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COMPOSITIONAL CHANGES OF DEVELOPING LOCAL WHEAT GRAINS.

BY;

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D E C L A R A T I O N

I hereby declare that this thesis is a record of my own research work and has not been submitted for the award of a degree at any other University. Information and excerpts from both published and unpublished works of others have been acknowledged in the text.

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C E R T I F I C A T I O N

This project thesis entitled "Compositional Changes of Developing Local Wheat Grains" by Mrs. Roseline Aliyu meets the regulations governing the award of the degree of M.Sc. in Biochemistry of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my husband Sarki Aliyu and all my children, with gratitude and affection.

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ABSTRACT

Three varieties of developing wheat kernels were analysed for their chemical and biochemical properties with the objective of improving their yield and quality. Moisture contents of the three cultivars ranged from 6.86 - 70.61% at different stages of development. Frond had the highest proein and crude fibre contents at maturity (14.43% and 13.83% respectively) while Siete cerros had the lowest protein and crude fibre contents at maturity (9.54% and 6.25% respectively). Mineral elements show no definite pattern but all decreased as maturity progressed. Lipid contents also decreased with development (1.13%, 0.91% and 1.19% for Frond, Pavon 76 and Siete cerros respectively at maturity).

Carbohydrate contents ranged from 51.01 - 18.79% for the three varieties showing decrease with development. 51.01%, 54.90%, 54.50% were gotten for Frond, Pavon 76 and Siete cerros respectively at maturity.

Glutamate dehydrogenase was observed to show the least activity of 3.6 x 10-2 um/ml/min while Pavon 76 showed the highest activity (4.8 x 10 um/ml/nun) at maturity.

Glut:amate synthase was also determined and it was observed that the activity increased as maturity progressed (3.6 x 10 um/ml/min, 9.8 x $10^{\sim 2}$um/ml/min, 4.0 x $10^{\sim 2}$um/ml/min for Frond, Pavon 76 and Siete cerros respectively at maturity).

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Food for humanity has over the years been an important determinant of the rise and endurance of a nation. It is estimated that between 400-500 million people in the modern world suffer widespread and severe food shortage (Nigerian Standard 1988).

Recently in Nigeria, the Federal government is giving increased attention to food production particularly the intensification of wheat production in an attempt to have national food security (Nigerian Standard 1988).

Until the late sixties, Nigeria was self sufficient in food production and was a major exporter of such agricultural commodities as groundnut and palm produce, both of which are also major staples (Andrae and Beckman, 1985). However, the rate of growth of per capital food production is not keeping pace with the rate of growth of the population, so there is need for increased food production.

Wheat was introduced by early European explorers, colonialists and traders to various countries either as a crop or as a commodity of trade. It was regarded as food of the ruling European colonialists and became desirable and sought after by the native population (Litzeberger 1974). According to Andrae and Beckman (1985), consumption of wheat bread was introduced to Nigeria on a modest scale during the early colonial period. The urban population in Nigeria has increasingly used bread as one of its main staples because of

its convenience as ready-cooked, easily transported good.

The Federal government of Nigeria in 1959/60 decided to develop irrigation schemes in the nothern parts of the country (between latitudes 12-14^{o}N) for the cultivation of wheat for flour production to substitute annual flour importation of over 60,000 tons (Jones, 1968). Since then, scientists have been testing lines (or varieties) of wheat which would be quick-maturing, responsive to fertilization, resistant to diseases and of good grain quality. The first recommendation was in 1965 when 'Tousson' was recommended for its high grain yield and Florence 'Aurore 8195' for quality (Jones, 1968) 'Tousson' on the average yields 15% more than the local control. However, new lines (or varieties) are being identified and ytested and wheat production is increased annually (Jones, 1968).

Wheat contributes more calories and more protein to the world's diet than any other food crop (Hanson, et al, 1982). While it may often be passed off as a starchy food crop, Hegsted (1965) reported that wheat is a very nutritive crop that contains notable amounts of protein, minerals, and vitamins. He also reported that its amino acid yield per acre far exceeds that of animal product for every one of the essential amino acids; wheat protein, when balanced with other foods that supply certain amino acids such as lysine, is an efficient source of quality protein. Wheat is commonly regarded as the best of cereals and its availability for food is considered a sign of civilisation. Nations with adequate supply of wheat for centuries have been considered

nutritionally well-off (Baols, 1948).

The universal emphasis on wheat reflects not only its food value but its relative ease of cultivation. Of all the cereals, wheat and barley offer the advantages of being highly nutritious and easily stored, more so, the return in usable form is relatively high in relation to the effort expended in raising them (Reitz, 1967). In addition to its abundance wheat has the greatest advantage in bread making than any other cereal because of the proteins (gliadin and glutenin) that form gluten, the elastic property of which contributes to the textural quality of bread (Shellenberger, 1971). In addition, a considerable amount of the wheat kernel removed in processing during milling is utilized fully as animal feed. Wheat has been cultivated in Northern Nigeria as a traditional crop for several centuries (Jones, 1968). It is grown during the dry season on the fringes of irrigated farms, and on the edges of minor irrigation channels. Production has been very limited and the entire crop is consumed locally in the form of unleavened wheat bread or local biscuits for special occasions (Jones, 1968).

Local wheat type is uniform and closely resembles durum. It matures early taking 45 - 50 days to flower, possesses awns and develops a flinty red-bumped grain. It has a weak straw and is susceptible to stem and leaf rusts and poor in protein quality (Andrew, 1968). Owing to its potential benefits, the temptation to successfully manipulate and upgrade the protein quality in local wheat varieties cannot be over-emphasized. The conventional white bread which is consumed by a large

proportion of Nigerians especially children, is made from 100% wheat flour but recent developments in the country has necessitated the mixing of wheat flour with flour from other cereals such as maize, sorghum and millet to produce composite flour for the purpose of breadmaking. This has led to the lowering of both the nutritional and rheological qualities of the resultant composite bread.

Based on this background, this work was initiated with the objectives of:

1. Evaluating the changes in levels of proteins, carbohydrate, lipids, mineral elements and crude fibre during grain development which differ in composition in their matured grains.
2. Determining changes in levels of glutamate dehydrogenase and glutamate synthase, enzymes of ammonia assimilation and subsequently protein synthesis, in developing seeds of wheat varieties which differ in protein content of their matured grains.

Wheat is the common name for cereal grasses of the genus Triticum (Sin, 1965). The exact place and time of the origin and earliest cultivation is not known. Records, however, show that it had been cultivated since prehistoric time (Shellenberger, 1971). The remains of "emmer" and "einkkorn", two of the three series classified by experts under the genus triticum, have been found by archaeologists on the sites in the Middle East, dating back to the 7th millenium BC (The Guardian, 1988).

Emmer which is regarded as the ancestor of today's common

wheat because it closely resembles the wild species of wheat found in the mountainous region of Syria and Palestine was grown in dynastic Egypt (Shellenberger, 1971). This made Percival (1921), to conclude that bread wheat originated from hybridisation of an 'emmer' type and a wild species of grass. There is also evidence that wheat was first used as a parched cereal and as time went on, the parched grains were probably ground and the coarse meal soaked in water and made into gruel.

This clearly indicates that wheat was cultivated by the primitive man. Further evidence of the origin of wheat was confirmed as carbonized grains of wheat were discovered by archaeologists in Turkey, Egypt, Iraq and in many other countries dating back to 6000 BC. Also in Jarmo (Site in the upland of Eastern Iraq), fossils of wheat kernels were found which dated back to 6750BC.

The first cultivated wheat type was the primitive hulled types firmly enclosed in the glumes. These were believed to have spread over most of the Western Europe during the stone age through colonization and trades to other parts of the world (Jones, 1968). The introduction of wheat to West Africa was as a result of Arabian influences from the 12th Century onwards (Lewicki, 1979). This wheat must have originated in North Africa and spread via Sudan to lake Chad. The earliest reports of wheat cultivation in Nigeria dates back to the 18th Century in Borno and 19th Century in Kano (Jones, 1968).

Since 1959, researches into wheat and irrigation schemes have been set up in the North (between latitudes 12 - 14°N) for the cultivation of wheat for bread flour production to

substitute flour importation of over 60,000 tons/year and also to arrest the dwindling foreign reserve position. The importation of wheat grew steadily at an average rate of close to 20% per annum, and by early 1980, it had reached the level of one and half million tonnes per annum, making Nigeria a major buyer in the world market (Andrew and Beckman, 1985). Today, the wheat cultivation area in Nigeria covers about 6,000 hectares with an average of about 2,000 kg/ha. yield (Andrew and Beckman, 1985).

1.2 CLASSIFICATION

Wheat is commonly classified according to species, commercial types and growth habit. There are sixteen species, two principal commercial types and three distinctive growth habit types. Wheat belongs to the grass family the Gramineae which is further classified under the genus Triticum under which sixteen species of wheat are commonly recognised but only two species Triticum aestivum and Triticum durum are cultivated on large scale (Hanson et al, 1982). Growth habit classification includes:

(a) Winter wheat - This is sown in autumn. Seedlings usually emerge and tiller before winter then they pass through an inactive stage during the cold weather. The plants resume rapid growth in spring and mature in summer after a total growing period of nine to eleven months.

