STUDIES ON THE PROXIMATE AND ANTINUTRITIONAL FACTOR COMPOSITIONS OF MILLED FRACTIONS OF A LOCAL VARIETY OF WHEAT (Triticum aestivum)

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DECLARATION

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. All quotations are indicated and sources of information are specifically acknowledged by means of references.

27/1/93

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CERTIFICATION

This thesis entitled 'Studies on the Proximate and Antinutritional Factor Compositions of Milled Fractions of a Local Variety of Wheat (Triticum aestivum) by 'Efe Mouson Omwirhiren meets the regulations governing the award of the Degree of Master of Science (M.Sc.) in Biochemistry of Ahmadu Bello University and is approved for its contribution to knwoledge and literary presentation.

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DEDICATION

This work is dedicated to 'Oseme' Oritse and 'Omonime' Otemu my father and brother whose encouragement has meant so much to me.

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Many things one do in life are meant for posterity to judge based on the compelling force behind them.

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EMO

ABSTRACT

The proximate composition, and antinutritional factor levels of local variety of wheat (Triticum aestivu and its commercially milled fractions (flour, dunst, germ, bran, fine middling and semolina) which were obtained from a local mill industry were determined.

The objective of the study were to determine the proximate and antinutritional factor composition of the wheat grain and its commercially milled fractions so as to ascertain the nutritional value of the various fractions as well as the profitable employment of the Nigerian growth wheat flour in bread making.

Proximate data revealed that the germ fraction was significantly higher ($P<0.05$) in crude protein and lipid contents than those of the other fractions with a value of 18.36% and 5.79% respectively while the bran fraction was significantly higher ($P<0.05$) in ash and crude fibre contents than each of the other fractions with a value of 7.16%. The flour fraction was observed to have the highest concentration of soluble sugar and starch with a partial distribution within the other fractions. The in vitro protein digestibility showed that the fine middling had the highest (96.43%) among the fractions while the flour had highest (76.79%) postcooking digestibility.

The level of oxalate, tannin, phytate-phosphorus and trypsin inhibitors was significantly higher ($P<0.05$) in the fine middling (52mg/100g), Semolina (3.6mg/100g), germ (9.5mg/100g) and dunst fractions

(329mg/100g) while the concentration of these antinutritional factors were higher in wheat bran than all fractions. This profile compares favourably with other commercially recovered wheat. The potential advantage of employing Nigerian wheat in bread making is discussed.

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Fig. 1. Standard Catechin Curve

Fig. 2. Standard Phosphorus Curve

Abbreviations, Definitions, Glossaries and Symbols

A.B.U.	=	Ahmadu Bello University
et al	=	et alia, and others
e.t.c.	=	et cetera, and so on
e.g.	=	for example
g	=	gramme(s)
GIT	=	Gastrointestinal tract
pH	=	Negative log of hydrogen ion concentration in moles
IVPD	=	In vitro protein digestibility
m/v	=	Mass to volume
mg	=	milligramme
ml	=	millilitre
U	=	Micron
M	=	Molarity
N	=	Normality
ppm	=	Part Per million
S.D.	=	Standard Deviation
1b	=	pound
bu	=	equivalent
C.E.	=	Catechin equivalent
v/v	=	Volume to volume
wt	=	weight
w/v	=	weight to volume
OSU	=	Oklahoma State University, Stillwater, Okla.

1.0 INTRODUCTION

The unique role of cereals as a major source of raw materials, for industries (e.g. beer industry) have resulted in the mass production and cultivation of these food crops annually. Of all known cereals, wheat has received quality considerable attention because of its utilization as a major source of food in developed countries. Available evidence however indicates that the cultivation of local varieties of wheat have received very little scientific attention in Nigeria.

The cereal wheat has been identified as a member of the grass family Gramineae. At present, fourteen species of wheat which belongs to the genus Triticum has been identified. Wheat has been classified into a 3 subgroups namely diploid, tetraploid and hexaploid based on the number of chromosomes in their reproductive cells. Essentially, the hexaploid form known botanically as Triticum aestivum remains the most widely grown variety of wheat (Reitz, 1967). In its physical form, the wheat grain is either hard or soft in texture and may appear as brownish red or white.

Among all cereals, wheat has remained the most widely cultivated cereal crop, especially in developed countries. Reitz (1967) reported that 3.2 billion tons of wheat are grown annually. Its economic importance can be traced to its wide consumption as food in developed countries. The primary use of wheat is in its form of milled or baked products which has resulted in the establishment of two giant industries, the flour milling and commercial baking (Inglet, 1974).

The process of flour milling involve a detailed separation of wheat into fractions. These constituent includes semolina, which refers to the pure endosperm of the wheat grain. It is often used as thick porridge and use in making pasta products such as macaroni. Others include the wheat bran which refers to the coarse outer coat of the wheat grain, the wheat middling, which is the immediate layer before the wheat bran and the wheat germ which constitutes the embryonic part of the kernel. The dunst is the coarse flour still attached to the bran and germ parts while the flour is the pure endosperm. Nutritionally, wheat flour appears to be the most widely used form of wheat. It is used principally for bread making, although other foods such as crackers, cookies, biscuits, cakes, doughnuts, pancake, ice-cream, sphagetti, pudding and various hot and ready to make breakfast foods.

All over the world, bread has been recognised as a traditional food. The introduction of bread by Nigerian soldiers returning from the Second World War, for instance, marked a new trend in wheat consumption in Nigeria (Olugbemi,1973). The estimate of wheat flour importation into Nigeria amounted to 57,000 tons in 1954 and increased to 1,258 million tons (Nigeria Trade Journal, 1961; Fed. Min. Agric. (FMA Bulletin, 1984). Because of huge amount of money lost annually to developed countries through wheat importation, the Nigerian government had to ban the importation of wheat in 1979. This singular factor has led to an orientation of values which forced the Nigerian baking

industry to explore the possibility of production and cultivation of local wheat varieties, especially since all other cereal crops had not been able to maintain such a unique characteristics exhibited by wheat in bread production. The government's concern for the improvement in the yield of the local variety, the baking quality and the nutritional value of the Nigerian growth wheat, led to the establishment of the Samaru Wheat Breeding Programme of the Institute for Agricultural Research, Samaru (Kubiczek, et al, 1987).

The need for local varieties resulted in direct investigation by breeders into the possibility of producing suitable strain of wheat and so far present many varieties have been identified with good milling properties among which are: Florence Aurore 8193, UQ 105, Sonora 63, Anza and 301 SON - 64 Pi 62 (Olugbemi, 1980).

It is known that the chemical composition of wheat and its desirable quality for bread making is markedly influenced by environmental conditions, grain yield and available nitrogen as well as the genotype of the variety (Schmidt, 1974). The present study was therefore designed to:

1) determine the proximate composition of the different commercial milled fractions of locally produced wheat.
2) determine the in vitro protein digestibility of wheat grain and its industrial milled fractions.
3) determine the level of some antinutritional substances in the wheat grain and their distribution in the milled fractions.

2.0 LITERATURE REVIEW

2.1 Structure of Wheat Kernel

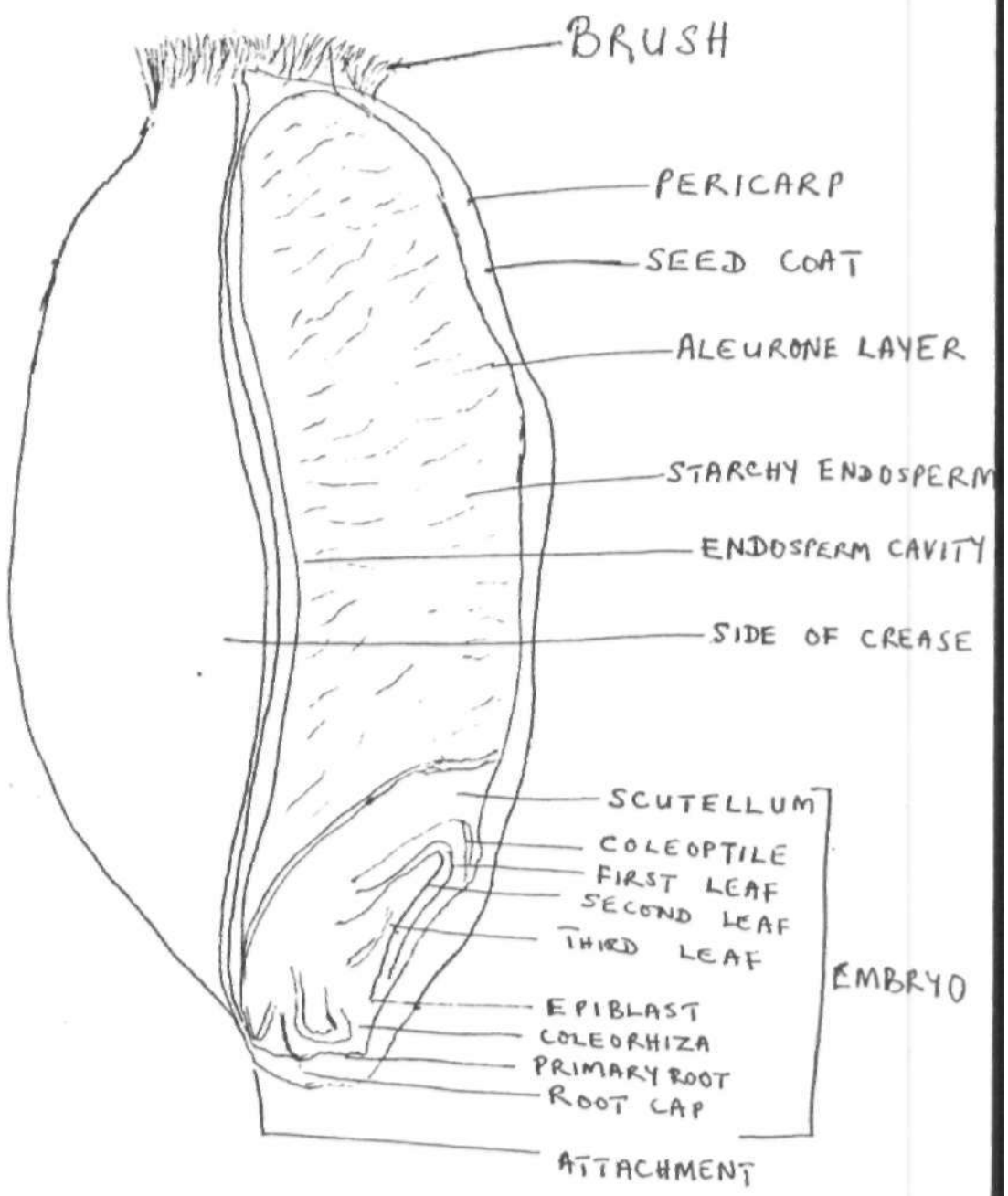
A wheat kernel is often referred to as one seeded fruit or botanically the caryopsis of the common wheat plant, Triticum aestivum (Reitz, 1967). Kernel composition varies more widely in wheat than any other cereal grain. Although the usual range for protein is from 8-15%, values as high as 24% have been reported (McCance et al, 1945). Genetic differences and climate conditions, such as temperature, rainfall, cultivation and soil characteritics, are mainly responsible for compositional variations (Johnson et al, 1973).

The anatomical structure of mature hard red winter wheat was extensively studied by Bradbury et al (1956) and the total microscopic structure of the grain tissues are: pericarp (5-8%), aleurone layer (6.0-7.0%), endosperm (81.0-83.0%), embryo (1.0-1.5%) and scutellum (1.5-2.0%).

Surrounding the kernel is the pericarp which can be subdivided into the epidermis and the inner pericarp or endocarp. Beneath the pericarp tissue is the seed coat or testa and a hyaline layer, so called because it appears bright when seen through a microscope (McMasters et al, 1971). The aleurone layer is one cell thick and morphologically is the outermost part of the endosperm. The endosperm including the aleurone tissue is responsible for nearly 90% of the total weight of the kernel. Inside the aleurone layer, the

endosperm consists of cells tightly packed with starch granules embedded in a protein matrix. Its structure is not homogenous, but varies depending upon location. The cells underlying the aleurone layers are small and cubical in shape while those closer to the centre are larger and polygonal. The outer part of the endosperm is usually vitreous or translucent, often called horny or flinty while the inner part is opaque (Bradbury et al, 1956b); The cells of the subaleurone layer of USA hard red winter wheat (13.7% protein) has a protein content of about 50% (Kent, 1966). The inner endosperm of same wheat has a protein content of about 8%. Evers (1970) attributed this difference to the inner endosperm. Endosperm texture is one of the most constant characteristics associated with different classes and varieties. Soft red winter wheat has either a hard endosperm or a combination of vitreous and soft endosperm (D'Appolonia, 1971).

