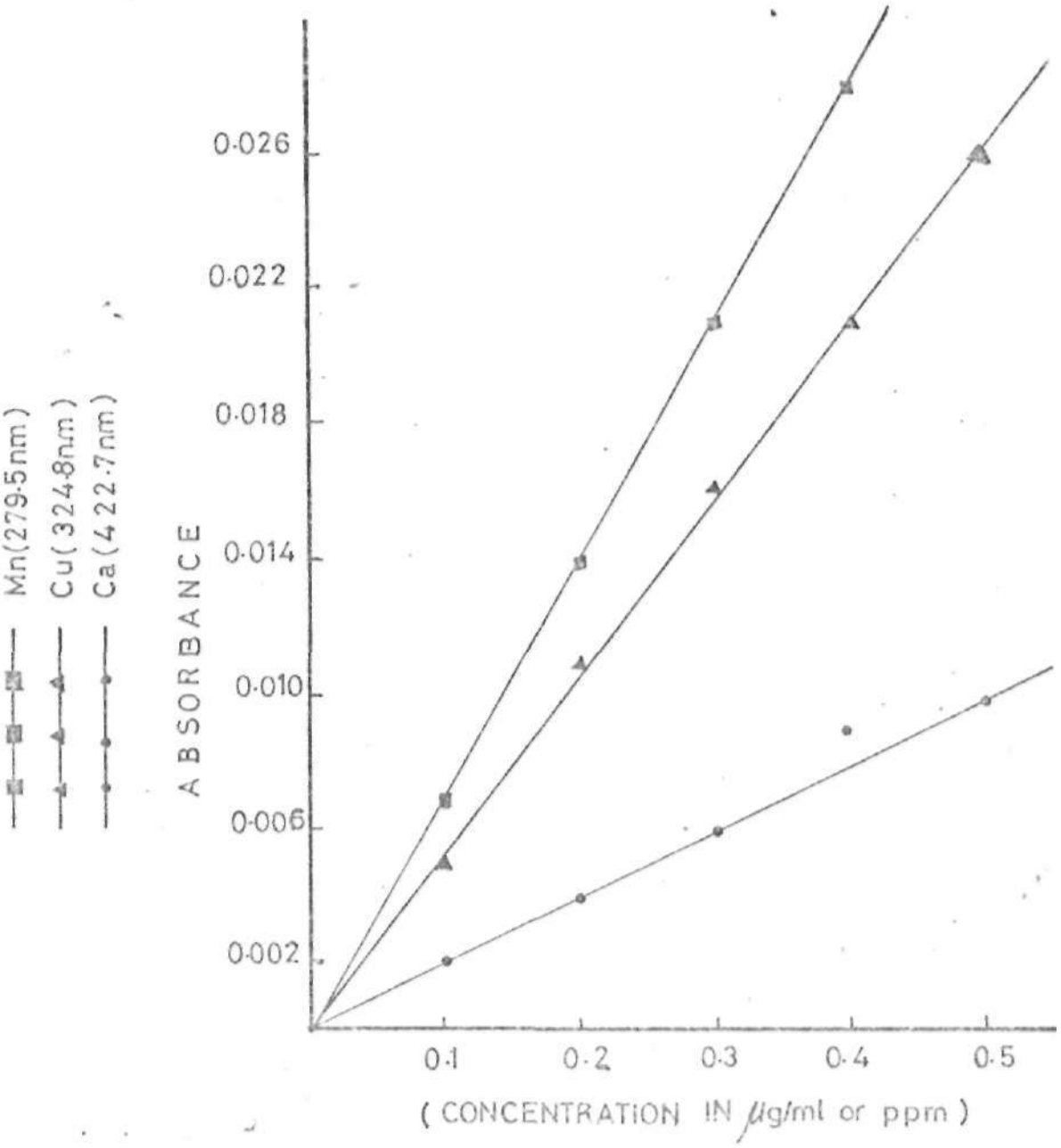

Mn(279·5nm)
Cu(324·8nm)
Ca(422·7nm)
ABSORBANCE
0·026
0·022
0·018
0·014
0·010
0·006
0·002
0·1
0·2
0·3
0·4
0·5
(CONCENTRATION IN µg/ml or ppm)