(b) Spring wheat - This has a continuous growth cycle with a growing period of three to six months. It has no inactive period and cannot survive sustained low temperatures. Finally,

(c) The facultative wheat which is an intermediate of winter and spring wheat (Hanson et al, 1982).

1.3 USES OF WHEAT

Wheat, like other cereal grains, has many natural advantages as a food crop. It is nutritious, concentrated, readily stored, transported and easily processed to give highly refined raw foods (Reitz, 1967, Harlan, 1981). Through the centuries wheat has been intimately associated with food uses for man. It is used principally in the form of fine flour for baked products like bread, sponge cakes, cookies, noodles, meat pie, biscuits or in form of crushed wheat for coarse brown bread. This crushed wheat is also mixed with Semolina ot produce paste products like macaroni, spaghetti and vernicidi (Shellenberger, 1971).

In Northern Nigeria, wheat is consumed in the form of unleavened wheat cakes or local biscuits. Other traditional dishes prepared with wheat include taliya, finkaso, puff-puff, chin-chin, alkaki, gurasa, alkubos and waina (Ajakaiye, 1979).

The use of wheat as livestock feed is limited because its price is higher than that of the feed grains (Shellenberger, 1971). However, flour milling by-products or offal which are germ, bran and pollard which amounts to 25% of the whole grain are utilised almost entirely for livestock feed (Ziegler and Greer, 1971).

Wheat is also utilised in the brewing industry in the production of malt drinks and distilled spirits. It is also utilised in the manufacture of starch for laundry and textile

industries. The use of wheat in the production of syrup, sugar or industrial alcohol cannot be over-emphasized. The gluten component of wheat is used in the production of gluten breads for diabetics and for the production of Monosodium glutamate, a flavour enhancer. Also flour produced from low quality wheat are used in the manufacture of pastes for wall papering and plywood adhesives (Shellenberger, 1971).

1.4 NUTRITIVE VALUE

Wheat is one of the most important of all cultivated cereals with respect to human nutrition (Harian, 1981; Anderson, 1974). Bread, made from wheat has been the staff of the life for countless numbers of human beings, right from dawn of history when human being first turned from an aboreal or nomadic life to settled agriculture (Reitz, 1967).

The protein content of wheat varies from 7 to 22% with an average value of 14% which far exceeds the protein content of other cereals (Shellenberger, 1971, Anderson, 1974). Wheat compares favourably in the distribution of essential amino acids (Wu and Dimler, 1963). However, it is poor with regards to lysine but when supplemented with lysine-rich foods wheat is a good protein source (Anderson, 1974). It is also very rich in sulphur - containing amino acids.

Wheat has 10%, 1.9%, 3.4%, and 1.8% content of moisture, fat, crude fibre and ash respectively (Shellenberger, 1971). The starch content (53.0-70%) of wheat is similar to that of other cereals and is readily digestible. Wheat has no particular advantage or disadvantage with respect to lipids,

however, they make substantial contribution to dietary requirements (D'Appolonia et al, 1971). Processing changes and reduces the nutritional content but vitamin rich foods are prepared from the by-products which contain lot of B, and B_2. Wheat consist of 20.9%, 8.0%, 0.73%, 0.26%, 0.21%, 0.13%, 0.05% potassium, magnesium, manganese, iron, zinc, calcium and copper respectively. These values vary considerably depending on environmental factors. Wheat has no particular advantage or disadvantage with respect to mineral content compared to other cereals, however, it makes substantive contribution to dietary requirements (Knowles and Watkins, 1931).

Apart from its nutritional advantage wheat has an edge over the other cereals because of its water insoluble protein which gives its dough unique cohesive properties that are very important in bread-making. The amounts of all the nutrients in wheat depend on the availability of soil nutrients, stage of growth and various other factors. Most of the mineral matter except nitrogen is absorbed before or slightly after anthesis (Knowles and Watkins, 1931).

The bran and pollard (offal) are very useful materials in animal nutrition, being well supplied with protein, lipids, vitamins and minerals. The bran particularly which contains high fibrous material helps to keep the animal system open.

1.5 CHEMICAL COMPOSITION

A knowledge of the chemical composition of foods, is essential in understanding the chemistry and technology of

food. Also improvement of the protein quantity and quality requires the basic understanding of processes involved in protein accumulation during development of the grains.

1.5.1 MOISTURE:

Moisture is one of the most important factors that affects the quality of wheat. Dry matter is inversely related to the amount of moisture it contains. Moisture content of wheat ranges from 8% for drier areas to 14% for wet areas. Moisture contents is of direct economic importance as this affects storage. Also very dry wheat has its disadvantages because it tends to be brittle and breaks easily (Zeleny, 1960). The basic method for determining moisture in wheat is the one hour air-oven drying at 130^{o} (AACC, 1962).

1.5.2 PROTEIN AND AMINO ACIDS

Protein content of wheat varies from 7 to 22%, and this depend in part on variety and class but more largely on environmental factors during growth. Abundant rainfall during the period of kernel development usually results in low protein content whereas dry conditions during the period favours high protein content. Heavy nitrogen fertilization increases the protein content of wheat (Zeleney, 1960). Protein in wheat is distributed non-uniformly among the morphological tissues of the grain, the highest concentration occurring in the outermost part of the starchy endosperm, the germ and aleurone containing some amount while pericarp contains little or nothing (Kent, 1969).

Apart from having valuable nutrients in the edible

portions, wheat protein perform important functions which are dependent on their chemical and mechanical properties. These functions are hinderance and disruption of the endosperm cell contents during the roller milling of hard wheat. Wheat protein also confer upon the endosperm a texture which may be hard or soft. They have a lot to do with the precise way in which the endosperm shatters during the milling process and also with the sizes and types of particles that result. Their mechanical properties together with those of starch granules are paramount on the processes of fine grinding and of protein shifting in flour, and of course, they play an essential role in bread-making (Kent, 1969). Many studies have shown that wheat protein alone are capable of maintaining high rates of growth in children for a long period. Wheat protein is deficient in lysine but provides 16% or more of the dietary intake of each of the other essential amino acids (Moran and Pace 1964; Kent, 1960). Wheat protein is an important and cheap contributor to the nutritive value of a diet , its relative deficiency of lysine being obscured because of lysine supplementation by other constituents of the diet (Kent, 1969).

Wheat owe its uniqueness in bread making to the protein-gluten, a principal storage protein formed in the proteoplast. Gliadin and glutenin form about 80-85% of the total endosperm protein (Mechan et al, 1981). They are insoluble in alcohol, dilute acids or alkali while albumin and globulins are soluble. Mechan et al, (1981) further reported that gliadin synthesis begins 10-15 days post anthesis.

Work on their amino acid composition by Wu and Dimbler, (1963) showed unusually high contents of glutamic acid and proline which confers strong aggregation tendency to wheat protein. The covalent, ionic, dipole, hydrogen and hydrophobic bonds which are as a result of amino acids interaction in the protein, contributes greatly to the cohesiveness, elasticity and plasticity of the dough structure (Pomeranz, 1964). Lysine has been shown to rise as protein content is lowered (Hepburn and Bradley, 1965). This inverse relationship is valid only when protein level is less than 13.5% (Nx5.7) (Lawrence et al, 1958). Arginine and some amino acids show other similar inverse relationship with protein; glutamic acid, phenylalanine proline have been reported to show a positive correlation with protein (Hepburn and Bradley, 1965).

The protein content of wheat, one of its principal quality parameters is influenced primarily by the enviromental conditions during growth and also by the genotype and interaction of genotype with growth environment. There are high protein wheat cultivars which consistently yield grains of higher protein content than low-protein wheat grain under the same condition (Donovan et al, 1977). Differences in the grain nitrogen content(% dry weight) between a high and a low protein wheat has been attributed to higher amounts of nitrogen per kernel in the high protein cultivar and partly to its lower kernel weight, also associated with these higher amounts of grain nitrogen were higher levels of non-protein nitrogen particularly in the early stages of grain development

(Donovan et al, 1977). It has been suggested that the higher levels of non-protein nitrogen reaching the grain by translocation from other parts of the plants may lead to a higher rate of grain protein accumulation and thus, a higher final grain protein content, provided that the duration of synthesis is maintained.

A study of the changes in protein fractions during grain development have shown that the proportions of gliadin I and II and glutenin II increased while those of albumin and globulin decreased as the wheat grains matured (Kapoor and Heiner, 1982). It was then concluded that immature wheat grains contain proteins with a higher nutritive value than mature grains. The increase or decrease in the content of certain amino acids might be due largely to changes in the proportion of these protein fractions during grain development. Chemical score, essential amino acids index and calculated biological value declined during grain maturation. This might be due to decreased albumin which contains high quantities of essential amino acids and increased gliadin which contains small amounts of essential amino acids (Kapoor and Heiner, 1982).

Glutamate has been reported to be the major transport form of nitrogen (Sodeck and Dasilva, 1977). However, Asparagine's contribution becomes more important in some cases as the plant matures. A large amount of either amide is probably incorporated directly into the appropriate prolamine the major storage protein in cereals. Presumably, excess asparagine or glutamine would be catabolized to supply both carbon and nitrogen for the synthesis of other amino acids and perhaps of

sugar as well.