The germ or embryo is composed of two major parts, namely, the embryonic axis, which during germination develops into the seedling and the scutellum which provides nutrients to the seedling. The embryonic axis is composed of the shoot (plumule) and the primary root (Evers, 1970). This is as shown in figure 1.



1 LONGITUDINAL SECTION OF A WHEAT GRAIN (Triticum aestivum) x 25

2.2 Flour Milling

The object of milling is to separate the three main parts of the wheat kernel into products commonly known as flour bran and (Cerning and Guilbot, 1974). The milling process varies largely among flour mills within one country and the differences are even greater when compared to processing technigues in another country. Furthermore the mill- mix obtained depends on the origional wheat and great difficulties are encountered in terminologt and translation of the various milling fractions.

Comparative study on typical America millings by Sannders *et at* (1969) showed that when the wheat kernel is milled to produce flour, a complex series of roller mills and sifters are used to separate out the maximum amount of flour which comprises at the least 72% of the wheat kernel is termed wheat millfeed. Mill streams combined to make a series of products of varying composition as follows: (Crampton and Harris, 1969)

Wheat bran is a mixture of the coarse outer covering of the wheat kernel flour and some fine ground weed seeds and other non- wheat material. Wheat milling comprises the layer of the kernel just inside the the outer bran covering , flour bran particles and other non- wheat materials.

Wheat shorts are those fractions which comprise the layer of the wheat kernel just inside the inner bran covering and near the outer endosperm. Wheat germ meal is the enbryonic portion of the kernel and can be separated out during the milling process. Commercial germ contain some bran and flour.

2.3 Nutrient Composition of Wheat and its Milled Fractions

2.3.1 Wheat Proteins

Wheat proteins are of special significance in providing the characteristic gluten which is so well known to millers and bakers. It is this particular substance which provides the structrual framework for the familiar spongy cellular texture of bread and baked products (Bushuk, 1972).

Essentially, wheat contains five different class of proteins which include: albumin, globulin, protease, prolamin (gliadin) and glutelin (glutenin). The gliadin and the glutenin are of special importance as these proteins together with water and salts, form the well known gluten (Bailey, 1941).

Glutenin imparts rigidity to the gluten while gliadin which is soft is responsible for the binding. The gliadin sticks to the glutenin and so prevents it from being washed away in the process of washing out of the gluten (Webb et al, 1971). The interaction between gliadin and glutenin and the influence of different proportions of these two proteins upon the baking quality of flour have been the subject of many investigations. Studies on the chemical nature of these two proteins in flour have shown that they are differently constituted (Lee and MacRitchie, 1971). The wheat proteins are not however evenly distributed throughout the whole grain as the bran and germ are richer in protein than the endosperm (Hinton, 1971).

The protein contents of American wheats have been extensively surveyed and is reported to vary approximately from 7 to 24% (Hegsted et al, 1954). Crude protein of hard red spring wheat ranged from about 10 to 20%. Soft red winter and white winter wheats are reported to be low in protein content and average about 10+0.7%. Protein content of India wheat was reported to range between 8.0 and 10.8% (Report of Wheat Marketing in India, 1937). The protein content of the sample of whole wheat grain is generally about 1% higher than that of the flour milled from the grain, because protein content is slightly higher in the germ, bran and outer endosperm layer of the grain than in the endosperm as a whole (Hinton and Redman, 1971). The embryo, bran and aleurone layers combined accounted for 18% of the weight of the grain and contain about 28% of its protein. Although these components (embryo, bran and aleurone layer) are important nutritionally, their chemistry has not been extensively studied.

Gluten: This is that fraction of wheat flour which is responsible for providing the framework for the spongy texture of bread and other baked products (Pal, 1966). Inspite of extensive investigations on the chemical nature of gluten, no satisfactory explanations has emerged for its physical behaviour that appears responsible for the variation in different wheats. On dry weight basis, gluten consists of 75 to 85% protein, 5 to 10% lipid, the remainder being starch. The lipid appears to be in combination with protein in a manner typical of lipo-proteins. The

lipids are reported to participate to a considerable extent in determining the dough structure (Hoseney et al, 1970; Daniel et al, 1971; Simmonds and Wrigley, 1972).

Gluten is generally regarded as a mixture of equal parts of gliadin and glutenin (Finney, 1971). But these terms are not appropriate since they do not signify any definite chemical substance as they have been retained for the sake of convenience. Glutenin refers to that portion of gluten which is insoluble in neutral solvents but soluble in acidic or alkaline solvent while gliadin is the remainder which is soluble in 60% aqueous alcohol (Pal, et al, 1966). Studies on the amino acid composition of gluten shows that the amino acid, glutamine, account reasonably well for the the nitrogen present (Catsimpoolars et al, 1971). The high level of nitrogen, has the practical consequence that cereal chemists must use a lower factor (5.7) for converting nitrogen content to protein level in wheat than the factor 6.25 used for most other proteins (Bushuk and Wrigley, 1974).

For man's purposes, the high glutamine content makes gluten a particularly suitable protein for bread making. The fact that about one amino acid in three is this neutral polar residue ensures that gluten is water-insoluble, but is sufficiently hydrophilic to compete strongly with starch for the absorption of water in dough. Furthermore, the strong tendency of glutamine residue to hydrogen-bond together contributes to the viscoelastic properties of gluten

necessary for a dough to retain gas bubbles during bread baking (Beckwith et al, 1963).

It has been shown recently that some of the Nigerian grown wheats compare favourably with the imported one (Kubiczek et al, 1987). These workers showed that Florence Aurore 8193, one of the highest in grain protein content, 15.0%, exhibited the highest gluten content of 42.2 and 14.9% for wet and dry gluten respectively. They also reported variety/line 3696(354-154) with 40.1 and 14.4% and UQ105 with 39.2 and 13.3% of wet and dry gluten respectively.

2.3.2 Wheat Carbohydrate

It is the most abundant constituent of the wheat kernel, forming about 83% of the total dry matter (Bruckner, 1966). In an early study (Builey, 1944), the major cabohydrate components of wheat were identified. However most investigators have reported carbohydrate as the remainder left after accounting for crude fibre, nitrogens and lipid compounds and mineral matter (Bishop, 1964; Destefanis and Ponte, 1960). The results obtained by available methods vary widely. This is probably due to the complex nature of some of the carbohydrates and also the variety. Growing, harvesting and storage conditions have considerable influence on the chemical composition of wheat (Tollier et al, 1968). Furthermore the total alcohol-soluble sugar contents from the same year in different regions was reported to vary from 2.72% to 3.72% of the grain dry matter. A value of 3.23% has been reported for

alcohol soluble sugars by Cerning (1970) as determined after extraction with boiling 80% ethanol. Vaisy and Unrau (1964) reported 1.67% and 0.06% for total surgars and maltose in flour respectively. This is in close agreement with 1.0 and 0.8% reported by Koch et al (1951).

Starch is a stored polysaccharide laid down in the plant in the form of water soluble particles called starch granules (D'Appolonia, 1971). Wheat starch has an average amylose content of 23% and contains minor constituents (Nitrogenous and phosphorus compounds, lipid, and various minerals) Greenwood (1972). The determination of starch has been widely studied by various authors (Bondarenko and Smirnor, 1966, Richter and Schiervaum, 1968; Banks et al, 1970, Libby, 1970; Meuser and Kempf, 1970). Generally, starch content in wheat kernel has been reported to be inversely related to the protein content (Hopkins and Graham, 1935). Studies on some American and European varieties appears to have a comparative higher mean starch content (62.0 to 75.0%) and a comparative lower protein content to the Canadian varieties respectively (Pomeranz and Macmasters, 1968). American varieties contain from 61.2% to 71.9% starch (Tollier et al, 1968). The effect of growing conditions on the chemical compsotion of wheat have been reported by Tollier et al (1968). These workers analysed 10 French wheat varieties cultivated during the same year in three regions of France which differed in climate. This investigation showed that the starch content of the same variety

grown in different places varied from 71.4 to 75.0%. The differences noted in the kernel were also found in the various flours. Starch content ranged from 74.8 to 85.3% (Pomeranz and MacMaster, 1968).

Audider et al (1966) reported that the soluble sugars are highest ian the protein rich fraction - the germ (20.55%) and the concentration lowest in thse middle fraction - the flour (2.6%).

2.3.3 Wheat Lipids

The lipid constituent of wheat and its products are extensively reviewed by Mecham (1971). The lipid content of wheat kernel, regardless of variety and grade is nearly 2% (by the ether-extraction procedure) with extreme variation between 1% and 3.9% (Miller, 1958). Lipid contents in typical milled products of hard red spring wheat by ether extraction are 1.15% patent flour, 1.88% clear flour, 3.51% low grade flour, 6.25% shorts, 4.76% bran, 10.81% germ and 2.3% wheat kernels (Sullivan and Near, 1928). The uneven distribution of liquids in all cereals kernels is a general characteristics which reveals itself in the liquid content of their various milled fractions. The content of ether extract in Indian wheats has been found to vary from 0.97 to 2.28% (Ann. Rep. I.A.R.I., 1957). This variation which had earlier been reported for common bread wheat grown under irrigated conditions, showed a higher lipid content compared to those grown under normal conditions (Das et al, 1954;

Barnagee and Das, 1957). It was also shown that the lipid concentration is principally high in the germ fraction.

2.3.4 Wheat Minerals

The wheat kernel has 1.8% mineral content distributed in the different parts of the kernel as follows: Pericarp, 20.0%, testa and hyaline layer 30.0%, aleurone layer, 32.0%, outer endosperm, 2.4%, Inner endosperm, 1.6% and embyo and scutellum, 14 %. These data show considerable differences in the various tissues of the kernel (MacMaster et al, 1971).

2.3.5 Wheat Fibre

Analyses for starch, crude fibre and sugars in commercial bran showed that the results epend on the wheat sample and the milling process as well as the methods used for the determination. Studies have shown that thae structural polysacharides known as crude fibre are concentrated in the bran and a measurablle amount of starch (10 - 19%) remain associated with commercial bran (Richter, 1961; Salo and Kutilainen, 1970; D'Appolonia et al, 1971)

The crude fibre content was 1.7 to 5.5% and this was essentially due to the contamination with varying amount of bran (Linko et al, 1960).

2.4 In Vitro Protein Digestibiliyty of Wheat Fractions

The digestibility of various fractions aprepared from a single sample of hard red spring wheat as determined by thse methods of Kohler et al (1969) have been reported by Miladi et al (1972). Their work showed that the flour fraction had the highest protein digestibility 99.0%) while the bran no relationship between protein content and digestbility as the germ and the wheat protein concentrate with protein contents of 25.25 and 27.40% respectively, showed a digestibility of 91.6 and 96.40% respectively, whilme the flour with a protein conent of 14.39% showed a digsestibility of 99%. These workers alos showed that the bran with a sprotein contnet of 17.2% and digestibility of 69.4% cannot be reconciled with whole garain whose protein contnet is 16.67 and sdisgestibiliyty of 91.0% (Miladi et al, 1972). An attemapt made by these workers to evaluate thase digestibility and the net protein utilization of the same sample with rats as well as other samples like br4an, shorts and millings gave digestibility values substantially below those obtained from the chemical method.

Recent in vitro studies on cooked and uncooked cereals using the ensume pepsin were carried out by Hamaker et al (1987). Their work showed that the raw wehat grain has a protein digestbiliyty of 91.3% while the cooked grain has 85.9%. The decrease in digestibiliyty of 5.4% was attributed to the

inaccessibiliyty of the protein bodies to enzymatic attack./ Electron swcanning microscopy revealed that some protein bodies remain intact after cooking and were even presrent after pesin digestion (Arum (1984) in Hamaker et al, 1987). Another explanation put forward for the lowered digestibility after cooking was that the proteins surrounding the interior protein bodies had been toughened by the formation of disulphide bonds. Other researchers have shown that heat treeatment can enhance the formation of cystine disulphide linkages in cysteine rich proteins which in turn produces less diges5tible protein (Opstevedt et al. 1984).