FIG I

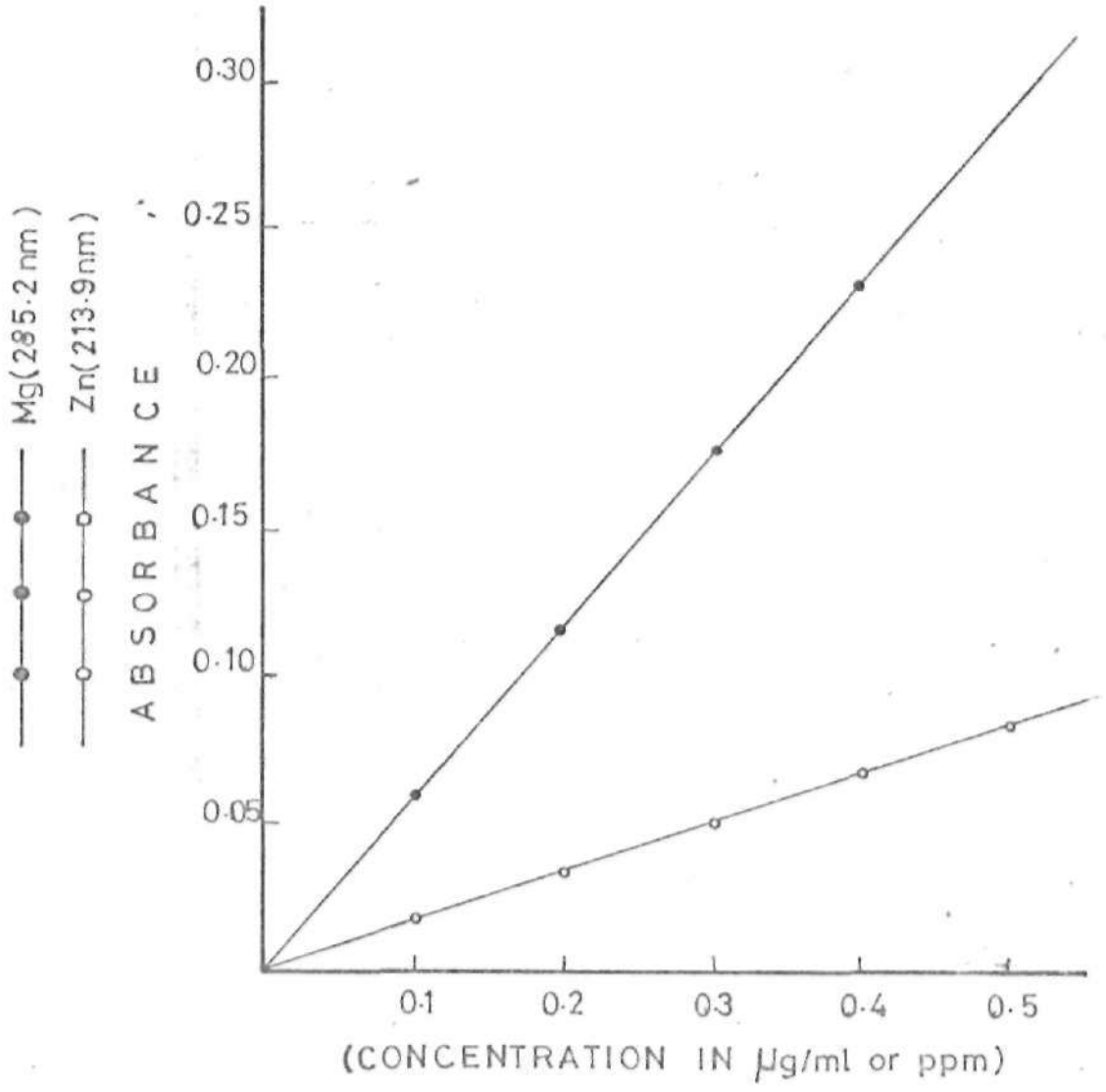

Mg(285·2 nm)
Zn(213·9nm)
ABSORBANCE
0·30
0·25
0·20
0·15
0·10
0·05
0·1
0·2
0·3
0·4
0·5
(CONCENTRATION IN µg/ml or ppm)