De novo synthesis of a number of amino acids from radioactive glutamine, glutamate or aspartate in developing wheat seeds before the discovery of glutamate synthethase in bacteria and later in plants, ammonia was considered to be assimilated solely through the route involving glutamate dehydrogenase. However, the demonstration of glutamate synthase in plant tissues (Stewart and Rhodes, 1978) has changed the whole concept of ammonia assimilation and it is now generally agreed that ammonia is assimilated in roots as well as in shoots of higher plants via the glutamine synthase - glutamate synthase pathways. These two enzymes have been demonstrated in developing grains but it is still not known with certainty as to which pathway of ammonia assimilation is operative in these tissues. The developing grains are also known to transform extensively glutamine and asparagine, translocated from the leaves through the phloem before being incorporated into storage proteins. The enzymes of glutamine and asparagine metabolism thus appear to play an important role in determining the extent of storage of proteins accumulation in grains during development in wheat, although, a barrier to the transport of nitrate from the phloem sap to the developing grain has been suggested. (Nair and Abrol, 1977), the presence of active reductase in this tissue has still not been demonstrated. It is now generally agreed that primary assimilation of ammonia into organic nitrogen does take place in the grain in situ. Demonstration of glutamate dehydrogenase, glutamate synthase

and glutamate-oxaloacetate transaminase activities in developing grains does not indicate that both the glutamate dehydrogenase pathway and glutamate synthase cycle may be operative in the primary assimilation of ammonia in the grain, but this has been proved that ammonia assimilation in the roots as well as in the shoots of higher plants is via glutamine synthetase, glutamate synthase pathway (Miflin and Lea, 1980). Developing grains do not have catabolic activity to any appreciable extent, and the constantly increasing activity of glutamate dehydrogenase (GDH) during development strongly suggests that glutamate dehydrogenase might be actively associated with ammonia assimilation (Garg et al, 1984).

1.5.3 CARBOHYDRATES

Carbohydrates are the most abundant and widely distributed food component in wheat. They are basically divided into two groups:

(1) The structural polyssacharides which include cellulose, hemicellulose and lignin which are part of a rigid mechanical cell wall, and

(2) Nutrient polyssacharides which comprises of starch, a metabolic reserve in plants (D'Appolonia et al, 1971).

Early workers identified the major carbohydrate component of wheat. Majority of such studies dealth with the proximate chemical composition by which carbohydrates are expressed as the difference between 100% and the sum of the percentage of moisture, protein, fat, ash and crude fibre (D'Appolonia et al, 1971). However, there are new quantitative methods which

include colorimetric, chromatographic, optical, physical and enzymic methods. Starch is the major component of flour prepared from wheat. Its role in food products prepared from flour has received relatively little attention in comparison to that of gluten-forming proteins. This is because its proportions and functions are substantially equal with other cereals. However, many workers have shown that wheat starch has an important function in baked goods as well as in those food products in which it is used for its thickening properties (Hopkins and Graham, 1935). Starch is inversely related to protein and is an important factor in determining grain yield.

Starch is synthesized from sucrose and a direct relationship exist between the concentration of sucrose in endosperm cells and the rate of starch synthesis (Haunold et al, 1962; Pepe and Heiner 1975; Kummar and Singh, 1981). It has also been suggested that starch synthesis is regulated by the concentration of sucrose. Denovan et al (1977) further confirms this by reporting on the relationship between he sucrose content of wheat grain at the initiation of starch synthesis and starch content of matured grains.Similarly, reports on reduced starch accumulation in mutant corn Notch -2 compared to NP 113 parent is due mainly to the rate of starch synthesis or an enhanced degradation of synthesised starch in Notch-2. A lower rate of starch synthesis therefore appears to be more probable cause for decreased starch deposition in Notch - 2 grain as compared with NP 113. A recent study of the enzymes of starch metabolism in mutant Notch - 2 and its parent suggested that the lower level

of Adenine Diphosphate Glucose Pyrophosphorylase (ADGP) corresponds with the low level of starch.

Free sugars, total sugars, reducing sugars, non-reducing sugars and sucrose per grain in developing wheat grain followed closely the changes in the rate of starch accumulation and in general were maximum at the 14 day stage allowed by a decline up to 28 days with no significant alteration afterwards. Reducing sugars in ripening grains may arise due to the hydrolytic action of invertase and sucrose synthase on sucrose and may be stored in the endosperm either as sucrose or in the form of glucofructosans (Kumar and Singh, 1981). Their accumulation is grains until the 14th day would indicate that they are not utilised at the stage as active starch synthesis starts from 14 days onwards. A sharp decline afterwards indicated their utilization in the synthesis of starch. The presence of sugars in grains even when starch synthesis is complete might be due to the translocation of surplus sugars which are not converted into starch. This indicates that the termination of starch accumulation as grain matures is not due to ceasation of the supply of precursors but to loss in the synthetic capacity of the endosperm.

Adenine diphosphate glucose pyrophosphyorylase is most active during the period of maximum starch synthesis and it may be considered a rate - limiting step in starch accumulation in developing wheat grains. Similarly, Dickinson and Preiss (1969) reported that the conversion of glucose-1-phosphate by the action of this enzyme controls the rate of starch synthesis in corn endosperm. 3- phosphoglycerate is the positive effect

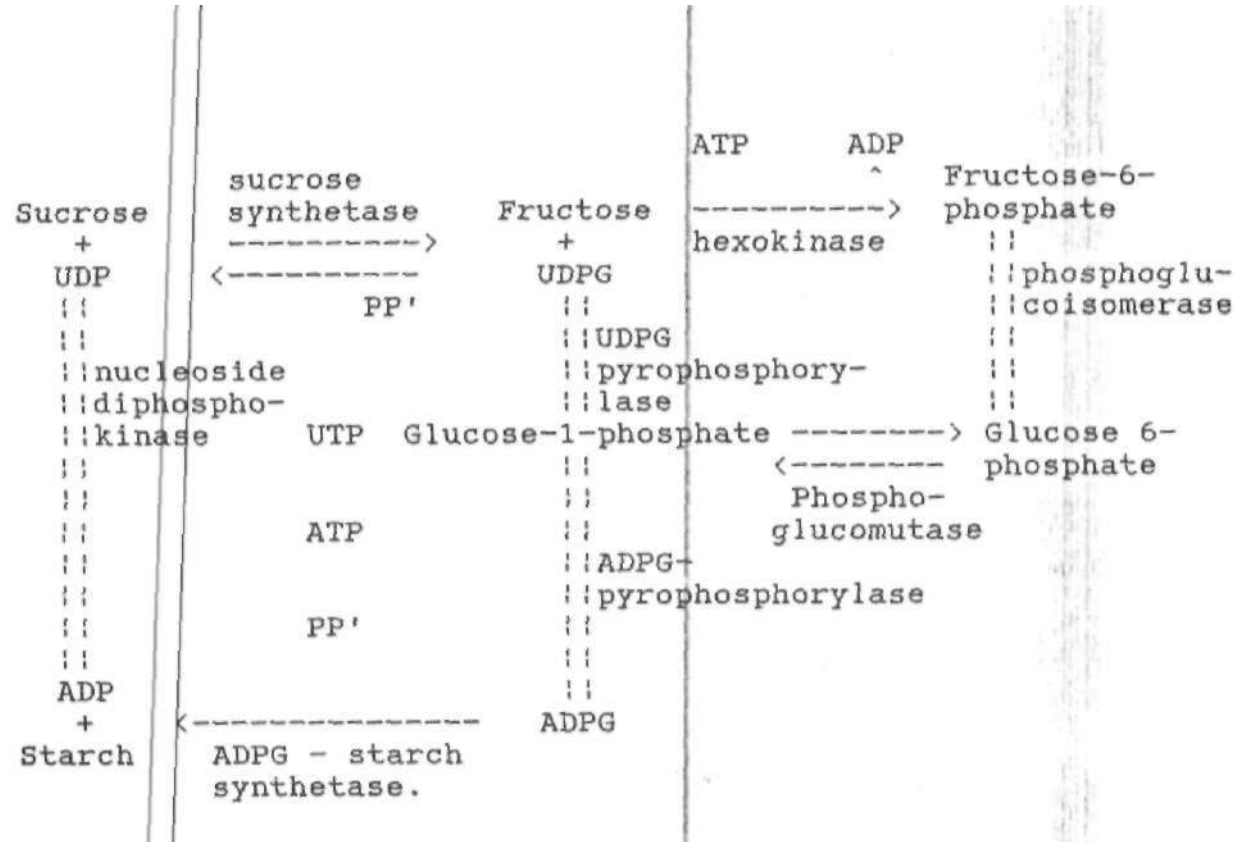
of this enzyme. Its level closely followed the changes in the rate of starch accumulation in rice grains (Villareal and Juliano 1977). It was assumed that 3-P-glycerate is concentrated in the pericarp as its level followed the level of chlorophyll which is found exclusively in pericarp. This was confirmed by Sin (1965) who found that the decline in the rate of starch accumulation in rice grains followed closely the visual disappearance of chlorophyll in the pericarp. Immature green barley and wheat have been shown capable of active photosynthesis and to contain ribulose-5-diphosphate and phosphoenol pyruvate carboxylases. 3-phosphoglycerate may occur in pericarp as a primary product of carbon fixation. Based on this, it was hypothesised that as long as the pericarp remains green, 3-phosphoglycerate is available to activate adenine diphosphate glucose pyrophosphorylase (ADPG) which further controls starch synthesis. Also there is a reverse relationship between starch content, reducing and non-reducing sugar contents in developing wheat grains (About-Guendia and D'Appolonia 1973).