2.5. **Antinutritional Factors**

Apart from thse analyses of the nutrient composition of wheat and its milled fractions, work has alos been done on some of the toxic or antinutritional substances associated with the grain and its fraction

2.5.1 Phytic acid

This is a compound formed by the combination of one molecule of inositoal with six moleculles of phosphoric acid (Stewart, 1974). If phytic acid is ingested it combines with calcium and iron ions in the diet to form insoluble phytates that are not raeadily absorbed in the gastrointestinal tract (Pal, 1966). High phytic acid content is thaerefore undesirable in

foodstuffs. The total quantity of phytin (magnesium salt of phytic acid) and othe4r salts of phytic acid praesent in the wheat varies with the varieyt aof wheat and other factors. A major portion of the sail of phytic acid is prsesent in the bran and germ and relatively little occur in the endosperm (Wheeler et al, 1971). Phytic acid content of flour incraeases as the extraction rate is raised. pasternak (1903) in Pal (1966) reported that phytic acid phosphorous constitute 70 to 90% of the total phosphorus in wheat grain. Similar observations were reported by Andrews and Bailey (1932). Knowles and Walk (1952) have reported the praesence of ovaer 90% total phytic acid in the aleurone layer which consist entirely of phytin, the potasium - magnesium salt of phytic acid (steven 1971).

Phytic acid has been shown to immobilizle the Ca and Fe present in the flour itself and in other ingredients of the diet. iron absorption from brsead decreased fairly regularly as the bran contasent of the bread was raised to 40%; as little as 3.3% of bran in the bread had an effect while 10% of bran decreased iron asborption by half (Kent, 1982). The prevaloence of rickets among Asian immiglrants in the UK has been ascribed by some workers to their diet, in which whole meal chapatties predominate, bohth fibre and phytate contributing to the effect (Kent, 1982).

Phytate content of Indian wheat was shown to vary from 250-376mg/100g (Final, Rep. I.A.R.I. 1957). The

phytin content of wheat was reportedly increased by appalication of phosphoric fertilizers. The grains from plots treated with superphosphate in conjunction with ammonium sulphate were reported to show the highest content of phytate phosphorus (Bains, 1949). The content in wheat grain from crops raised with different manure are found to be loowest for plots receiving green manure and highest for the plots receiving mineral fertilizers (Srivastava et al, 1955).

2.5.2 Tannin

Tannins are high molecular weight polyphenolic compounds present in a variety of foods (Maxson and Rooney,1972). A report on tannin content of foods and diets showed that in most legumes and cereals, the seed coat or outer potions contains fairly high amount of tannin (Rao and Prabhavathi, 1982). For bread wheat, 41mg/100g has been reported.

Tannin has been reported to cause growth depression in rats and in poutry whose effect was attributed to a reduction in the digestibility of dietary protein and other nutrients (Mosley et al,1979). Tanninalso inhibits the activities of trypsin, chymotrypsin, amylase and lipase (Rao and Deosthale, 1982). Tannin content of cooked grain was significantly lower than that of the raw grains and loss as high as 70% has been reported (Rao and deosthale, 1982)>

Tannin has been shown to affect the growth of animals in two ways: (1) their astringent taste decrease feed consumption and (2) their ability to bind proteins to form indigestible complexes. The depressing effect of condened tannins seems to depend on their degree of polymerisation (Martin-Tanguy et al,1977).

2.5.3 Oxalate

Oxalates are salts of oxalics acid and are found in plants.The presence of oxalate has been demostrated in rice strw (Oyenuga, 1968)> Excesssive feeding of the straw produced harmful effects due to its oxalic acid which acts as an inhibitor in the absorption of calcium. Spinnach has been reported to have such a high oxalic acid content as to render all of the its calcium unavailable while the calcium of Kale (a particular fruit in INdia) with practically no oxalic acid is assimilated as that of milk (Maynard and Loosli, 1962).

Plants harvested at the leafy stage of growth would have higher oxalate content, this being the stage at which oxalate content is highest (Cookand Stoddart, 1953). These workers have shown that cocoyam corms contain oxalic acid which are irritating to the digestive tract and even be poisinous. Soluble oxalate are converted into carbnate and bicarbonate in the body and if it is produced in sufficient quantities a severe alkalosis could occur. The first evidence of poisoning was shown by dullness, lowering of the head, loss of appetite and progressive incoordinayion (osanniyi, 1977).

2.5.4 Trypsin Inhibitors

They are naturally occuring protease inhibitors found in microorganism, plants and animals which inhibit the activity of trypsin and other serine proteases in the gastrointestinal tract (Richardson, 1977).

The occurence of proteinase inhibitor in plants has been known since 1938 and they are extensively widespread in their distribution throughout the plant kingdom (Vogel and Werle, 1968; Leiner and Kakade, 1969; Dechary, 1970).

The seeds of legumes have long been recognised as excellent sources of protein hence it is not surprising that a great deal of early work on protease inhibitors of trypsin and chymotrypsin from the widely grown soya (Glycine max) and Lima bean (Phaseolus lunatus) (Puztai, 1968; Leiner and Kakade, 1969).
Tubers of potato (Solanum tuberosum L.) are a notable source of a wide variety of trypsin inhibitors. In tissues, at least seven types are distinguishable based on their molecular weight, amino acid composition, N- and C- terminal amino acids, stability during heating and specificity (Hojima et al, 1973). Other fleshy tubers such as those of the taro (Colocasia antiquorum) and the banana (Musa sapientum) are also know to contain trypsin inhibitors (Ryan and Huisman, 1968).

The proteinase inihitor represents a quantitatively important fraction of the protein found in most cereal grains. Mikola and Kirsi (1972) have

demonstrated that several different inbibitors are present in high concentrations (5 - 10% of the water - soluble protein) both in the embryos and endosperm of grains of barley, wheat, oat and rye. They are also well known in the grains of rice, sorghum (Filhol, 1974) and maize (Chu and Chi, 1965).

A recent survey for trypsin inhibitors in plant tissues which are commonly found in human diets revealed that the potato and sweet corn contains the highest levels and that moderate levels are found in spinnach, raddish and cucumbers while low or negligible amount are present in fruits of peach and avocado (Chen and Mitchel, 1973).

The physiological function of the trypsin inhibitors are poorly understood despite the fact that they often form an important part of protein in tissues. In grains of Gramineae, it was reported that they comprise some 5-10% soluble protein (Mikola and Kirsi, 1972) whereas in legume it was recorded variously as:

0.25g/kg for mung bean (Chu and Chi, 1965).

1.5g/kg for chicken peas (Hojima et al, 1973).

and 3.6g/kg for kidney bean (Puztai, 1972).

Confirmation of the exact location of the proteinase inhibitor is clearly important for our understanding of the in vivo physiological function. Three physiological roles are suggested:

1) they are as storage proteins and because of their peculiar properties regulate the endogenous

protease enzyme and hence control protein turn over and metabolism (Ryan, 1973),

2) they may be part of the plants defence mechanism against attacks of insects and microorganisms (Ryan, 1973); and

3) they play important role in the endozoic dispersals of seeds. Birds and other animals which eat fruits or whole plant are known to subsequently excrete some seeds in a viable condition. Their high concentration of trypsin inhibitors increases their chances of passing through the alimentary canal unaffected (Marshal and Landa, 1975).

The exact nutritional significance of trypsin inhibitor is difficult to asses (Puztai, 1968). The observation of growth inhibition when animals are fed with high level of trypsin inhibitor led to their implication in nutritional disorder. Experiment with rat and chicks feds with raw soybean product led t pancreatic hypertrophy and excessive enzyme secretion (Vogel and Werle, 1968; Birk and Gertlelr, 1970). It was also reported to cause growth retardation in ruminants (Kakade, et al, 1967). Metabolic disturbance in cystine and methionine utilization was also suggested (Vogel and Werle, 1968). The nutritive value of legumes is improved on heat treatment as this reduces the level of the trypsin inhibitor which is an important parameter in assessing the quality of proteinaceous food ingredients (Leiner and Kakade, 1969; Richardson, 1977).

2.6 Utilization of Wheat and its milled fractions

2.6.1 Food Uses of Wheat

The primary use of wheat in the form of milled and baked products for food, has evolved over the years into the establishment of two giants industries - flour milling and commercial baking (major, 1974).

The food use of wheat includes the manufacture of breakfast foods, macaroni, spaghetti, noodle, and related products; and snack foods.

Production of the milling and baking industries is based on specialised set of processing and quality standards, a separate set involving both raw materials and finished products for each industry. Such rigid requirements were developed for the necessity of: (1) Satisfying the desire of a multitude of baking-goods consumers in terms of appearance, taste, texture and convenience of use; and (2) meeting efficiencies in large-scale production of flour and baked goods required to market products within an acceptable price range.

The baking industry put the wheat flour principally into bread-making which is produced with the desired attributes for a given market (Pomeranz and Shellenberger, 1971). All processes leading to the baking of yeast-leavened bread are aimed at combining flour and other ingredients into dough and to modify the physical properties of the dough in order to optimize its ability to hold gas during fermentation

and baking (Marz, 1972).

The production of rolls and buns form an important segment of the baking industry; During the past twenty years per capita consumption of rolls has essentially doubled (Euverard, 1970). Typical products of this type include different types of dinner rolls (hard and soft) and hamburger and hot-dog rolls.

Another important product of interest is the English muffin which is made from learner dough for a short-time producers of ancient lineage, it has in recent years enjoyed a resurgence in popularity and was reported to be the fastest-growing item in bakery production in the early seventies (Noel, 1971).

Another use of the wheat flour is in the production of fermented foods such as sweet doughs which are basically bread doughs supplemented with additional sugar, shortening, flavor and yeast. It is worked into one of many products, often topped with an icing and/or a fruit jelly. Sweet dough item are often utilized by consumers as a snack item (Cotton, 1964).

Crackers are made by mixing a sponge and allowing an 18 to 20 hr fermentation. After this time, the dough that is made soda is added. The dough is allowed to ferment for about 4 hr after which it is rolled out, docked, band oven, cooled and packaged.

2.6.2 Feed uses of Wheat and its Products

Uses of wheat in livestock and poultry feeds have been reviewed at an international sympsoium sponsored

by the Oklahoma State University (Anon, 1970). Recent studies have shown that the use of wheat as an indicator plant in mineral supplement of horses and ruminants (Mocsenyi et al, 1987) was viewed under two main subheadings which are (1) ruminant and (2) non-ruminant feeding.

2.6.2.1 Wheat in ruminant feeding Beef Cattle Wheat has been used sucessfully in high concentrate beef - fattening rations (Brethour, 1970). In most cases, wheat appear to give about the same performance on a pound-for-pound basis as maize, barley or stream-flaked. Wheat is most fed into beef cattle in combination with another feed grain. This was due to the fact that wheat may lead to rumen malfunction and development of acidosis (Dunlop, 1970; Oltjen, 1970). The basic problem was that wheat carbohydrate was more readily fermented in the rumen than other carbohydrate of other feed grains which lead to lower ruminal pH values and greater concentration of volatile compounds.

Dairy Cattle - Much of the work on feeding wheat to lactating cows was done in the 1930's and '40's when wheat was cheap. Two studies were conducted at the Washington State University (Mcpherson and Waldern, 1969; Tomerrik and Waldern, 1960). In the first study, the rations proved satisfactory in a short term trial for palatability, consumption, performance and milk composition. The second trial revealed that the cows fed with 96% wheat ration performed as well as those on any other ration.

Sheep - According to Morrisons (1956) wheat is a very satisfactory grain for sheep but care must be taken here to avoid acidosis problems as with cattle.

2.6.2.2 Wheat in non-ruminant feeding Swine - Wheat has proved to be an acceptable swine feed, but has not been used as much as maize for this purpose.

Poultry - Wheat is an excellent poultry feed that has been fed extensively in areas where it is cheap. Report on the use of wheat in broiler, and layer showed that digestibility of the various nutrients is similar to that of other grains (Bolton, 1955; Carlson, 1970; Sullivan, 1970; Thayer, 1970). High wheat diets for broilers tend to lower vitamin A content and minerals, but inexpensive commercially prepared supplements are available to handle these problems (Nelson, 1970).