FIG II

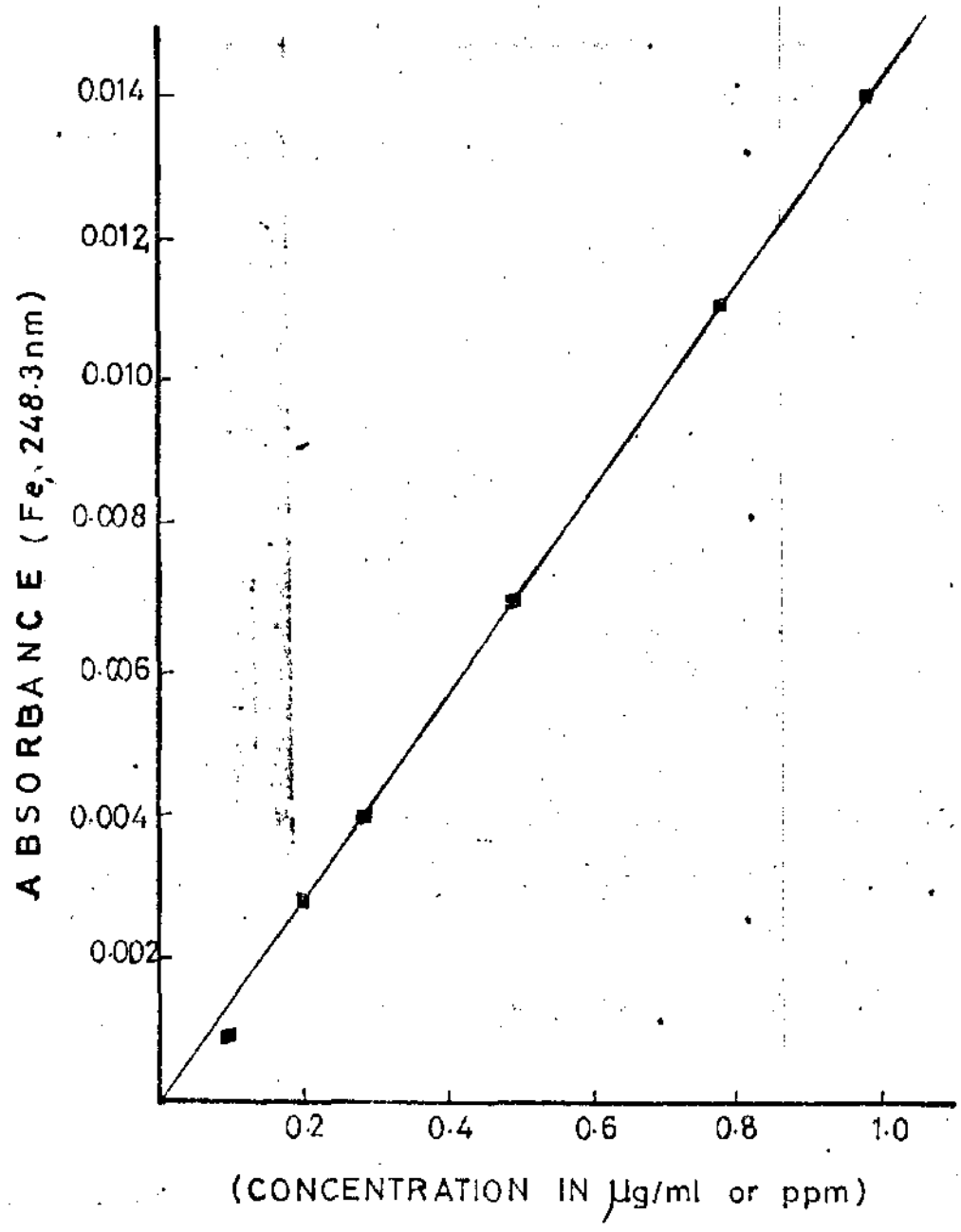

0.014
0.012
0.010
0.008
0.006
0.004
0.002
ABSORBANCE (Fe, 248.3nm)
0.2
0.4
0.6
0.8
1.0