Donovan et al (1977) also reported that Adenine Diphosphate glucose Pyrophosphorylase (ADPG) could be used as a critical determinant of the ceasation of starch accumulation. Turner (1969) suggested that these enzymes were under regulatory control by nucleotides.

The activity of uridine diphosphate glucose pyrophosphorylase in wheat has been reported to be much greater than that of ADPG pyrophosphorylase. However, both enzymes increased in activity during the phase of starch synthesis and

a sharp rise in ADPG pyrophosphorylase was associated with the onset of rapid starch formation. Adenine diphosphate glucose (ADPG) pyrophosphorylase activity decreased to a very low level when starch formation in the grain ceased and some with UDPG pyrophosphorylase. There was a sixfold increase in ADPG pyrophosphorylase on the 5th day period which coincided with the onset of the phase of rapid starch synthesis. Starch accumulation effectively ceased between 33 and 38 days from flowering and during these periods both ADPG and UDPG pyrophosphorylases activity declined (Turner, 1969).

Kumar and Singh (1981) reported on the relationship of starch metabolism and grain size in wheat grain. They showed that active starch synthesis started from 14 days onward and continued till 35 days after anthesis. Invertase was also reported to be active only during the initial stages of grain development. Sucrose-UDP glucosyltransferase had maximum activity at the 14th and 21st day stage and was present throughout the period of grain development. UDP-and ADP-glucose pyrophosphorylases and amylase were most active during the period of active starch synthesis and at the same time tended to parallel grain size and starch content at different stages of grain development (Kumar and Singh, 1981)



Scheme 2: Mechanism of starch synthesis (Turner, 1969).

1.5.4 LIPIDS

Jamieson and Baughman (1930), Sullivan and Bailey (1936), and many other cereal chemists worked on the fatty acid composition of wheat. Though values and composition vary from other cereals considerably, lipids of wheat have no particular nutritional advantage but make substantial contribution to dietary requirements.

The principal location of lipid is the germ (Shellenberger, 1971). Linolenic acid accounts for nearly half the total unsaturated fatty acids and palmitic acid is the major saturated fatty acid (Mechan, 1964). Lipid content ranges from 1.4% to 3.8% depending on the method of analysis, Kernel size, with variety having least effect (Pomeranz, 1968).

Processes involved in lipid degradation during storage is poorly understood. However, the functional effects of the many lipids in wheat on flour processing are many. Flour lipids are involved through a lipoxygenase system in the absorption of oxygen which occurs when a dough is mixed, and the lipoxygenase fatty-acid system may be involved in the maturing of dough by the oxidation of sulphurhydry (SH) groups (Summer, 1943) evidence that free fatty acids are oxidised by enzymes other than lipozygenase in dough mixing has also been reported (Bloksma, 1959; Cookson et al, 1957; Morrison and Maneely 1969). Wheat-germ fat such as unsaponifiable material and acylglycerol have little or no effect on the rheological properties of a patent flour but unsaturated fatty acids increase dough development time (Sullivan et al, 1936).

CHAPTER TWO
MATERIALS AND METHODS

Sample Collection

Three varieties of wheat, Frond, Pavon 76, and Siete cerros, were grown in open fields at the irrigation site of the Institute for Agricultural Research Samaru, Ahmadu Bello University, Zaria.

All varieties were harvested at 7, 14, 20, 30, and 34 days after anthesis. The samples were shelled and analysed immediately for moisture while the rest were kept in airtight containers of $0^{o}C$ prior to analyses.

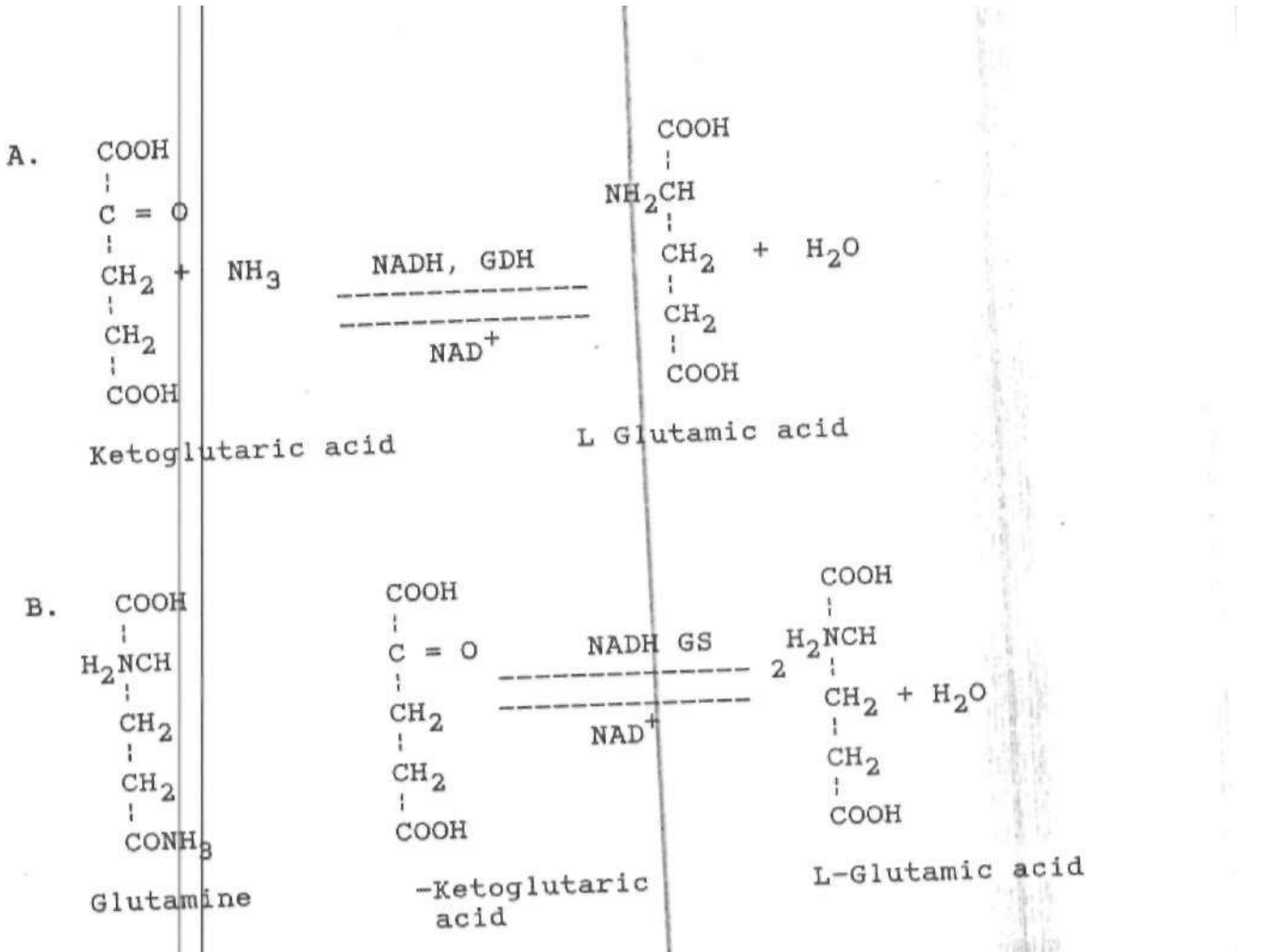
Sample Preparation

Stored grains (10g) were ground in a mortar with chilled (50ml) 0.1M tris-HCl buffer (pH 7.6).

The suspension was filtered and the filtrate centrifuged at 10,000g for 40mins. The resulting supernatant was decanted and used as source of crude enzyme for glutamate dehydrogenase (GH) and glutamate synthase (GS) analyses.

Enzyme Assays

Glutamate dehydrogenase and glutamate synthase were assayed spectrophotometrically using a Beckman DB-GT spectrophotometer to follow the oxidation of NADH to NAD^{+} at 340nm by the enzymes (scheme -2).



Scheme 2: A: Reaction sequence for the formation of L-glutamic acid from -Ketoglutamic acid and ammonia.

B: Reaction sequence for the formation of L-glutamic acid from glutamine and -Ketoglutamic acid.

The reaction mixtures for GH contained 0.1M tris-HCl buffer (pH7.6, 1.4ml), 3M NH_4Cl (o.1ml), 0.33M -Ketoglutamic acid (neutral, 0.1ml) 1mM NADH (0.2ml) and enzyme extract (0.1ml) all mixed together in a cuvette.