2.6.2.3 Millfeeds in Ruminant Feeding

Cattle - Wheat millfeed are excellent sources of protein for cattle and usually contain about twice as much protein as barley or maize. They are also excellent source of phosphorus and can be used to advantage in balancing ration of lactating cows fed with high-legume roughages. Millfeeds are usually fed to cattle in combination with other cereal grains and supplements. For example bran is often added to concentrate rations to improve the palatability of ground grain meals (Waldern, 1970). Little information exists on the value of millfeeds in cattle feed when they constitute the major part of the concentrate

mixture. In a previous experiment (Waldern and Cedeno, 1970) a 98% wheat mill run ratio was fed to dairy cattle and compared with a 98% barley ratio and a control mixed-grain ration. Dry matter digestibility, energy, and total digestible nutrients were lowest in the millrun feed. Cows fed millrun meal lost weight compared to the other feeds but when the millrun was pelleted, weight change was comparable to other rations. Thus wheat millrun, after pelleting can be used as the only cereal ingredient in a concentrate ratio for lactating cows.

Millfeeds could possibly be used to a greater extent as part of a complete ration for early weaned calves, though supplementation with Vitamins and amino acids is, of course, necessary (Moran and Summer, 1970).

2.6.2.4 Millfeeds in nonruminant feeding

Horse - Bran is a useful feed for horses because of its bulky nature. Dense feed tend to produce colic in horses if fed in too large amounts. A periodic feeding of bran is often recommended to avoid constipation. In situations where oats are unobtainable, bran is recommended as a constant item of diet. Most horse rations contain about 10% wheat bran (Cunha, 1963).

Swine - Wheat millfeed are popular ingredients in swine feeds (Morrison, 1956). When wheat midds and shorts are used in mixed feeds for swine, they produce

good results along with milk by-products, soybean meal, meat scraps or fish mills, which supplement the protein of the grains. In several experiments cited by Morrison (1956) in which midds were added to a ration of grain and tankage for pigs, the midds increased the rate of weight gain slightly. On the average, each 100lb of midds replaced 88lb of maize and 10lb of tankage.

Poultry - Before the advent of high-energy feeds, it was common to use 20 to 25% millfeeds in all poultry formulars. Millfeed are also used in layer replacement, starter, grower, and breeder rations for both chicks and turkeys (Enochian et al, 1972). They are not recommended for broiler at any growth state, because the energy requirements are usually too high.

Considerable research has been done on the use of millfeeds in chick ration during the past decade. The general improvement in performance of poultry with pelleted diets containing millfeeds has been well documented even though the mechanism of improvement is not fully understood (Care et al, 1965). Millfeeds of course, are high in protein which is of better quality than wheat-flour protein (Miladi et al, 1972). Other uses of millfeeds are in pet food and fur-bearing animal food industries to which the wheat germ is put to use as it is rich in protein (Inglett, 1974).

2.6.3 Industrial Uses of Wheat and its Products

For centuries, wheat has been primarily a food grain and a major item in the diet of man. As food,

wheat has a higher market value than maize, sorghum and other cereal grains similar in chemical composition. The status of wheat as a food has resulted in low usage of wheat and wheat flour for industrial purposes except in periods of national emergency.

Three periods will be considered on industrial uses of wheat and flour namely: the past, present and future.

Before the World War II, industrial uses for wheat were insignificant (Miller, 1972). About 500 million bushels annually were milled for food and about 100 million bushels were consumed as feed (Regional Res. Lab, Ill. On wheat utilisation from 1940 - 1970). Between 1942 - 1945, however, major attention was given to wheat because it was readily available and the demands for maize and sorghum grain exceeded the supply for livestock feed, industrial alcohol starch, sweetening agents and many other necessary materials. Industrial consumption of wheat increased rapidly and in 1943 some 1200 million bu (Miller, 1966). The high price of wheat during this emergency period was of minor importance. The end products were required regardless of cost. About 33lb of starch was recovered per bu. Although the gluten produced was devitalized, it was a suitable protein for the manufacture of monosodium glutamate, a flavour enhancer. The byproduct fibre and oilmeal were marketed as feeds (Krull and Inglet, 1971).

During World War II, wheat served as a major raw material for the production of ethyl alcohol (Ethanol) by fermentation (Miller, 1972). Alcohol was essential for the manufacture of munitions and synthetic rubber. About 250 million bu of wheat was consumed during this period for the manufacture of ethanol.

At the end of the war, economics became a prime factor governing the use of wheat. Wheat and wheat flour lost most of their industrial markets and became of minor importance as industrial raw materials. The situation is still essentially true today. However, processes have been developed for the wet-milling of wheat; the fermentative production of ethanol; the fractionation of wheat flour into starch and gluten and the chemical modification of starch and gluten. When needs and economics warrant, industry can turn to any of these processes (Anderson, 1970; Miller, 1972).

There is only a small amount of total consummable wheat flour that is supplied industrially and utilized as follows: (Krul and Inglett, 1971).

1) As a component of hardwood, plywood urea-formaldehyde where a light colour is required to prevent staining or darkening of the wood surfaces. A white flour that contains about 13% protein has proved most satisfactory for this purpose. The protein in flour has effect on glue viscosity. Approximately 90lb of flour is used per 100lb of Urea - formaldehyde resins (65% concentrations).

2) As a component of composition - bours resins where wheat flour imparts properties similar to those required for plywood adhesives. Because flour is less expensive than the resins, it lowers total glue cost.

3) As an extender in soft wood exterior - grade phenolic resins. The flour is incoporated primarily for cost reduction. About 1516 of wheat flour is used per 10,000 sq.ft of finished plywood.

4) As a raw material for production of wheat starch and vital wheat gluten. Vital wheat gluten is consumed in foods, while almost all wheat starch goes into the manufacture of paper and plywood.

For many years the industrial market for wheat has shown relatively small growth and if its components are to serve industrial purposes, even though more expensive than other cereal grains, their premium and unique properties must be utilized.

So far consideration has been given to the chemical or mechanical processing or both, or representative wheats and flours for industrial uses. At present wheat varieties were bred for food use. The possibility of developing and growing certain wheat varieties specially for industrial use remains to be exploited. New high-yielding varieties could produce wheat at a lower cost and could make it more competitive with other cereal grains, such as maize and sorghum. The possibilities certainly warrant future investigation (Anderson, 1970; Krull and Inglett, 1971; Miller, 1974).

3.0 MATERIAL AND METHODS

3.1 MATERIALS

The wheat fractions for the experiments were obtained directly from the Northern Nigeria flour Mill, Kano which uses locally grown wheat grains for its operations. The samples were kept in tightly covered plastic containers till the commencement of the experiment.

3.1.1 SAMPLING

The various fractions obtained from the mill were of different particle size as shown below,

Fraction	Particle size range (microns)
1. Mixture of millings	2884 - 950
2. Germ	950 - 810
3. Bran	810 - 710
4. Medium Semolina	719 - 340
5. Dunst	340 - 265
6. Fine Middling	265 - 180
7. Flour	180 or less

Each of these fractions was milled in a Wiley Laboratory mill to pass through a 0.5mm sieve.

3.1.2 CHEMICALS

Pepsin (Activity: 1200 - 2000 units/mg protein) and Trypsin (Activity: 0.5 Anson Units/g) were supplied by Sigma Chemical Company, USA. N- -Benzoyl-DL-arginine-p-nitro-anilide hydrochloride, Ethanol, methanol, petroleum ether, D-glucose, ammonium

molybdate, Sodium tungstate and phenol were supplied by British Drug House (BDH) chemicals, Poole, England while Vanillin, catechin and Potassium dihydrogen orthophosphate were ;supplied by Hopkin and Williams, both in England.

3.1.3 EQUIPMENT

Spectronic 20
Desiccator
Bench top certrifuge, MSE
Analytical balance (accurate TO 1MG)
Water bath, Hot plate, and
Flask Shaker.

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3.2 METHODS

3.2.1 Determination of Moisture and Dry Matter Contents

This was carried out as specified by AOAC (1984) with some modifications.

Procedure: A clean crucible was dried to a constant weight in an air oven at 80°C, Cooled in a desiccator and weighed. Two gm of the finely ground sample was accurately weighed into a previously labelled crucible. The crucibles containing the sample was kept in the drying oven for 24 hrs. It was removed, cooled in a desiccator and weighed intermittently until a constant weight was obtained.

Calculation

$$\% \text{ moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100\%$$

where

W_1 = Wt of crucible (g)

W_2 = Wt of crucible + sample before drying (g)

W_3 = Wt of crucible + sample after drying (g).

% Dry matter = 100 - % moisture content

3.2.2 Determination of Crude Lipid Content

The method used was that of Stewart (1974) as modified specifically for grains by Guiragossian et al (1977).

Procedure: Exactly 2 gm powdered sample was accurately weighed into a clean dry previously weighed cellulose thimble and weighed again with degreased cotton wool as cover. 200 ml petroleum ether (b.p 40°C - 60°C) was poured into a clean dry 250ml round bottom flask. Some antibumping granules were then added. The flask was then filtered to the Soxhlet extractor and placed on heating mantle. The flask was subjected to moderate heat Just before the solvent started boiling, the water source (the tap) was turned on to ensure a prepared cooling of the apparatus. The extraction process was continued for 6 hrs after which the process was stopped and the apparatus left to cool. The thimble with its contents was removed and air dried for ten minutes. The loss in weight of thimble with content represent the ether extract (crude fat) content.

Calculation

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

3.2.3 Determination of Crude Protein

The crude protein contents of the samples were determined by the Kjeldahl method as modified by Concon and Diane (1973). The method entails the estimation of total nitrogen content after conversion of the protein nitrogen to Ammonium salt by digestion with sulphric acid.

The following reagents were prepared before use.

40% Sodium Hydroxide was prepared by dissolving 400gm of NaoH in distilled water in 1 litre beaker which was placed in a cold water bath. The mixture was stirred continously until all the NaOH pellets was dissolved.The solution was allowed to cool and then transfered to a 1-litre volumetric flask and made up to mark with distilled water.

Mixed indicator was prepared by placing 0.1g of bromocresol green and 0.1g methyl red into separate 100ml volumetric flasks each indicator was dissolved in ethanol and the solution made up to the mark with the same solvent. In another 100ml volumetric flask, 75ml bromocresol green solution was mixed 25ml methyl red solution to obtain the mixed indicator.

4% Boric acid solution: This was prepared by dissolving 80g of boric acid in 1-litre of boiling water and the solution allowed to cool before use.

Procedure for crude protein determination From a finely ground sample 0.2gm was sample weighed into a clean 50ml micro-kjeldahl flask including half tablet of selenium catalyst. Some antibumping granules were

added. 10 ml of concentrated sulphuric acid was added, swirled gently, and set up in a slanting position on a heating mantle in a fume cupboard. Low heat was maintained for the first 40 minutes before raising to medium heat for 1 hr as an index of the end of digestion. The digests were removed from the heating mantle and allowed to cool. Thereafter each digest was diluted to 100ml with distilled water and 10ml aliquots of the diluted solution taken for distillation. 7ml of 40% NoaH solution was gently added into the distillation flask. The distilled ammonia was trapped in a conical flask containing 10 ml of 4% boric acid and mixed indicator solution for about 5 - 10 minutes. At this stage, the tip of the condenser was immersed into the boric acid solution to avoid loss of NH_3 gas being liberated and this changed the indicator colour from red to blue. The trapped ammonia was back titrated with 0.1M hydrochloric acid according to standard procedure (AOAC, 1984a).

Calculation: Each ml of 0.1M HCL neutralized by ammonia is equivalent to 1.4mg Nitrogen.

Crude Protein = Crude Nitrogen x conversion factor.
(s/100g)

Type of fraction	Conversion Factor*
1. Mixture of millings	5.83
2. Flour	5.70
3. Dunst	5.70
4. Germ	6.31
5. Bran	6.31
6. Fine middling	5.70
7. Semolina	5.70

* Adopted from sorghum Quality Lab. Mannual for use in West Africa by Jeanette et al (1987).

3.2.4 Determination of ash content

This was carried out at 550°C with the aid of a muffle furnace (AOAC, 1984b).

Procedure: Exactly 2g of the finely ground sample were weighed into a previously weighed clean crucible which had been ignited in the muffle furnace at 550°C for 10 minutes and the crucible and content weighed. It was then transferred into the muffle furnace. The temperature of the furnace gradually increased until it reached 550°C. The samples were left for 8 hrs at this temperature so as to ensure proper ashing of the samples. Thereafter the samples were later removed from the furnace into a desiccator where they were cool.Each cooled cruciblewith its ash content was weighed. The weight of the residue in the crucible corresponds to the ash content of the sample while the loss in weight corresponds to the organic matter content.