(CONCENTRATION IN µg/ml or ppm)

FIG III

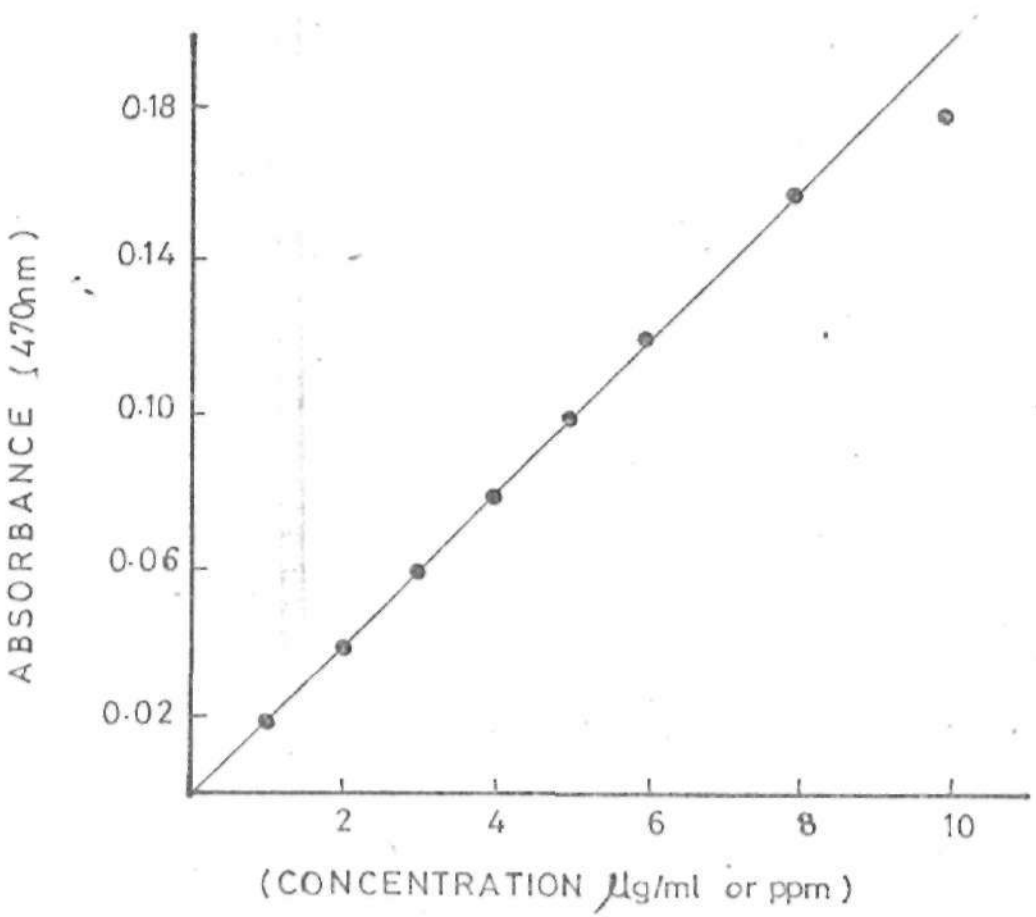

0·18
0·14
0·10
0·06
0·02
ABSORBANCE (470nm)
2
4
6
8
10
(CONCENTRATION µg/ml or ppm)