While the reaction mixtures for GS contained 0.1M tris-HCl buffer (pH.7.6, 0.5ml). 0.2M glutamine (0. 2ml), 0.2M - Ketoglutamic acid (neutral 0.2ml), Mercaptoethanol (1: 100v/v, 0.2ml) 0.1M KCl (0.3ml), 0.02M EDTA (0.2ml) 1mM NADH (0.2ml), and enzyme extract (0.1ml), all were mixed in a cuvette. The

rate of oxidation of NADH to NAD^+ was followed by monitoring the change in absorbance/min at 340nm using a Beckman DB-GT spectrophotometer. The activities of GH and GS were calculated as:

$$\text{Activity} = \frac{\text{A/min} \times V_t}{\text{ENADH at 340nm} \times \text{Va}}$$

Where A/min = change in absorbance per min.

Vt = Total vol. of enzyme extract

ENADH at 340nm = Extinction coefficient of NADH at 340nm.

Va = Volume of sample taken for assay.

Aliquots (0.1m) of the crude enzyme preparation were added to tubes containing 2% Sodium Carbonate (4.9ml), 2.7% sodium Potassium tartrate (0.05ml) and 1% copper sulphate (0.06ml) and thoroughly mixed using a votex mixer. The mixture was incubated for 10 minutes at room temperature. After incubation phenol reagent (0.5ml) was rapidly added to each tube, thoroughly mixed and incubated for 30 minutes at room temperature. The absorbance was read at 700nm in a Beckman DB-GT spectrophotometer. The protein content was calculated against bovine serum albumin standard.

CHEMICAL ANALYSIS

Moisture, ash and crude lipid were determined by the official methods of analysis (AOAC, 1980).

Total Available Carbohydrate

Aliquots (0.2g) of the finely ground dried samples were

weighed into 100ml beaker to which was added 10ml of distilled water. The mixture was stirred for 10mins to disperse all solids, followed by the addition of 13ml 52% Perchloric acid and again stirred for 20 ins. The samples were filtered and the filtrate transferred into 250ml volumetric flasks and made up to the mark.

The filtrate (0.1ml) was dispensed in triplicates into test tubes to which 5ml of freshly prepared anthrone-sulphuric acid reagent was rapidly added, thoroughly mixed and placed in a boiling water bath for exactly 20mins cooled rapidly to room temperature and the absorbance read at 630nm. The carbohydrate content was calculated against 0.1% D- glucose standard calculation in as follows:

$$\% \text{ Carbohydrate} = \frac{At}{Astd} \times Std \times \frac{100}{V}$$

where At = Absorbance of test

Astd = Absorbance of standard

Std = Concentration of standard

V = Volume of sample used.

Total Protein:

The total protein was determined by the Micro Kjeldahl method of AOAC (1970) and Purvis et al (1964).

Total protein concentration was calculated as follows:

$$\% \text{ Nitrogen} = \frac{(A - B) \times N \times 14.007 \times 100}{W}$$

% Protein = % Nitrogen x conversion factor (5.83).

where A = volume of HCl used

B = titre value of the blank

N = normality of HCl

W = weight of sample taken (g).

Crude Fibre

Crude fibre was determined according to the method of Shirlaw (1967).

Two grammes of dried, ground, defatted sample was boiled under reflux for exactly 30 minutes with 200ml of 1.25% sulphuric acid. The mixture was filtered under vacuum and then washed with boiling (or hot) water until neutral (pH = 7.0) the residue was boiled further in 200ml of 1.25% sodium hydroxide for 30 minutes, filtered and washed with hot water until neutral (pH = 7.00). The washing was continued with a little amount of ethanol. The residue was dried, cooled, weighed and transferred into a previously washed, dried and weighed crucible. The content of the crucible was incinerated in a muffle furnace at 450 - 500°C for four hours, after which it was cooled in a dessicator and finally weighed.

Calculations:

$$\% \text{ Crude Fibre} = \frac{\text{weight residue} - \text{weight of ash}}{\text{Original weight of sample}} \times 100$$

MINERAL ELEMENT

Mineral elements were determined according to the methods of AOAC (1970) and AMC (1960).

Wet Digestion of Sample

The method of the Analytical Method Committee, (1960)

which used nitric and perchloric acids as the decomposition reagent for organic matter was used. These acids have advantage of not producing insoluble sulphates which may absorb a considerable proportion of trace metals.

Sample Preparation

Two grammes of the dried ground sample was accurately weighed into a 200ml Kjeldahl flasks. To this was added 25ml of concentrated nitric acid and glass beads. The mixture was boiled slowly for 30 minutes under fume cupboard. This was cooled and 15ml of perchloric acid added. The mixture was boiled very gently for about one hour until it became colourless and dense white fumes evolved. This mixture was allowed to cool and quantitatively transferred to a 25ml volumetric flask and diluted to volume with distiled deionised water.

ELEMENTAL ANALYSIS

Atomic Absorption Spectrophotometry

The determination of elements, Magnesium, Copper, Iron, Zinc, Manganese, Cobalt and Calcium in the digested samples was carried out by the method of Osborne and Voogt (1978).

The solution obtained from the wet digestion was sprayed into the flame of an atomic absorption spectrophotometer SP-900 and the absorption of each metal was measured at a specific wavelength using appropriate standards (see Appendix 1).

Calculations

As for copper (see Appendix).

MANGANESE

Manganese was determined at 279.48nm with a maximum lamp current of 12mm and slit setting of 0.10 - 0.15mm. The flame system used was air-acetylene. The concentration of manganese in the sample solutions were read off from the calibration curve plotted from the readings of the standard solutions.

Magnesium, Calcium and Cobalt were determined and calculated as the rest of the elements.

RESULT AND DISCUSSION

ENZYME ASSAY

GLUTAMATE SYNTHASE (GS) ACTIVITY

Presented on table 1 is activity of GS during development. The activity of the three varieties of wheat kernel range from 5.9×10^{-3} - 10.5×10^{-3}, 4.8×10^{-3} - 9.6×10^{-3}, 3.2×10^{-3} - 9.05×10^{-3}, 3.0×10^{-2} - 8.2×10^{-3} and 3.6×10^{-2} - 9.8×10^{-2} uM/ml/min at 7, 14, 20, 30, and 34 days post anthesis respectively. The three cultivars showed significant ($P< 0.001$) difference as maturity was attained with Pavon 76 having the maximum activity of 9.8×10^{-2}uM at 34 days post anthesis. However, all varieties showed increase in activity as maturity progressed. This agrees with the work of Garg et al, (1984) who showed that glutamate activity per grain increased sharply from a very low value at day 10 after anthesis to a peak value at day 24 (0.86 - 1.22 nMole/min at 10 and 24 days respectively). However, the lower activity observed in this study could be as a result of varietal difference or non purification of the enzyme extract. It could be suggested that this increase in activity of glutamate synthase (GS) in developing wheat kernels could be an indication that the GS cycle is operative in the primary assimilation of ammonia in wheat kernel (Miflin and Lea, 1976; Sodek and Dasiva, 1977).

Glutamate Dehydrogenase (GH) activity (uM/ml/min) of the three wheat cultivars during development is presented in table 2. The three wheat cultivars showed no significant difference ($P<0.001$). However, the three wheat varieties showed significant ($P<o,001$) difference as maturity progressed, with

Table 1: Glutamate synthase (GS) activity (uM/ml/min) of developing wheat grains

Variety	Days Post Anthesis				
	7	14	20	30	34
Frond	5.9×10^{-3} $\pm 0.4^{ab}$	4.8×10^{-3} $\pm 0.02^{ab}$	3.6×10^{-3} $\pm 0.01^{ab}$	3.0×10^{-3} $\pm 0.3^{ab}$	3.6×10^{-3} $\pm 0.03^{ab}$
Pavon 76	10.5×10^{-3} $\pm 0.01^{ab}$	9.6×10^{-3} $\pm 0.01^{ab}$	9.05×10^{-3} $\pm 0.3^{ab}$	8.2×10^{-2} $\pm 0.5^{ab}$	9.8×10^{-2} $\pm 0.9^{ab}$
Siete-cerros	7.5×10^{-3} $\pm 0.02^{ab}$	7.2×10^{-3} $\pm 0.04^{a}$	3.2×10^{-3} $\pm 0.001^{ab}$	3.25×10^{-2} $\pm 0.5^{ab}$	4.0×10^{-2} $\pm 0.2^{ab}$

Results are expressed as mean ± standard deviation (n=4).
Figures bearing the same superscript letter are significant.
a = $p < 0.001$
b = $p < 0.05$

Table 2: Glutamate dehydrogenase activity (uM/ml/min) of developing wheat grains.