Calculation

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

$$\% \text{ Organic matter} = \frac{\text{Weight loss (g)}}{\text{Weight or sample (g)}} \times 100$$

3.2.5 Determination of crude fibre content

This was carried out by the AOAC method for each crude fibre (AOAC 1984c). The AOAC (1984) method involves sequential digestion of the sample with dilute acid and alkali solutions. The residue obtained was ignited to obtain crude fibre.

Procedure: 1g of the finely ground sample was put into a round bottom flask, 100ml of 1.25% (0.25N) sulphuric acid solution was added and the mixture boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. It was then transferred quantitatively into the flask and 100 ml of hot 1.25% (0.312N) sodium hydroxide solution was added and the mixture boiled again under reflux for 30 minutes and quickly filtered under suction. The insoluble residue was washed with boiling water until it was base free. It was dried to a constant weight at 100°C in an oven, cooled in a desiccator and weighed (C_2). The weighed sample was then incinerated in a cabolite muffle furnace at 550°C for 2hr, cooled in a desiccator and reweighed (C_3).

Calculation

The loss in weight on incineration = $C_2 - C_3$

Weight of original sample = W

$$\% \text{ fibre} = \frac{C_2 - C_3}{W} \times 100$$

3.2.6 **Determination fo non-protein nitrogen (NPN)**

Essentially this determination involves the rapid extracted of the non-protein nitrogen with ethanol (Pearson, 1976) followed by the conventional micro-kjeldahl analytical procedure for nitrogen estimation (AOAC, 1984a).

Procedure: 1.0g of the fine sample was shaken with 25ml of 80% v/v ethanol in a flask shaker for 30 minutes at room temperature and then filtered into a 250ml Erlenmeyer flask using Whatman No. 41 filter paper. The filterate which contains the non-protein was left to stand while the residue was re-extracted again with 80% ethanol for exhaustive extraction. The filterate obtained from the second extraction was added to the first and the volume made up to 50ml with 5.0 ml aliquots were taken for nitrogen-estimation according to the standard AOAC procedure described earlier in subsection 3.2.3.

Calculation

Same as that of crude nitrogen estimation.

3.2.7 **Estimation of true nitrogen and protein**

This was determined by the method of difference

% True Nitrogen = % crude Nitrogen - % NPN

True protein = % True Nitrogen x Conversion factor

3.2.8 **Determination of tannin content**

Tannin was determined by the vanillin - HCL procedure which was based on an acid catalysed addition

of vanillin to flavonols and their polymers as well as addition to other polyphenolic compounds such as dihydro chalcone and flavones. These reactions are determined colorimetrically at 500nm (Earp et al, 1981).

The following reagent were prepared immediately before use:

A. **Concentrated HCL in Methanol (1%) (v/v):** This was prepared by pipetting 1ml of conc.HCL into a 100ml volumetric flask and making up to mark with methanol.

B. **Concentrated HCL in methanol (8%) (v/v):** This was prepared by pipetting 8ml of conc.HCL into a 100ml volumetric flask and making to mark with methanol.

C. **Vanillin Methanol(1%) (w/v):** Prepare by dissolving 1g of vanillin in 100ml of methanol.

D. **Vanillin-HCL reagent:** this was prepared by mixing together equal volumes of reagents B and C.

E. **Con.HCL in methanol (4%) (v/v):** This was prepared by pipetting 4ml of con.HCL into a 100ml volumetric flask and making up to mark with methanol.

F. **Standard solution of catechin (Sigma):** This was prepared by dissolving 100mg of catechin in 100ml of methanol to give it a concentration of 1mg/ml.

Working standards of catechin: These were prepared by pipetting out 0.2, 0.4, 0.6, 0.8 and 1ml of catechin stock solution into separate tubes and their volumes

made to 10 ml with methanol. This was done specifically to obtain a calibration curve (Fig. 2). The pipetted standards gave concentrations of 0.02, 0.04, 0.06, 0.08 and 0.10mg/ml of catechin respectively.

Procedure for determination of tannin content: From the ground sample 500 g was accurately weighed into a centrifuge tube. To each tube, 10ml of 1% HCL in methanol was added and shaken for 20 minutes on a flask shaker. The tubes were then centrifuged for 10 minutes at 10,000 g and the supernatant transferred into a 25ml volumetric flask. To the residue in each tube, another 5ml of 1% HCL in methanol was added and shaken as was done earlier. The tubes were centrifuged for 20 minutes at the same speed and the resultant supernatant added to the first extract in the 25ml volumetric flask and the volume was made up to mark with the 1% HCL-methanol reagent. The contents of the flasks were thoroughly shaken to ensure proper mixing of the contents.

In order to estimate the tannin content of the samples, 1ml aliquote of the extract was pipetted into a test-tube and 5ml of vanillin-HCL reagent was added to each of the catechin standards alongside with the samples and standard preparations. Individual sample blanks were prepared by adding 5ml of 4% HCL in methanol to 1ml of the sample extract. The absorbance of the sample and their respective blanks as well as the standard catechin of different concentrations were

determined at 500nm in a spectronic 20 spectrophotometer.

Calculation: The concentrations of catechin in the samples were determined on the standard curve by extrapolation in which catechin equivalent (C.E) was determined according to the following equation.

$$\% \text{ C.E.} = \frac{\text{Mg Catechin/ml} \times \text{volume made up} \times 100}{\text{Volume of extract taken} \times \text{weight of sample(mg)}}$$

3.2.9 Detemination of Phytic Acid Content

Phytate phosphorus was determined by the colorimetric method of Stewart (1974). The acidified extract was used to dissolve the phytin present in the sample. Phytic acid was then isolated as Iron (III) phytate. It was recovered, digested and estimated as phytate phosphorus.

Standard Phosphorus solution: This was prepared by dissolving 0.4391gm of dry potassium dihydrogen phosphate (KH_2PO_4) in distilled water and the volume made to 1 litre. This stock solution contain 100ppm phosphorus. 1ml of the stock solution was pipetted into 50ml volumetric flask and diluted to mark with distilled water thereby obtaining 2ppm phosphorus.

Ammonium molybdate-sulphuric acid reagent: This was prepared by dissolving 12.5gm ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$] in 100ml of warm distilled water in a beaker. Meanwhile 140ml of concentrated sulphuric

acid was carefully added to 200ml of distilled water in a 500ml volumetric flask. The molybdate solution in the beaker was filtered into the acid mixture, mixed thoroughly, allowedto cool and made up to 500ml.

Stanous chloride Reagent: This was prepared by dissolving 0.10gm stanous chloride ($SnCl_2.2H_2O$) in 50ml of 2% (v/v) hydrochloric acid. A piece of tin was kept in the solution to preserve it. This reagent was used immediately after preparation.

Working standard of Phosphous: Five standard solutions were prepared by transferring a given of the 2ppm phosphorus solution into a 100ml volumetric flask and made up to mark with distilled water. The volumes were: 10, 15, 20, 25 and 30ml respectively and the corresponding concentration were 0.4, 0.6, 0.8, 1.0 and 1.2 ppm phosphorus respectively (Fig. 3).

Procedure for Determination of Phytic Acid: Exactly 2.5g dry sample was extracted for 3 hours with 100ml of 0.5M HCL on a flask shaker. Thereafter the mixture was filtered and 15ml portion of the filtrates neutralised with 0.5M NaOH solutions. The resultant solution was made slightly acidic with few drops of 0.17M HCL and was diluted to 50ml with distilled water. 10ml of this solution was pipetted into a centrifuge tube and 4 ml 0.25%(w/v) $FeCl_3$ solution was added and the mixture heated for 15 minutes at 100^{o}C. On cooling, it was centrifuged and the supernatant liquid discharded. The residue was washed with 0.5M and 0.17 HCL respectively. It was centrifuged again and the liquid portions

discarded. 2ml of distilled water was added to the residue and the mixture heated for 5 minutes at 100°C. 2ml of 0.5M NaOH solution was added and the mixture heated and filtered into a 100ml Kjeldahl flask. The residue was washed with distilled water and the washings added to the flask. 0.5ml conc.H_2SO_4 was added to the filtrate in the digestion flask and boiled to white fumes in a fume cupboard. 1ml 60% perchloric acid and 5ml conc.HNO_3 were added and the flask swirled gently. It was then digested slowly for 15 minutes after the appearance of white fumes and was set aside to cool. The digest was transferred to a 50ml volumetric flask and diluted to mark with distilled water. 10ml aliquots of the sample solution were pipetted into 50ml volumetric flasks. Each of the standard phosphorus solution was transferred to a 50ml volumetric flask as well as the sample solution and was diluted to 2/3 full with distilled water. To each was added 2ml. Ammonium molybdate-sulphuric acid reagent and shaken followed by 2ml stannous chloride reagent and the volume made to mark with distilled water. The absorbance was then taken at 700nm using a spectronic 20 instrument with distilled water treated as for sample preparation serving as a reference.

<u>Calculation</u>

The neutral and digest solutions are evaluated to give:

$$\% \text{ Phytate-P} = \frac{(\text{cmg}) \times 3.75}{\text{Sample wt (mg)}}$$

C is the concentration of phytate-phosphorus in the sample which were determined on the standard curve by extrapolation.

3.2.10 **Determination of Total Oxalate**

The method used was that of Dye (1956) as modified by Oke (1965).

Procedure: Exactly 1.5g aliquot ample was weighed into a 250ml volumetric flask and digested with a mixture of 190ml distilled water and 10ml of 6N hydrochloric acid. It was incubated in a water bath at 90^{o}C for 5 hrs. Thereafter the mixture was centrifuged and filtered into a 280ml volumetric flask. and the filtrate made up to mark with distilled water. 50ml aliquot of the extract was mixed with 25ml 6N HCL and the mixture evaporated to about 25ml on a hot plate. The brown precipitate formed was filtered off and washed with hot distilled water. The combined solution and washings were titrated with concentrated ammonia until the pink colour of the methyred indicator changed to faint yellow. The solution was then heated on a hot plate to about 90^{o}C. The oxalate was precipitated with 10% (w/v) calcium chloride solution. After keeping the mixture overnight, it was filtered and the precipitate washed with distilled water until calcium free as test with bench sodium hydrxide.

The precipitate was then washed with 25% (v/v) sulphuric acid solution and diluted to 125ml with

distilled water. It was warmed to 90°C and titrated with 0.05N potasium permanganate solution.

$CaC_2O_4 + H_2SO_4 \longrightarrow CaSO_4 + H_2C_2O_4$

$2MnO^-_4 + 5C_2O_4^{2-} + 16H^+ \longrightarrow 2Mn^{2+} + 10CO_2 + 8H_2O$

Calculation

1ml of $0.05NKMnO_4$ = 2.2mg oxalte.

3.2.11 Determination of Trypsin Inhibitor Levels

The trypsin inhibitor levels were determined by the method of Kakade et al (1974) as modified by Smith et al (1980).

The following solutions were prepared:

Tris-Buffer (0.05M, pH 8.2) containing 0.02M $CaCl_2$: This was prepared by dissolving 6.05g Tris (hydroxymethylaminomethane) and 2.94gm $CaCL_2.2H_2O$ in 900ml distilled water. The pH of the resulting solution was adjusted to 8.2 with the volume brought to 1 litre with distilled water.

Substrate Solution: This was prepared by dissolving 40mg of N- α -benzoyl-DL-arginine-P-nitro-anilide (BAPNA) hydrocloride in 1ml dimethylsulphoxide in a 100ml volumetric flask. The volume was made up to mark with the tris-bufer previously warmed to 37°C. The BAPNA solution was prepared daily and kept on a hot plate at 37°C while in use.

Trypsin solution: This was prepared by dissolving 4mg of trypsin in 200ml of 0.001MHCL. The solution was

preserved in the refrigerator for 3 weeks without loss of activity.

Procedure for Determination of trypsin inhibitor: 1 g of the finely ground sample was extracted with 50ml of 0.01N NaoH for 1hr. The pH of the suspension was adjusted to between 9.5 and 9.8 with 0.01N HCL. 2ml portion of each extract was pipetted into a 50ml volumetric flask and made up to mark with distilled water (giving it a dilution factor of 25.)