FIG IV

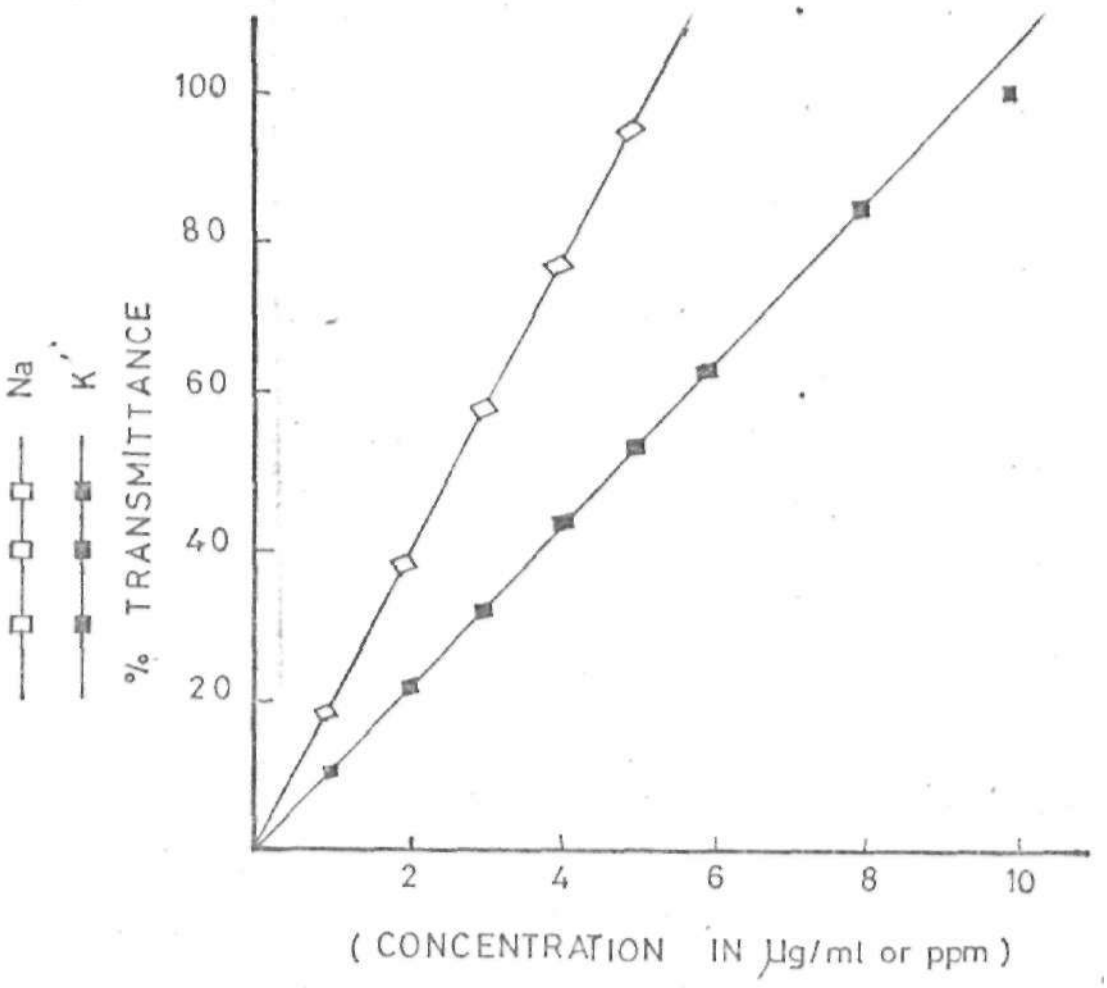

Na
K
% TRANSMITTANCE
100
80
60
40
20
2
4
6
8
10
(CONCENTRATION IN µg/ml or ppm)