Variety	Frond	Pavon 76	Siete cerros
Days post anthesis			
7	$^{a}3.40 \times 10_{3}^{-3}$ ±1.7	$^{a}3.55 \times 10^{-3}$ ±0.15	$^{a}3.35 \times 10^{-3}$ ±0.05
14	$^{a}1.44 \times 10^{-2}$ ±0.53	$^{a}1.64 \times 10^{-2}$ ±0.04	$^{a}2.84 \times 10^{-2}$ ±0.04
20	$^{a}1.05 \times 10_{3}^{-2}$ ±0.01	$^{a}1.40 \times 10_{3}^{-2}$ ±0.11	$^{a}2.23 \times 10_{3}^{-2}$ ±0.05
30	$^{a}1.00 \times 10^{-2}$ ±0.35	$1.16_{a} \times 10_{3}^{-2}$ ±0.10	$^{a}2.37 \times 10_{3}^{-2}$ ±0.01
34	$^{a}3.7 \times 10_{3}^{-2}$ ±1.25	$4.8 \times 10_{3}^{-2}$ ±0.26	$^{a}3.6 \times 10_{3}^{-2}$ ±0.01

Results are expressed as mean ± standard deviation (n=4)
Figures bearing the same subscript number and superscript letter are significant.

3 = $p < 0.001$
a = $p < 0.001$

Pavon 76 showing the maximum activity at 4.8 x 10^{-2}uM/ml/min at 34 days post anthesis. However all varieties showed increased activity as maturity was attained. Garg *et al* (1984) showed that GH activity increased during kernel development (1.78 - 2.50 nMole/min at 10 and 31 days post anthesis respectively) with variety playing significant role.

It is also evident from the results obtained in this study that all of the wheat cultivars examined have at least the enzymic potential to assimilate ammonia via both the GH and GS pathways. The ability of the grain GS and GH of the three cultivars to utilise NADH is evidenced though the enzymes have not been sufficiently purified and characteristised to permit utilisation solely due to GS and GH. This infact could perhaps explain the non-correlation between the level of protein and activities of the enzymes.

The moisture content presented in table 3 of the three varieties of wheat kernel range from 66-70%, 53-59%, 42-45%, 9-29% and 6-8% for 7, 14, 20, 30, 34 days post anthesis respectively. There is no difference in the moisture content of the three varieties of wheat until at 30 days post anthesis, when the moisture content of Pavon 76 was significantly ($P<0.001$) lower than that of Frond and Siete cerros. There is, however, a significant ($P<0.05$) difference in the moisture content as maturity progressed in each variety. This difference could probably be attributed to the accumulation of dry matter in the wheat kernel, a process which is inversely relaed to the amount of moisture (D'Appolonia *et al*, 1971). Matured wheat kernels have been eported to have moisture

contents of between 10-12% and 14% (Kumar and Singh, 1981, Turner, 1969) respectively. The lower values observed in this study can possibly be due to the dry harmattan period at which the wheat varieties were cultivated. Low moisture content of some wheat varieties have beene related to its quality because it becomes too brittle and breaks easily, thus low quality flour (Ziegler and Greer, 1971).

The moisture content at 34 days post anthesis showed a precipitous drop from that of 30 days especially in the Frond and Siete cerros varieties. This change was probably due to the sudden stoppage of irrigation water from day 30 post anthesis onwards.

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Protein

The total protein content of the three varieties of developing wheat kernels showed significant ($P<0.001$) at 30 days post anthesis (Table 4 and 5). The protein content of Frond was significantly ($P<0.001$) higher than that of Pavon 76. However, all varieties showed similar increase in protein content from 7 days post anthesis with a maximum protein content at 30 days post anthesis for Frond and Siete cerros, and 14 days post anthesis for Pavon 76 (Table 4).

Increase in number and size of protein bodies during development of wheat kernel has been documented (Garg et al, 1984; Morton and Raison, 1964). The influence is that the soluble proteins (albumin and globulins) are being converted to storage proteins as evidenced in the progressive decrease in soluble protein content of whet kernels (Table 5). Protein

Table 3: Moisture content of developing wheat grains (%)

Variety	Days post anthesis 7	14	20	30	34
Frond	70.61[a] ±0.03	53.57[a] ±0.03	45.47[a] ±0.04	20.50[a*] ±0.01	6.86[a] ±0.01
Pavon 76	69.44[y] ±0.22	59.25[y] ±4.86	42.66[y] ±0.32	9.08[a*] ±1.95	7.08[a] ±0.05
Siete cerros	66.89[y] ±1.67	59.23[y] ±5.39	45.65[y] ±0.25	29.04[a*] ±7.45	7.73[a] ±0.10

Results are expressed as mean ± standard deviation (n=3)
Figures bearing the same superscript letter and or asterick are significant.

a = $p < 0.001$
y = $p < 0.05$
a* = $p < 0.05$

Table 4: Total protein (%) of developing wheat grains.

Variety	Days post anthesis 7	14	20	30	34
Frond	$11.78^{*c}_{\pm 0.07^{b}}$	$14.62^{*c}_{\pm 0.50^{*b}}$	$15.92^{c}_{\pm 0.01^{b}}$	$17.97^{c}_{\pm 0.01^{b}}$	$14.43^{c}_{\pm 0.50^{*b}}$
Pavon 76	$14.62^{c}_{\pm 0.01^{b}}$	$16.65^{c}_{\pm 0.40^{*b}}$	$16.37^{c}_{\pm 1.22^{*b}}$	$11.98^{c}_{\pm 0.61^{b}}$	$12.18^{c}_{\pm 0.60^{b}}$
Siete cerros	$11.78^{*c}_{\pm 0.41^{b}}$	$14.62^{*c}_{\pm 0.01^{*b}}$	$14.15^{c}_{\pm 0.11^{*b}}$	$14.62^{c}_{\pm 0.01^{*b}}$	$9.54^{c}_{\pm 1.10^{b}}$

Results are expressed as mean ± standard deviation (n=3)
Figures bearing the same superscript (vertical) and subscript (horizontal) letter are significant while those with asterick are not significant.

c = $p < 0.001$
b = $p < 0.005$

Table 5: Soluble protein (%) of developing wheat grains.

Variety	Days post anthesis 7	14	20	30	34
Frond	$1.64^{c}_{\pm 0.03^{b}}$	$1.63^{*c}_{\pm 0.06^{b}}$	$0.65^{*c}_{\pm 0.01^{b}}$	$0.53^{*}_{\pm 0.60^{b}}$	$0.51^{c}_{\pm 0.02^{b}}$
Pavon 76	$1.47^{c}_{\pm 0.02^{b}}$	$1.56^{c}_{\pm 0.05^{b}}$	$0.66^{*c}_{\pm 0.03^{b}}$	$0.34^{*}_{\pm 0.03^{b}}$	$0.34^{c}_{\pm 0.05^{b}}$
Siete cerros	$1.41^{c}_{\pm 0.01^{b}}$	$1.69^{*c}_{\pm 0.02^{b}}$	$0.60^{c}_{\pm 0.02^{b}}$	$0.32^{*}_{\pm 0.04^{b}}$	$0.28^{c}_{\pm 0.01^{b}}$

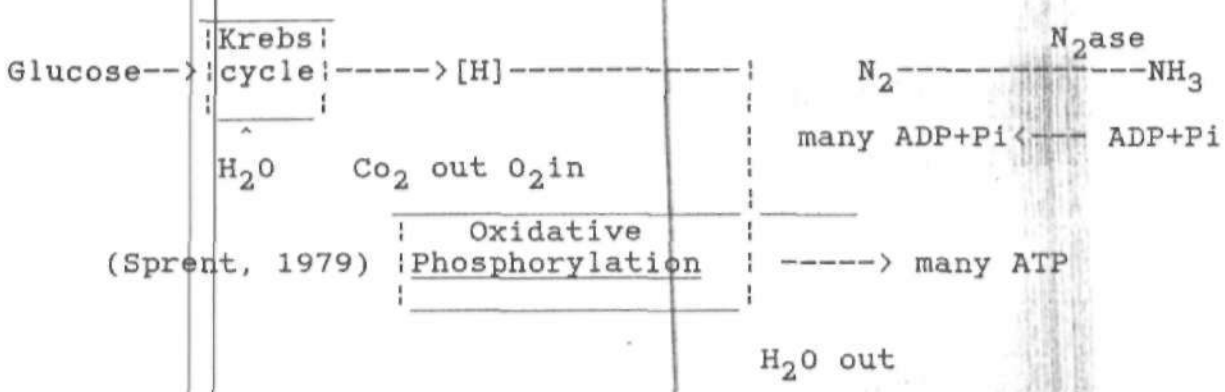
Results are expressed as mean ± standard deviation (n=3)
Results bearing the same superscript (vertical) and subscript (horizontal) letter are significant while those with asterick are not significant.

c = $p < 0.001$ *c and *b are not significant
b = $p < 0.05$

content varies from about 6 - 20%. This depends on variety and class but more largely on environmental factors during growth (Kasarda et al, 1971). Protein content is also influenced considerably by the available soil nitrogen; heavy nitrogen fertilisation increases the protein content of wheat kernels. The significant ($P<0.05$) decrease of the protein content in Pavon 76 and Siete cerros could be as a result of the heavy glood of irrigation water at this period. Since abundant rainfall has been documented to result in low protein content during kernel development (Kasarda et al, 1971).