In determining the trypsin inhibitor level, the following additions were pipetted into a series of 10ml test-tubes.

a) Reagent blank: This was prepared by pipetting 2ml distilled water in a test-tube marked A

b) Working standard trypsin (40ug): prepared by adding 2ml standard trypsin solution to 2ml of distilled water in a test-tube marked B.

c) Sample blanks were prepared by adding 1ml of diluted sample extract to 1ml distilled water in test-tube marked C.

d) Samples were also prepared by adding 1ml diluted sample extract to 1ml diluted water and 2ml standard trypsin solution in a test-tube marked D.

All the tubes were thoroughly mixed and heated to 37^{o}C in a water bath for 10 minutes 5ml of BAPNA solution previously warmed to 37^{o}C was added to each tube and mixed. The reaction was stopped after 10 minutes by the addition of 1ml 30% (v/v) acetic acid solution to tube A, the reagent blank and to sample

blanks. The tubes were thoroughly mixed and filtered through Whatman No. 41 filter paper. The absorbance of the sample blanks, standard trypsin and samples respectively were taken at 410nm in a spectronic 20 Spectrophotometer using the reagent blank as a reference.

Calculation

Change in absorbance (A_1) due to trypsin inhibition per ml of diluted sample extract is (A_b - A_a) where (a - d) is as shown above.

$$\%T1A = \frac{100A_1}{(A_b - A_a)}$$

1 g pure trypsin gives A_{410} = 0.0190. Weight of pure trypsin ihibited per ml is

$\frac{A_1}{0.0019}$ μ g (that is $\frac{50A_1}{19mg50ml^{-1}}$).

From the above value, the Trypsin inhibitor activity (T1A) is therefore calculated in terms of mg pure trypsin per g sample as weighed (mg/g).

$$T1A = \frac{2.632.D.A_1}{S}$$ mg pure trypsin inhibted per g sample

where D is Dilution factor

A_1 is change in Absorbance due to trypsin in hibition per ml of diluted sample extract

S is sample weight.

2.632 constant factor obtain from $\frac{50}{19}$ as weight of pure trypsin inhibited per ml.

3.2.12 **Estimation of In Vitro Protein Digestibility**

The method used was developed by Mertz et al (1984) in which proteins are digested by enzymatic reaction at pH 2.0 using pepsin and the percentage protein solubilized at 37°C for a period of 3hr calculated.

Pepsin reagent: This was prepared by dissolving 13.6gm KH_2PO_4 in 800ml of distilled water. The pH was adjusted to 2.0 with concentrated HCL before dissolving 2.0gm of pepsin in the solution. After vigorous shaking to dissolve, it was diluted to mark with distilled water.

Procedure for the Determination of in vitro protein digestibility: 200mg aliquot of uncooked sample was weighed in a centrifuge tube and 20ml pepsin reagent added to each tube and shaken vigorously. Cooked samples were also prepared by weighing 200mg aliquot of dry sample in 25ml beakers. To the sample in the beaker was added 3ml distilled water before cooking on a burner for about 10 minutes. After cooking, 2ml distilled water was added again to ensure total transfer of sample into the centrifuge tubes. The centrifuge tubes containing the cooked fractions were placed in a boiling water bath for 230 minutes. The tubes were removed and cooled and 20ml of pepsin reagent added to each tube. Duplicate pepsin blank was

also treated as for uncooked and cooked samples respectively. All the tubes were placed in a water bath at 37°C for 3 hr with constant stirring every 15 minutes. After 3hr, the digestion was stopped by removing the tubes from the water bath and placing them in ice bath for 30 minutes. The cooled samples were filtered though Whatman No. 41 filter paper and the residue washed with distilled water. The residue on the filter paper was enveloped and placed in a 50 ml micro-Kjeldahl flask and analysed for nitrogen by micro-Kjeldahl digestion. The indigestible nitrogen was subtracted from total nitrogen of sample, to obtain digestible nitrogen.

Calculation

Digestible N (mg) = Total N in sample - mgN in residue

Digestible Protein = Digestible N x Conversion factor.

Total protein = mg N in sample x conversion factor

% *in vitro* digestibility =

$$\frac{\text{Digestible protein}}{\text{Total protein in the sample}} \times 100$$

3.2.13 Determination Of Amylose Content

The method adopted is based on the fact that amylose in starch is released by treatment with dilute alkali and the addition of a triiodide ion gives a blue colour which can then be measured colorimetrically (Williamson *et al*, 1958).

The following reagents were prepared for this experiment.

Amylose standard: This was prepared by dissolving 100mg of amylose in 100ml distilled water in a 100ml volumetric flask giving it a concentration of 1mg/ml.

Iodine reagent: This was prepared by dissolving 2mg of potassium Iodide and 0.2g of iodine in 75ml of distilled water and making up to 100m in a 100ml volumetric flask.

Procedure for determination of amylose 100mg of defatted sample by ether extraction was weighed into a 100ml volumetric flask and 1 ml 95% ethanol and 9ml 1N NaOH were added to the flask and swirled gently to dissolve the sample. The flask was incubated overnight at room temperature overnight. Thereafter the solution was made up to 100ml with distilled water, shaken vigorously and allowed to stand for 30 minutes to allow particles to settle.

5 ml of the supernatant was pipetted into a 100ml beaker and 30ml of distilled water was added. This aliquot in the 100ml beaker was adjusted to pH 10.2 with 0.1NHCL. To the aliquot 1ml of Iodine reagent was added and the resulting mixture thoroughly mixed and allowed to stand for 30 minute before it was made up to 100ml with water. The amylose standard prepared was treated as precisely described. The absorbance of the blue complex was then read at 600nm against a blank solution

Calculation

$$\% \text{ Amylose} = \frac{1 \times 100 \times 100 \times A_{sample}}{A_{standard} \times 5 \times \text{wt of sample (mg)}}$$

where 1 is concentration of amylose (1mg/ml)

100 is the total volume made up

100 is percentage

5 is Aliquot volume

A_{sample} is absorbance of sample

$A_{standard}$ is absorbance of standard

3.2.14 Determination of soluble starch

The procedure was the same as that of amylose determination except that the standard used was not amylose but soluble starch and was treated according to the procedure of Williams *et al*, (1958).

3.2.15 Estimation of Total Soluble Sugars

The method used was that described by Dubois *et al* (1956) in which the soluble sugars are extracted with hot ethanol in aqueous phase and these sugars on treatment with phenol-sulphuric acid, produces a stable and sensitive yellow colour which can then be measured colorimetrically.

The following reagents were prepared.

80% Ethanol: This was prepared by mixing 800ml of ethanol in 1 liter volumetric flask with 200ml of water.

5% Phenol: This was prepared by dissolving 5g phenol in water in a 100ml volumeric flask and the resulting solution made up to mark with distilled water.

Glucose standard: 100mg of D-glucose was dissolved in distilled water in a 100ml volumetric flask and made up to mark.

Working standard: 100 g glucose/ml was prepared from glucose stock solution by pipetting 10ml into a 100ml volumetric flask and then made to mark with distilled water. From this working standard, 0.1ml to 0.5ml was pipetted in duplicate into various test-tube and made to 1ml with distilled water.

Procedure for estimation of total soluble sugars: Exactly 100mg of the defatted sample by ether extraction was weighed into a boiling tube and 25ml of hot 80%(v/v) ethanol was added and the mixture was shaken on a flask shaker.

The content was allowed to stand for 15 minutes and then was filtered into a beaker through a Whatmen No. 41 filter paper. The extraction was The extraction was repeated to ensure complete removal of the soluble sugars. The solvent was evaporated off the extract by heating on a hot plate. The residual was dissolved with little distilled water and transfered into a 100ml volumetric flask and made to mark with distilled water. 1ml aliquot was pipetted into a test-tube and 1ml of 5% (w/v) phenol solution added to each tube mixture was added 5ml 96%(v/v) sulphuric acid and shaken vigorously. The resulting yellow complex was cooled in a cold water bath. A sample blank using 1ml distilled water and each of the 1ml standards were treated as in sample preparation. The aborbance of the resulting

golden yellow colour was read at 490nm with a spectronic 20 spectrophotometer using distilled water as a blank. The sample blank absorbance was also noted.

3.2.16 **Estimation of total starch**

The determination was done by digesting the sample with perchloric acid according to Osborne and Voogt (1978). The glucose release was measured *colorimetrically by the Dubois et al (1956) procedure* using phenol - sulplhuric acid reagent.

The followiwng reagents were prepared immediately before use.

5% Phenol solution: This was prepared by dissolving 5mg phenol in distilled water and the resulting soltuion made up to 100ml in a volumetric flask.

52% Perchloric acid: This was prepared by mixing 270ml perchloric acid with 100ml distilled water. The resulting solution was placed in a cold water bath for sometime to allow it to cool before use.

Procedure for total starch estimation: 100mg of dry sample was accurately weighed and transferred quantitatively into a 100ml volumetric flask and 10ml of distilled water added to and stirred with a long glass rod to disperse the sample thoroughly. To the dispersed sample in the flask, 13ml of 52% perchloric acid reagent was added using a measuring cylinder and the content stirred frequently and agitated by hand for 30minutes to ensure full hydrolysis. The glass rod was

washed down into the flask with distilled water and the solution made up to 100ml with distilled water. After mixing, the solution was filtered into a 250ml graduated flask and later diluted to mark with distilled water and thoroughly mixed. Duplicate determinations were made by pipetting 0.2ml of the diluted filterate into 10ml test-tube and 0.8ml of distilled water added. Duplicate blanks and standard containing 1ml of distilled water and 1ml standard glucose (100ug/ml) respectively were also pipetted into separate test-tubes To each the tubes, 1ml 5%(w/v) phenol was added and mixed, followed by 5ml 96% (v/v) H_2SO_4. The golden yellow coloured solution was allowed to cool in a cold water bath before the absorbance was taken at 490nm.

Calculation

Total volume = 250ml

Wt of sample = 100mg

Aliquot taken = 0.2ml

250ml contain 100mg of sample

$$0.2\text{ml will contain} = \frac{100 \times 0.2}{250} = 0.08\text{mg}$$

$$= 80\text{ug.}$$

Distribution factor =

$$\frac{\text{Concentration of standard (ug)}}{\text{Absorbance of standard}} \times \frac{0.9}{80}$$

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where 0.9 is conversion factor for glucose to starch.

% starch = Distribution factor x Absorbance of sample x $\frac{100}{1}$

3.2.17 **Determination of Maltose content**

This determination was according to the method of Berliner (1980) which essentially involved the treatment of sample with for 1hr followed by filtration and boiling of filtrate with 1N NaoH solution and finally measuring the maltose content colorimetrically.

Procedure: Exactly 1.5g of the finely ground sample was weighed into a test-tube and 5ml of distilled water previously warmed to 30°C was added. The test-tube was closed with a rubber bung and shaken vigorously to get a uniform suspension.

The tubes were then placed on a tube rotator and the motor switched on to drive the tubes for exactly 1hr at room temperature. During this period, the enzyme amylose present in the sample hydrolyzes starch in maltose. After 1hr, the test-tubes were taken out and 2ml concentrated H_2SO_4 were added and shaken thoroughly. The suspension was then filtered through a filter paper (Whatman No. 41) which was placed in glass funnel. The filtrate which contains maltose was collected in a beaker and allowed to stand for 30 minutes.

After 30 minutes, 3ml alequot was pipetted in a test-tube and 1ml NaoH solution added and mixed

together. The tubes were placed in boiling water for 5 minutes until a colour change from yelllow to dark brown confirming the presence of high maltose content. The tubes were then removed from the boiling water bath and allowed to cool in a water bath at room temperature. The absorbances of the cooled tubes were measured colorimetrically at 550nm using a spectronic 20 spectrophotometer with distilled water as the blank.

The maltose content was calculated using the following equation:

$$\% \text{ Maltose} = A_{550} \times 6.734 + 0.0609$$

where 6.734 and 0.0609 are standnard constants obtained from maltose extinction curves.

3.2.18 Determination of crude gluten

This was done according to the method specified by AACC (1982).

Procedure: Exactly 25g of flour was weighed into a porcelai cup, sufficient water (about 15ml) was added and worked into dough with a spatula ensuring that no material adhered to the utensil. The dough so formed was allowed to stand in water for exactly 1hr at room temperature. After 1 hr, the dough was kneaded in a stream of tap water with careful and thorough washing so as to remove starch and all soluble substances. In order to asertain that the gluten was approximately starch free, 2 drops of washed water obtained by

squeezing was allowed to fall into a beaker containing clean water to see whether there was cloudiness which is an index of the presence of starch in it. The gluten obtained was allowed to stand again in tap water for another 1hr after which it was pressed as dry as possible and rolled into a ball. This was placed in a weighed flat bottom dish and weighed as wet gluten. The wet gluten was transferred into an oven and dried to constant weight at 100°C for 24 hr and cooled in a desiccator before weighing as dry gluten.