FIG V

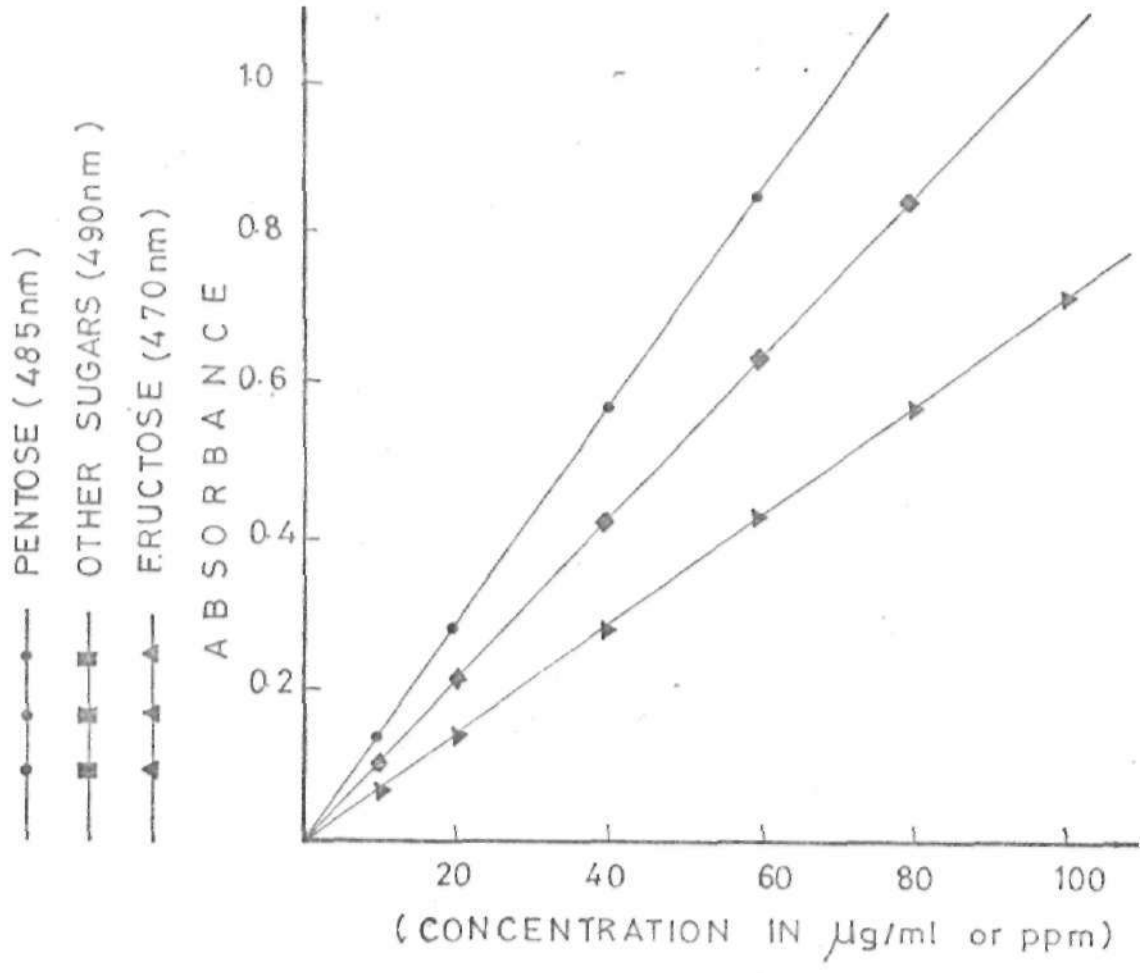

PENTOSE (485nm)
OTHER SUGARS (490nm)
FRUCTOSE (470nm)
ABSORBANCE
1.0
0.8
0.6
0.4
0.2
20
40
60
80
100
(CONCENTRATION IN µg/ml or ppm)

FIG VII

Fig VII.

Calibration curves for the estimation of Pentoses, Fructose and Other Sugars in the maize samples.