Total Carbohydrates

The carbohydrate contents in the three cultivars of developing wheat grains was significantly ($P< 0.05$) higher at 7 days post anthesis (Table 6). The carbohydrate content of all the cultivars decreased as maturity progressed. There was also significant ($P< 0.05$) difference between the carbohydrate conent of Pavon 76 and the other two varieties (Frond and Siete cerros). Increase in carbohydrate content during kernel development have been documented (Kapoor and Heiner, 1976; Donovan et al, 1977; Jennings and Morton, 1963). Conversely, result obtained in this study showed decrease in carbohydrate content during development. The inference could be either that the soluble carbohydrate content are actively mobilised and used for the synthesis of ATP used in Nitrogen fixation as shown below:



OR that it can possibly be due to the non hydrolysis of the non-reducing sugars by the acid extraction procedure.

A massive accumulation of amides glutamine and asparagine was accompanied by rapid lose of tissue carbohydrate (Miflin and Lea, 1980). It could also be deduced that since only soluble fraction of total carbohydrate was assayed, perhaps there is conversion to structural carbohydrates thus the decrease.

Crude Fibre

The crude fibre content (presented in Table 7) of the three varieties of wheat kernel range from 0.41-0.61%, 1.40-3.08%, 6.17-13.29% and 6.25-13.83% at 14, 20, 30 and 34 days post anthesis respectively. There is significant ($P<0.05$) difference between the three varieties at all stages of development Siete cerros differ significantly ($P<0.05$) from Pavon 76 and Frond having the least crude fibre content at all stages of development.

Both crude fibre and ash content of wheat kernels are related to the amount of bran in wheat, small or shriveled kernels usually have more bran on a % basis and therefore more crude fibre and ash. Crude fibre content is usually in the

Table 6: Total available carbohydrate (%) of developing wheat grains

Variety	Days post anthesis				
	7	14	20	30	34
Frond	$81.79^{*c}_{\pm 6.79^{b}}$	$70.65^{*c}_{\pm 4.33^{b}}$	$65.62^{c}_{\pm 0.02^{b}}$	$59.59^{*c}_{\pm 0.06^{b}}$	$51.01^{c}_{\pm 0.01^{b}}$
Pavon 76	$71.18^{c}_{\pm 5.30^{b}}$	$63.36^{c}_{\pm 2.48^{b}}$	$59.65^{c}_{\pm 0.55^{b}}$	$60.00^{c}_{\pm 2.50^{b}}$	$54.90^{c}_{\pm 0.39^{b}}$
Siete cerros	$79.80^{*c}_{\pm 5.28^{b}}$	$70.11^{*c}_{\pm 5.82^{b}}$	$62.51^{c}_{\pm 0.02^{b}}$	$58.19^{*c}_{\pm 1.70^{b}}$	$54.50^{*c}_{\pm 1.00^{b}}$

Results are expressed as mean ± standard deviation (n=3)
Figures bearing the same superscript (vertical) and subscript (horizontal) letter are significant while those with asterick are not significant.

c = $p < 0.001$
b = $p < 0.005$

Table 7: Crude fibre (%) of developing wheat grains.

Variety	Days post anthesis				
	7	14	20	30	34
Frond	Nd	0.59^{c} $\pm 0.06^{b}$	2.35^{c} $\pm 0.06^{b}$	13.29^{c} $\pm 1.59^{*b}$	13.83^{c} $\pm 2.01^{*b}$
Pavon 76	Nd	0.61^{c} $\pm 0.01^{b}$	3.08^{c} $\pm 0.03^{b}$	10.52^{c} $\pm 0.24^{b}$	11.02^{c} $\pm 0.24^{b}$
Siete cerros	Nd	0.41^{c} $\pm 0.02^{b}$	1.40^{c} $\pm 0.19^{b}$	6.17^{c} $\pm 0.13^{b}$	6.25^{c} $\pm 0.13^{b}$

Nd = not detected.
Results are expressed as mean ± standard deviation (n=3).
Figures bearing the same superscript (vertical) and subscript (horizontal) are significant while those with asterick are not significant.

c = $p < 0.05$
b = $p < 0.001$

range of 2.0 to 2.7% and the ash 2.4 to 2.0%, both calculated to 14% moisture basis (D'Appolonia, 1971). However, results obtained in this study for the Crude Fibre content is much higher than that reported. This could probably be due to the difference in variety. Also the increase in Crude Fibre content as the wheat kernel developed could be attributed to the increased structural complexity of the wheat kernel.

Total Lipid

The total lipid content (presented in Table 8) of the three developing wheat cultivar showed significant ($P<0.001$) difference as maturity was attained. Also the three cultivars differ significantly($P<0.05$) at all stages of development. The total lipid content of the three varieties obtained in this study ranged from 23.3-27.7%, 17.8-20.2%, 18.7-19.6%,n0.45-0.81%, and 0.91-1.19% at 7, 14, 20, 30 and 34 days post anthesis respectively.

Total lipid content of 2.5-3.8% in 20 North American wheat of widely different character and origin were documented, Sullivan and Near (1927); averages of 2.52% lipid content, with a range of 2.1 to 3.3% in 32 samples was reported (Pomeranz et al, 1966).

Fat extractable petroleum ether was reported to range from 1.4 to 2.6% (Mechan, 1971). Total fat content obtained in this study is comparable with that of Mechan, (1971). However, the slight discrepancy could be as a result of varietal diffeerence, as documented by Sullivan and Near, (1927). It could also be suggested that because of the loation of lipids

Table 8: Crude lipid (%) of developing wheat grains.

	Days post anthesis				
Variety	7	14	20	30	34
Frond	27.72^{*c} $\pm 5.10^{b}$	20.17^{c} $\pm 6.20^{b}$	19.67^{c} $\pm 4.51^{b}$	0.68^{c} $\pm 0.01^{b}$	1.13^{c} $\pm 0.06^{b}$
Pavon 76	23.33^{*c} $\pm 0.16^{b}$	17.86^{c} $\pm 0.31^{b}$	18.71^{c} $\pm 0.13^{b}$	0.45^{c} $\pm 0.16^{b}$	0.91^{c} $\pm 0.09^{b}$
Siete cerros	25.33^{c} $\pm 1.29^{b}$	19.34^{c} $\pm 1.29^{b}$	19.14^{c} $\pm 0.09^{b}$	0.81^{c} $\pm 0.02^{b}$	1.19^{c} $\pm 0.23^{b}$

Results are expressed as mean ± standard deviation (n=3).
Figures bearing the same superscript (vertical) and subscript (horizontal) are significant while those with asterick are not significant.

c = $p < 0.005$
b = $p < 0.05$
*c = not significant.

in structures (starch granules, aleurone cells) or protected films (gluten matrix) extraction solvents are impermeable. Thus the lower values observed (Mechan and Mohammed, 1955). Also because the proess of Nitrogen fixation is energy consuming, and carbohydrate components are actively utilised, it could be suggested that lipids through gluconeogenesis are being converted to carbohydrates. Thus the decrease in lipid content during development of Kernel size could also influencee t4he lipid content (Chiu and Pomeranz, 1967).

The mineral content of whole wheat grain is poorly documented. However, reports in mineral content as it affects flour quality are numerous.

Iron

The iron content of the 3 developing wheat grains showed significant ($P<0.001$) difference as maturity progressed (Table 9). Frond and Siete Cerros showed no significant ($P<0.05$) difference at 7 days post anthesis. No particular pattern was followed by the three cultivars, but all showed lower values at 34 than 7 days post anthesis.

Iron content range from 39 - 67 (ppm) in Pakistani wheat varieties (Khan and Eggom, 1976). However, the high values obtained in this study could be attributed to the diffeerence in variety and soil availability of Iron to the plant. Iron, aside from its role in the various enzyme system, plays no particular role in the final product of wheat grains.

Calcium

The calcium content presented in Table 10 showed significant ($P<0.001$) difference as maturity progressed except

Table 9: Iron content (ppm) of 3 cultivars of developing wheat grains.

Variety	Days post anthesis				
	7	14	20	30	34
Frond	125.00^{*c}_{*b} $\pm 0.3^{*b}$	32.50^{c}_{b} $\pm 0.01^{b}$	202.50^{c}_{b} $\pm 0.6^{b}$	125.00^{c}_{ab} $\pm 0.7^{ab}$	31.25^{c}_{b} $\pm 0.01^{b}$
Pavon 76	200.00^{c}_{*b} $\pm 0.20^{*b}$	176.55^{c}_{b} $\pm 0.75^{b}$	103.60^{c}_{b} $\pm 0.90^{b}$	200.00^{c}_{*b} $\pm 0.50^{*b}$	111.50^{c}_{b} $\pm 1.5^{b}$
Siete cerros	125.00^{*c}_{*b} $\pm 0.57^{*b}$	200.00^{c}_{b} $\pm 3.45^{b}$	30.60^{c}_{b} $\pm 5.73^{b}$	251.20^{c}_{*b} $\pm 8.02^{*b}$	127.20^{c}_{b} $\pm 11.48^{b}$

Results are expressed as mean ± standard deviation (n=3).
Figures bearing the same superscript (vertical) and subscript (horizontal) are significant while those with asterick are not significant.

c = $p < 0.05$
b = $p < 0.001$

Table 10: Calcium content (ppm) of 3 varieties of developing wheat grains.