Calculation

$$\%\text{Wet gluten} = \frac{\text{Wt of wet gluten}}{\text{Wt of flour}} \times 100$$

$$\%\text{Dry gluten} = \frac{\text{Wt of dry gluten}}{\text{Wt of flour}} \times 100$$

Ash, crude lipid, crude protein and crude fibre contents of the dry gluten were determined by the appropriate AOAC (1984) method. Starch was also determined as previously described in section 3.2.16.

4.0 RESULTS AND DISCUSSION

4.1 Proximate Composition

The result of the proximate composition of the wheat grain and its milled fractions are as shown in table 4.1. The result revealed that moisture content of the fractions ranged between 8.6% in flour and 12.3% for semolina fractions. The index level indicative of poor storing characteristics of wheat is 13.5% (Christian and Kaufman 1964). Other findings suggest that Triticum aestivum variety is suitable for use both as foods in flour mills or in animal nutrition where wheat products may need to be stored for a considerable period of time.

The crude protein concentration for whole wheat grain was 14.25% on dry matter basis (Table 4.1). Bailey (1944) had previously reported that wheat protein content of 10 - 20% is indicative of good baking properties. The values from the present study compared better than the crude protein level of 8 - 10% reported for Indian wheat (Report on Wheat marketing in India, 1937). It is also important to indicate that wheat germ fractions had significantly higher level ($P<0.05$) of protein than all other fractions. This findings are consistent with previous reports (McCance et al, 1945 and Miladi et al, 1972).

Mecham (1971) had earlier reported that regardless of the variety of grain, the lipid content of wheat

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Table 4.1 Proximate Composition of Wheat grain and Milled Fractions

Sample	Moisture %	Total Solid %	Ash	Crude fat	Crude protein	Crude fibre
Whole grain (Mixture of millings)	11.02 ± 0.18	88.98 ± 0.18	3.00 ± 0.38	2.12 ± 0.14	14.25 ± 0.01	1.10 ± 0.14
Flour	8.60 ± 0.14	91.40 ± 0.14	0.04 ± 0.02	0.21 ± 0.10	11.35 ± 0.01	0.25 ± 0.06
Dunst	10.50 ± 0.01	89.50 ± 0.01	3.02 ± 0.70	0.90 ± 0.01	11.23 ± 0.01	0.43 ± 0.06
Germ	9.35 ± 0.14	90.65 ± 0.14	4.69 ± 0.54	5.79 ± 0.53	18.36 ± 0.64	2.54 ± 0.28
Bran	11.13 ± 0.01	88.87 ± 0.01	13.77 ± 1.23	4.34 ± 0.32	16.96 ± 0.64	7.16 ± 0.72
Fine Middling	11.59 ± 0.11	88.41 ± 0.11	1.77 ± 0.35	0.24 ± 0.03	11.32 ± 0.01	0.21 ± 0.05
Semolina	12.33 ± 0.14	87.67 ± 0.14	0.51 ± 0.01	0.43 ± 0.02	10.97 ± 0.57	0.15 ± 0.06

Values are means ± S.D of duplicate determinants

grain was always 2%. In this report, the lipid crude portion constitute about 2.12% of whole wheat of the various fractions analysed which are not at variance with the findings of Mecham (1971). The germ fraction had significantly higher levels than the flour moiety ($P<0.05$). The low fat level of the flour produced from the local variety also attest to the suitability of the wheat strain for bread production.

Results from this study also indicate that the crude fibre content obtained for the whole grain was 1.10%. This is lower than the 1.71 - 2.6% fibre content obtained for some European wheat varieties (Thomas, 1972). The bran fraction had the highest crude fibre content (7.16%) which differed significantly ($P<0.05$) from all other fractions. This observation is of particular importance in human nutrition as many investigators have postulated the role of crude fibre in the prevention of colon cancer (Davidson et al, 1975).

It is evident from this study that the mineral composition (as determined total ash levels) for all the fractions are relatively low (Table 4.1). It is therefore suggested in the study that what is not a good food source of dietary minerals. This suggestion have ealier been indicated from studies on European and American wheat varieties by independent investigators (Neuman and Pelshenke, 1954; Fraser and Holmes, 1959; Richter, 1961, Salo and Kutilainen, 1970). The bran fraction of wheat may however be considered as an important source of minerals in

ruminant nutrition except that the level of antinutritional factors (e.g tannin) in the bran have been implicated in the binding of protein to form indigestible complexes in ruminant (Martin - Tanguy et al, 1977) and in the induction of Colic in horses if fed in too large amount of bran (Cunha, 1963).

4.2 Carbohydrate Composition

Table 4.2 shows the carbohydrate composition of wheat and its milled fraction. It is well established that the ability of wheat flour to form highly acceptable dough is dependent on protein starch interactions within the wheat four. Kent (1982) showed that the starch moiety serves to gluten, in providing sugar through diastates forming a bond with gluten in making gelatinization flexible. The total starch reported for wheat in present study was (68.70%). This is higher than values reported by Cerning (1970) and Tollier et al (1968) for wheat in other geographical regions. Of all fractions (Table 4.2) the wheat flour fraction had the highest soluble starch [and total soluble sugar] levels. This is of utmost important in the baking industry as sugars increase loaf volume after fermentaion. Also the total soluble sugar level is high in the bran than all other fraction The findings is consistent with previous report on wheat from other geographicalregions (Audider et al, 1966). The amylose content of the flour was also

Table 4.2 Carbohydrate Composition of Wheat Grain and Milled Fractions (Dry matter basis)

Sample	Total soluble Sugars %	Maltose %	Amylose %	Soluble Starch %	Total Starch %
Whole grain (Mixture of millings)	1.92 ± 0.10	0.25 ± 0.03	19.65 ± 0.77	13.10 ± 0.51	68.70 ± 5.30
Flour	2.03 ± 0.26	0.161 ± 0.004	29.73 ± 1.08	19.82 ± 0.72	80.63 ± 4.26
Dunst	0.33 ± 0.04	0.136 ± 0.007	27.69 ± 3.63	18.46 ± 2.40	27.72 ± 0.04
Germ	N.D	0.069 ± 0.003	1.01 ± 0.03	0.68 ± 0.01	2.44 ± 0.58
Bran	2.26 ± 0.90	0.065 ± 0.007	0.76 ± 0.07	0.51 ± 0.04	10.15 ± 2.29
Fine Middlings	1.81 ± 0.04	0.099 ± 0.004	28.63 ± 0.35	19.09 ± 0.25	78.34 ± 15.65
Semolina	0.96 ± 0.13	0.114 ± 0.005	28.51 ± 1.44	19.01 ± 0.96	72.69 ± 7.20

Values are Means ± Standard Deviation of duplicate determinati

N.D = Not determined

significantly higher (P<0.05) than other fractions. The low value obtained for germ fraction is expected since the concentration of carbhydrate is principally in the endosperm (Cotton and Ponte (Jr.), 1974).

The maltose content of Triticum aestivum, the local variety of wheat from table was 0.25% which is slightly higher than that reported by Menger (1961). In general, the study confirms previous reports that the carbohydrates in wheat grain are pricipally concentrated in the endosperm.

4.3 **Gluten of flour fraction**

Table 4.3. shows the gluten content of flour fraction. In the present study, the gluten protein obtained was 66.52% (on dry weight basis). This indicates an appreciable level of purity and also a higher potential in bread-making. The gluten content was 39.2 and 19.4% for wet and dry gluten, respectively. This was lower than the levels previously reported four another local variety of wheat growin in Nigeria (Kubiczek et al, 1987). Schmidt et al (1979) have reported that the gluten content is an important determinant of the bread making quality of wheat flour., The findings from the present study is of primary significant to the baker and miller who uses the wheat flour on account of its gluten content for bread baking and confectionary products. The result also indicate a higher keeping quality of the product as lower lipid content of gluten is known to increase shelf life of products, The present study therefore confirms kubiczek et al (1987) that the local variety compare favourably to foreign variety popular commercially recovered wheat.

Table 4.3 Crude Gluten Content and Composition of the flour fraction.

Parameter	Percentage
Wet gluten	39.20
Dry gluten	19.44
Protein*	66.52
Crude Lipid *	0.82
Starch*	4.11
Ash*	0.90
Crude fibre	ND

* Dry matter basis

ND - Not detected.

Table 4.4 In Vitro Disgestibility of of the Protein of Wheat Grain and its Milled Fraction

Sample	Non-Protein ***Nitrogen %	True ***Protein %	In vitro Protein digestibility (%) Raw	Cooked	Decrease
Whole grain (Mixture of millings)	0.15 ± 0.02	13.37 ± 0.13	90.63 ± 1.01	75.01 ± 4.42	15.62
Flour	0.10 ± 0.01	10.75 ± 0.06	91.08 ± 2.52	76.79 ± 2.52	14.29
Dunst	0.12 ± 0.01	12.17 ± 0.07	N.D	N.D	N.D
Germ	0.04 ± 0.001	18.09 ± 0.63	91.46 ± 2.11	60.53 ± 4.08	30.93
Bran	0.08 ± 0.01	16.42 ± 0.72	78.95 ± 0.05	60.53 ± 3.73	18.42
Fine Middling	0.30 ± 0.05	9.59 ± 0.27	96.43 ± 0.05	75.00 ± 0.03	21.43
Semolina	0.18 ± 0.01	9.91 ± 0.63	94.43 ± 2.62	75.93 ± 7.86	18.50

Value are Mean ± Standard Deviation of duplicate determination

- Not determined

*** Dry matter basis

4.4 In vitro protein digestibility

Table 4.4 summarizes the *in vitro* digestibility profile of wheat grain and the corresponding milled fractions. Hamaker *et al* (1987) had earlier reported *in vitro* digestibility value of 91.3 and 85.9% respectively for raw and cooked grain of United States wheat. The relatively lower values observed in our study relative to theirs may be due to variations in wheat and geographical factors during their cultivation. The high value observed for fine middling raw fraction ($P<0.05$) compared to other fractions is indicative that fine middling fraction is easily digestible. The low digestibility level observed for bran may be consequent to the high concentrations of antinutritional factors in the fraction which readily impairs protein digestion.

The cooked flour fraction gave a significantly higher ($P<0.05$) digestibility value compared to germ and bran fractions. These findings tend to support observations of Miladi *et al* (1972) who reported decreased digestibility of cooked wheat fractions. One possible reason for this phenomena may be inaccessibility of the protein bodies to enzymatic attack enzymatically since it has been shown from electron scanning spectroscopy that some of the protein bodies remains intact after cooking (Arum (1984) in Hamaker *et al*, 1987). The proteins surrounding the surrounding the interior protein body may have been formation of disulphide bonds (Hamaker *et al*, 1987). heat treatment to enhance the formation of cystine disulphide linkages in cysteine rich proteins which in

turn produces less digestible protein polymers (Opsteredt et al, 1984).

4.5 **Antinutritional Factors**.

The level of some antinutritional factors of whole wheat and milled fraction are presented in Table 4.5. The levels of oxalate in fine middling is significantly higher ($P<0.05$) than those obtained for other fractions. Oxalate has been reported to elicit deleterious effects in human nutrition by binding haemostatically to divalent minerals (trace element) such as Iron and calcium, thus reducing their bioavailability. However, oxalate concentration in wheat fractions are however lower than 2 - 5% oxalate level capable of exerting toxicological symptons in man (Oke, 1965). The low level of ash and oxalate levels also equally indicate that the effect of antinutritional factors on mineral availability may be minimized during baking of bread as high temperature have been observed to break the bond between the gluten stands.

Table 4.5 Level of Antinutritional Factors in Wheat Grain and Milled Fractions (Dry matter basis) **

Fraction	Total Oxalate	Tannin	Phytate-Phosphorus	Trypsin Inhibitor activity %
Whole grain (Mixture of millings)	93.00 ± 5	2.1 ± 0.2	2.8 ± 0.3	1.09 ± 0.14
Flour	4.00 ± 1	1.2 ± 0.2	5.3 ± 0.6	0.99 ± 0.01
Dunst	20.30 ± 3	0.7 ± 0.2	3.8 ± 0.5	3.29 ± 0.02
Germ	4.00 ± 1	0.4 ± 0.1	9.5 ± 0.1	2.83 ± 0.28
Bran	10.20 ± 2	1.5 ± 0.3	5.9 ± 0.3	1.32 ± 0.01
Fine Middling	52.00 ± 3	1.9 ± 0.2	4.5 ± 0.4	1.25 ± 0.09
Semolina	29.00 ± 5	3.6 ± 0.3	5.6 ± 0.2	1.22 ± 0.02

Values are Means ± Standard deviation of duplicate determinations expressed in mg/100g weight.