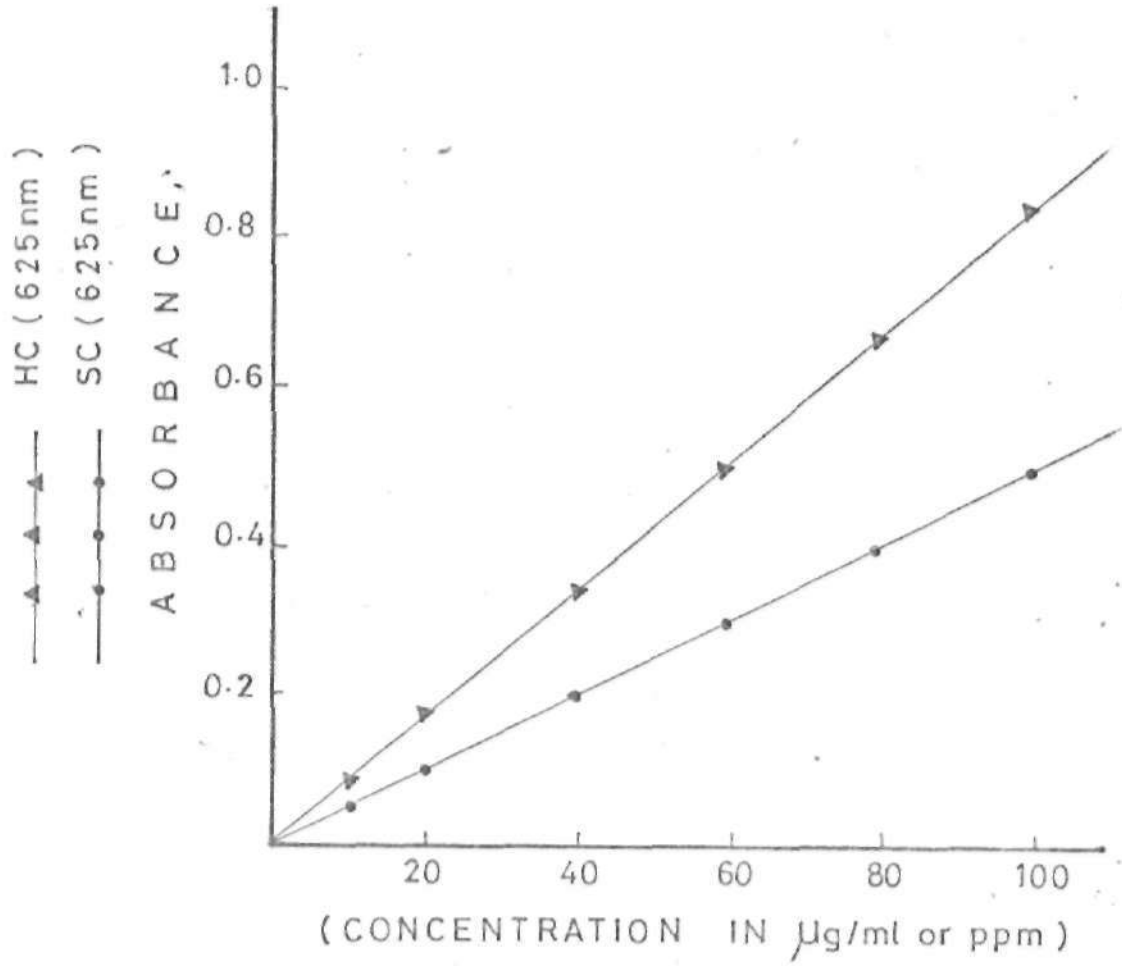

HC (625nm)
SC (625nm)
ABSORBANCE,
1.0
0.8
0.6
0.4
0.2
20
40
60
80
100
(CONCENTRATION IN µg/ml or ppm)

FIG VI

Fig VI.

Calibration curves for the estimation of Hemicelluloses (HC) and Soluble Carbohydrates (SC) in the maize samples.