Variety	Days post anthesis				
	7	14	20	30	34
Frond	$2026.50^{c}_{\pm 3.50^{b}}$	$752.50^{*c}_{\pm 1.25^{b}}$	$652.50^{c}_{\pm 0.01^{b}}$	$650.00^{c}_{\pm 0.01}$	$400.00^{c}_{\pm 0.51^{b}}$
Pavon 76	$1350.00^{c}_{\pm 6.01^{b}}$	$990.00^{c}_{\pm 3.20^{b}}$	$638.50^{c}_{\pm 0.50^{b}}$	$525.00^{c}_{\pm 0.01^{*b}}$	$525.00^{c}_{\pm 0.02^{*b}}$
Siete cerros	$1450.00^{c}_{\pm 4.00^{b}}$	$752.50^{*c}_{\pm 0.10^{b}}$	$610.50^{c}_{\pm 0.05^{b}}$	$825.00^{c}_{\pm 0.09^{b}}$	$652.50^{c}_{\pm 0.31^{b}}$

Results are expressed as mean ± standard deviation (n=3).
Figures bearing the same superscript (vertical) and subscript (horizontal) are significant while those with asterick are not significant.

c = $p < 0.001$
b = $p < 0.001$

Siete cerros which increased at 30 days post anthesis.

Calcium content range from 2.4 - 4 (ppm) in five varieties of wheat (Kartz and Querry, 1965). The outrageously high values reported in this study could be the reason why our local wheat varieties are not acceptable to bakers, since calcium exerts softening effect on gluten, resulting in some loss of dough quality.

Manganese

The manganese content presented in Table 11 showed significant ($P<0.001$) difference with Siete Cerros having the least value at 7 days post anthesis and highest value at maturity. However, all varieties showed decrease in the manganese content as maturity progressed. There is little or no reports on the role manganese plays on wheat quality, however, its role in chloroplast cannot be ovder emphasised. Therefore he decreasing trend in the manganese content could be attributed to the reduction in photosynthesis as maturity progressed.

Magnesium

The magnesium content presented in Table 12 showed significant ($P<0.001$) difference between the three varieties at all stages of maturity. However, all varieties decreased at 14 days except Siete cerros which increased and decreased at 20 days post anthesis.

There is a direct relationship between magnesium content and the strength of wheat as determind by Proein percent and gluten quality (Pomeranz, 1968). It could be observed that at some instances where total protein content decreased or

increased with maturity, so also did the magnesium content decrease or increase as the case may be.

Zinc

The zinc content presented in Table 13 showed significant ($P<0.001$) in all the varieties as maturity progressed. No particular pattern was shown by any variety but all the varieties showed a peak at 20 days post anthesis. Zinc is a cofactor in the glutamate dehydrogenase (GH) reaction, and it will be recalled that there was increase in GH activity between 14 to 30 days post anthesis which could be observed to correspond with the peak value of zinc at 20 days post anthesis.

Table 11: Manganese content (ppm) of 3 cultivars of developing wheat grains.

Variety	Days post anthesis				
	7	14	20	30	34
Frond	$127.50^{c}_{\pm 0.01^{b}}$	$87.50^{c}_{\pm 0.20^{b}}$	$37.50^{c}_{\pm 0.50^{b}}$	$50.00^{c}_{\pm 0.02^{b}}$	$55.00^{c}_{\pm 0.01^{b}}$
Pavon 76	$83.70^{c}_{\pm 0.71^{b}}$	$58.80^{c}_{\pm 0.44^{b}}$	$56.60^{c}_{\pm 0.49^{b}}$	$37.50^{c}_{\pm 0.09^{b}}$	$37.50^{c}_{\pm 0.01^{b}}$
Siete cerros	$65.00^{c}_{\pm 0.98^{*b}}$	$65.00^{c}_{\pm 0.21^{b}}$	$58.80^{c}_{\pm 0.01^{b}}$	$52.50^{c}_{\pm 0.82^{b}}$	$66.20^{c}_{\pm 0.45^{b}}$

Results are expressed as mean ± standard deviation (n=3).
Figures bearing the same superscript (vertical) and subscript (horizontal) are significant while those with asterick are not significant.

c = $p < 0.001$
b = $p < 0.001$

Table 12: Magnesium content (ppm) of 3 cultivars of developing wheat grains.

	Days post anthesis				
Variety	7	14	20	30	34
Frond	145.0^{c} $\pm 0.05^{b}$	63.30^{c} $\pm 0.06^{b}$	97.00^{*c} $\pm 0.09^{b}$	115.0^{c} $\pm 0.02^{b}$	107.5^{c} $\pm 0.03^{b}$
Pavon 76	137.50^{c} $\pm 0.90^{b}$	94.00^{c} $\pm 0.66^{b}$	113.20^{c} $\pm 0.66^{b}$	109.50^{c} $\pm 0.79^{b}$	123.70^{c} $\pm 0.01^{b}$
Siete cerros	127.50^{c} $\pm 0.06^{b}$	137.00^{c} $\pm 0.79^{b}$	96.50^{*c} $\pm 0.72^{b}$	110.00^{c} $\pm 0.64^{b}$	117.50^{c} $\pm 0.38^{b}$

Results are expressed as mean ± standard deviation (n=3).
Figures bearing the same superscript (vertical) and subscript (horizontal) are significant while those with asterick are not significant.

c = $p < 0.001$
b = $p < 0.001$

Table 13: Zinc content (ppm) of 3 cultivars of developing wheat grains.

Variety	Days post anthesis				
	7	14	20	30	34
Frond	$57.50^{c}_{\pm 0.05^{b}}$	$75.00^{*c}_{\pm 0.04^{b}}$	$45.00^{c}_{\pm 0.40^{b}}$	$42.50^{c}_{\pm 0.06^{*b}}$	$42.50^{*c}_{\pm 0.10^{*b}}$
Pavon 76	$75.00^{c}_{\pm 0.01^{b}}$	$63.80^{c}_{\pm 0.60^{b}}$	$41.50^{c}_{\pm 1.00^{*b}}$	$58.80^{c}_{\pm 0.83^{b}}$	$41.20^{*c}_{\pm 0.92^{*b}}$
Siete cerros	$60.20^{c}_{\pm 0.54^{b}}$	$75.00^{*c}_{\pm 0.90^{b}}$	$44.40^{*c}_{\pm 0.82^{b}}$	$48.50^{c}_{\pm 0.13^{b}}$	$46.50^{c}_{\pm 0.53^{b}}$

Results are expressed as mean ± standard deviation (n=3). Figures bearing the same superscript (vertical) and subscript (horizontal) are significant while those with asterick are not significant.

c = $p < 0.001$
b = $p < 0.001$
*c = $p < 0.001$

CONCLUSION

Evaluation of the compositional changes of developing wheat cultivars, Frond, Pavon 76 and Siete cerros revealed differece in protein, total carbohydrate, ash and lipid contents. Glutamate synthase and glutamate dehydrogenase activity was also observed that all varieties showed higher composition during the early stages of development. However, Frond showed highest contents of total protein, crude fibre and glutamate dehydrogenase activity and the least moisture and carbohydrate contents at maturity. Frond seem to show the best qualities for improvement.

Greater number of cultivars, extension of final harvest period to 40 days post anthesis, the effect of genetic component variability and the environmental effect on the various parameters have to be studied before any variety could be suggested for improvement.

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APPENDIX I: PRINCIPLE

ATOMIC ABSORPTION SPECTROPHOTOMETRY

In this technique a sample is aspirated into a flame and atomised. A light beam is directed through the flame into a monochrometer and into a detector that measures the amount of light absorbed. Absorption is more sensitive because it depends upon the presence of free unexcited atoms. In the usual flame, the ratio of unexcited to the excited atoms at a given moment is very high. Because each metallic element has its own characteristic absorption wavelength, a source light composed of that element is employed which makes the method free of spectral or radiation interferences. Thus, the amount absorbed in the flame is proportional to the concentration of the element in the sample.

To determine the concentration of a particular metal in an unknown sample, a calibration curve of concentration versus absorbance is usually prepared, using standard solution prepared from the stock solution.

METHOD

Copper was determined at 324.75nm with a maximum lamp current of 5mA slit setting of 0.15 - 0.20mm. The flame system was air-acetylene. The concentrations of copper in the sample solutions were read off from the calibration curve plotted from the readings of the standard solutions.

CALCULATIONS

The method employed is that of the American Water Works

Association, American Public Health Association and Water Pollution Control Federation (1985).

$$\text{Metal Concentration (ppm)} = \frac{A \times B}{C}$$

Where: A = Concentration of metal in digested solutions, (ppm) OR Graphical Reading.

B = Final volume of digested solution (ml) and

C = Sample size (gm).

IRON

Iron was determined at 248.33nm with maximum lamp current of 15mA and slit setting of 0.10 - 0.15mm of iron in the sample solution were off from the calibration curve plotted from readings of the standard solutions.

Calculation

As for copper.

ZINC

Zinc was estimated at 2139nm with a maximum lamp current of 10mA and slit setting of 0.10 - 0.15mm. The flame system was air-acetylene, the concentration of zinc in the sample solutions were read off from the calibration curve plotted from the readings of the standard solutions.