The table shows that the germ fraction had a significantly higher ($P<0.05$) phytate-phosphorus concentration which tend to suggest a considerable decrease in mineral mobilisation within the fractions (bran and germ) especially as the phytate have been shown to bind to divalent metals in food. The ash to phytate ratio is indicative of the fact that there is a considerable levels of ash and phytate in the bran fraction which tend to suggest a condition of reduced bioavailability of minerals from the bran fraction. Previous investigation reveal similar result Steven (1971) reported that iron absorption from bread decreased fairly regularly following the consuption of bread with 40% bran as the concentration of phytate in whole wheat was however low (2.8mg/100g) when it was compared to 25 - 37.6mg/100g values reported for Indian wheat (Pal, 1966). The findings from present study indicate the extent to which the local variety has been improved nutritionally.

The germ fraction had a significantly lower ($P<0.05$) tannin than other fractions. Available evidence (Chang and Fuller, 1964; Vohra et al, 1966; McGingty, 1969) have shown that high concentration of tannins in diets may result in growth retardation in chicks and a fishing or bitter flavour in broilers by reducing the digestibility and utilization of dietary protein and other nutrients. The tolerance level reported for bread wheat by Rao and Prabhavathi (1982) is 41 mg/100g. Owing to the low level of tannin in the wheat grain, the adverse effects described may not

be manifested in the local variety. The germ fraction is therefore recommended for ruminant and non-ruminant nutrition. Tannin content has also been shown to undergo significant reduction during cooking and a loss as high as 70% has been reported (Rao and Deosthale, 1982). This property therefore suggest a high potential of the local variety in breadmaking.

The level of trypsin inhibitor in wheat fraction were significantly lower in the flour compared to other fractions. In the whole grain, the level was 1.09%. This values are of significance as trypsin inhibitors have been shown to help in the regulation of endosperm protease enzyme and therefore control protein turnover and metabolism. The dunst and germ fractions had significantly higher ($P<0.05$) trypsin inhibitor level than all other fraction..

In summary, the proximate composition of Triticum aestivum compares favourably with popular commercial varieties of bread making wheat cultivated in other countries. The present work therefore confirms Kubiczek et al (1987) reports that locally grown wheat have the potentials in meeting the demand for wheat in Nigeria and other African countries.

5.0 CONCLUSION AND SUGGESTIONS

5.1 Conclusion

From the nutrient and antinutritional factor compsotion of locally grown wheat and commercial milled fructions, the following deductions can be made from the present study.

One is the distribution of nutrient within the wheat kernel as identified in the various milled fractions. The favourable comparison of these nutrient with foreign ones attest to the suitabiliyty of the various milled fractions in animal nutrition. The high digestibiliyty of the fine middling and semolina makes it a potent recipe for man and poultry. The high protein and lipid levels of the germ makes it an indispensable supplement in ruminant nutrition.

Another, is the low level of the autrinutritional factor composition which is an index of improvement in the nutritional value of the local variety. Analysis also indicate the efficiency of the Nigerian milling industry in the extraction of flour from wheat grains thereby reducing the level of antinutritional substances in the flour.

Finally the study shows that the commercial flour obtained from the locally grown wheat possesses comparable bread making qualifies to that of foreign flours as the gluten content indicates. Therefore, Nigerian grown wheat could suitably replace foreign wheat for the production of good and acceptable bread and other baking products.

5.2. Suggestions

1) Much work needs to be done by breeders to improve the baking quality and the nutritional value of the locally grown wheat so as to cut import bills on wheat. While pondering on the various crossing and breeding technique in the improvement of the protein content, particular attention should also be paid to the environment where a particular variety will thrive.
2) Research needs to be financially assisted by the government and industrialists so as to achieve the objective of self sufficiency in wheat production.
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3) In order to compare the effectiveness and quality of products from the Nigerian milling industry, different milled products from various mills need to be analysed.

FIQURE 12.

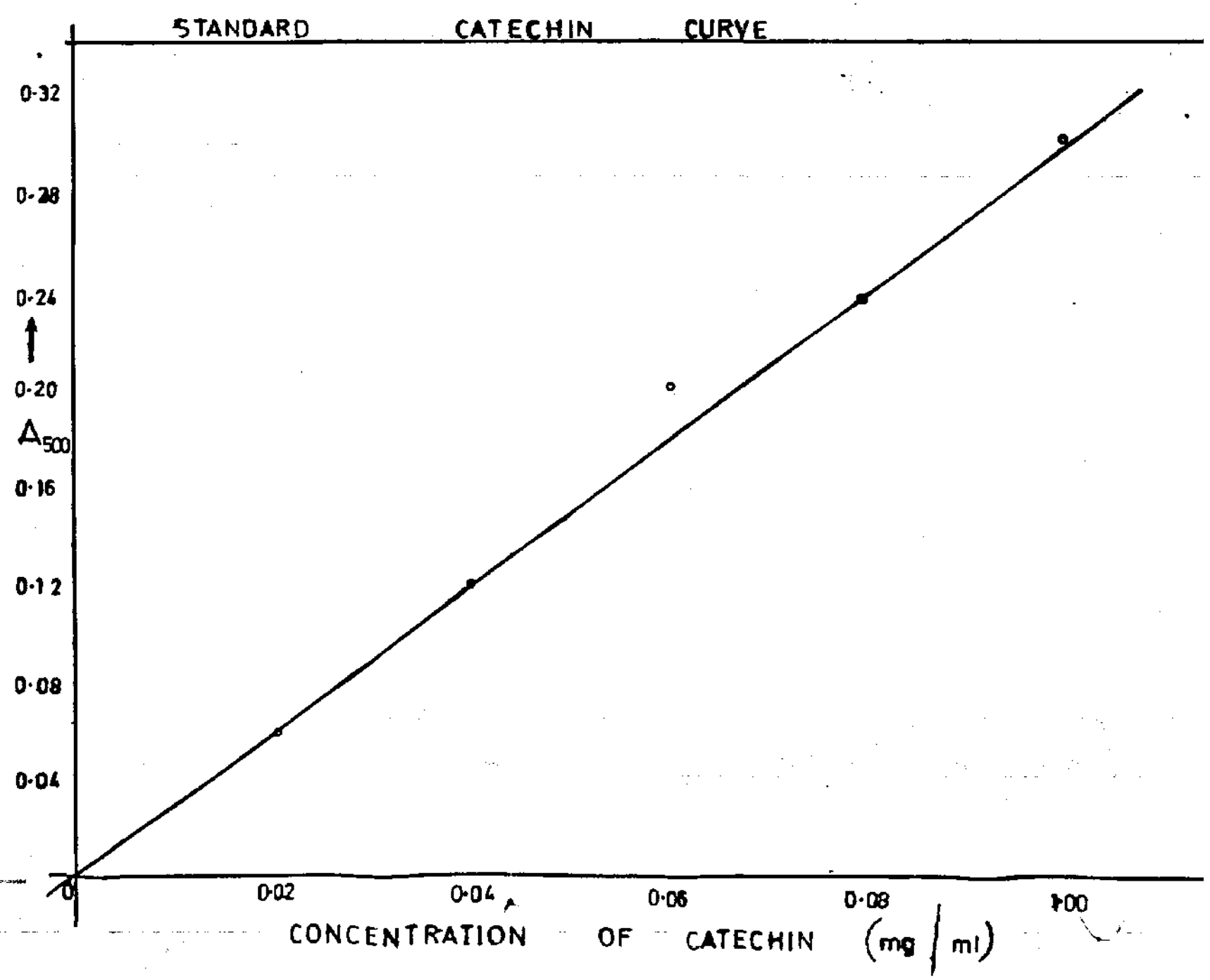
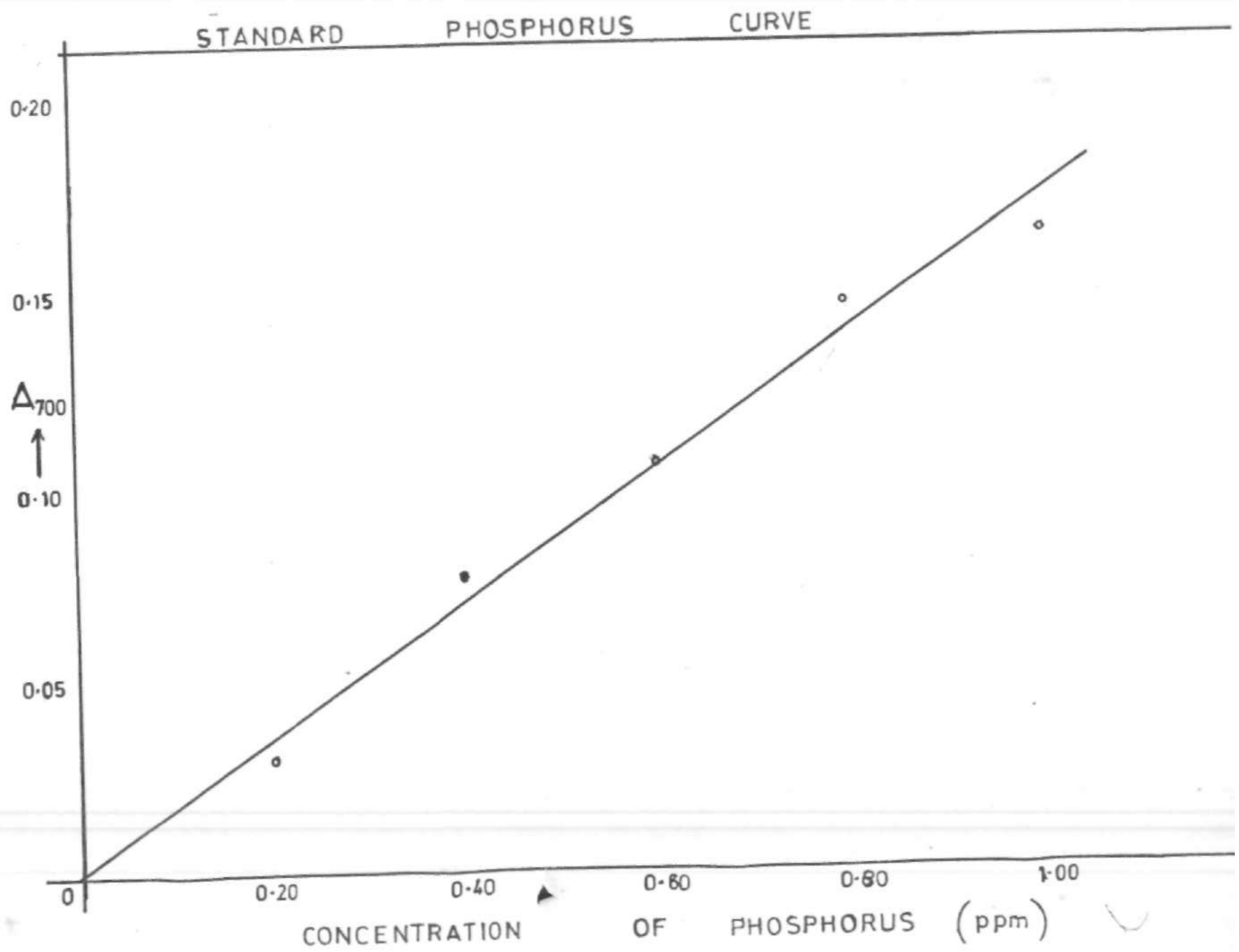

STANDARD CATECHIN CURVE
0·32
0·28
0·24
0·20
A500
0·16
0·12
0·08
0·04
0
0·02
0·04
0·06
0·08
0·00
CONCENTRATION OF CATECHIN (mg / ml)

FIGURE 3


STANDARD PHOSPHORUS CURVE
0·20
0·15
A_{700}
0·10
0·05
0
0·20
0·40
0·60
0·80
1·00
CONCENTRATION OF PHOSPHORUS (ppm)

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pp.211

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