KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

GROWTH AND BIOCONVERSION ABILITY OF OYSTER MUSHROOM (*Pleurotus ostreatus*) ON DIFFERENT AGRO-WASTES

By

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DECLARATION

I do hereby declare that this piece of work is the result of my own work towards the MSc.

Biotechnology, and that no previous submission for a degree has been done here or elsewhere. All authors whose works served as sources of information for my work have been duly recognized by references to their work.

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ABBREVIATIONS

ADF	Acid Detergent Fibre
AIBPs	Agro-Industrial By-Products
ANFs	Anti-Nutritional Factors
GS	Groundnut Shell
IVDMED	*In **vitro*** Dry Matter Enzymatic Digestibility

NDF	Neutral Detergent Fibre
RS	Rice Straw
RSGS	Rice Straw + Groundnut Shell
SCP	Single cell protein
SSF	Solid State Fermentation

ABSTRACT

The livestock industry in Ghana is challenged with high cost of quality feed ingredients. However, rice straw and groundnut shells are freely available in large quantities as agro wastes after rice and groundnuts have been harvested and processed. Though their use as animal feed is challenged with digestibility problems, through biotechnological means, these "wastes" could be turned into valuable animal feeds. This study pretreated and incubated rice straw (RS), groundnut shell (GS) and in equal proportions of rice straw and groundnut shell (RSGS) with *Pleurotus ostreatus* in a solid-state fermentation (SSF) for a period of five weeks. Proximate

analysis, mineral composition, fibre fractions, anti-nutrients and *in vitro* dry matter digestibility were determined and compared. The fungus was able to improve the protein content significantly ($P < 0.05$) above the control samples, and protein enhancement was highest in RS (132.98%), whereas GS and RSGS recorded 65.41% and 61.43% respectively. The crude fibre component of the fermented samples also significantly reduced ($P < 0.05$) with GS recording a highest of 42.25%, followed closely by RSGS (39.69%) and RS (39.07%). The fungus also delignified the "wastes" with significant ($P < 0.05$) reduction in the lignin, cellulose and hemicellulose contents. Lignin for instance declined by 48.12% in RS, 36.39% in GS and 39.78% in RSGS. The mineral content (P, K and Ca) also significantly ($P < 0.05$) improved in the fermented samples as compared to the control. *In vitro* dry matter digestibility studies indicated that the samples were over 100% more digestible than the control at the end of the optimum fermentation period of the various substrates. The tannin levels in RS reduced by 76.9%, 75% in GS and 73.8% in RSGS. The study demonstrated that SSF of these agro wastes with *P. ostreatus* increased the level of limiting nutrients e.g. proteins and minerals while at the same time decreasing the fibre levels to enhance their digestibility for monogastrics and ruminants.

TABLE OF CONTENTS

ITEM……..PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background

Several challenges confront the livestock industry in Ghana. One of the major challenges has to do with providing adequate quantities of quality feed all year round in the dry season, which impairs ruminant livestock productivity (Tuah, 1971). Meanwhile, successful management of the livestock industry requires the availability of nutritious feed at competitive prices for farmers. In the poultry industry for instance, feeding alone accounts for about 60-80% of the production cost (Tewe, 1997). Also, in the animal production industry, more than 70% expenditures are incurred on feed (Abrar *et al.*, 2002).

In Ghana, natural grasslands are the principal sources of available forages for small ruminants kept by small and medium scale traditional farmers. Livestock, however, cannot perform well on forages alone all year round due to the fact that grasses lose their crude protein content rapidly with time and their digestibility also decreases with age and they become fibrous. Plant cell wall alone accounts for about 70 to 80 per cent dry matter; cellulose content varies from 30 to 45 percent (Jayasuriya, 1985). Another reason for the poor performance lies in the seasonal inadequacies of the quantity and quality of feed available**.**

Crop residues and other agricultural by-products, once categorized as wastes have become major components of livestock feed in many parts of the world (Jayasuriya, 1985). The study added that some of the factors that call for the use of crop residues and agricultural by-products as animal feed include: the increasing demand for food, greater pressure for agricultural land use, rising cost of better-quality feed, pollution problems due to waste disposal, and the realization of the wasting of enormous quantities of potential sources of carbohydrates, among others**.** Most often than not, the wastes generated on the farms and industries are burnt, ploughed back into the soil or simply left on the field to their own fate. This can lead to massive environmental

pollution. This problem of pollution increases year after year due to the ever-increasing human population (and hence more wastes generation). It is obvious that world food production may not keep pace with the rapidly expanding population. Ruminants in the future will have to use more and more of these fibrous wastes, particularly, cassava peels, cocoa pod husks, coffee bean husks, coffee pulp, corn cobs, cotton seed cake, pulse husks, rice hulls, soybean straw, sugarcane bagasse, tea leaves, tobacco stalks, wheat straw, rice straw, water hyacinth, etc which are believed to give some fair amount of nutrients needed by animals for maintenance. Huge tonnages of these agro-wastes are produced annually after every harvest, and during the processing of these produce. They are readily available (Adebowale and Taiwo, 1996), and can supply a substantial part of the maintenance requirements of small ruminants (Jayasuriya, 1985).

The use of these by-products as feed for ruminants, however, is limited by the high levels of lignin and silica. These make it difficult for livestock to digest (Pathak, 1997). Most of these feeds also contain anti-nutritional factors (ANFs) such as tannins, theobromines and saponins (chemical constituents) which interfere with the normal digestion, absorption and metabolism of the feed (Wardlaw and Insel, 1995). The study also indicated that tannins are astringent and reduce iron absorption (Wardlaw and Insel, 1995). Also, saponins have the ability to form bonds with proteins (Livingston *et al.*, 1977) and could therefore conceivably bind digestive enzymes. Fresh rice straw for instance, has high energy content, but its use as feed for ruminants is limited by the high levels of lignin and silica in the straw (Pathak, 1997). The crude protein content of rice straw is generally between 3 and 5 per cent of the dry matter (Jayasuriya, 1985). However, any crop residue with less than 8 per cent crude protein is considered inadequate as a livestock feed because it is unlikely that such residues, without supplementation, could sustain the nitrogen balance in an animal.

This calls for an urgent need to convert safely, these “wastes” into very useful products of feed,

fuel and fertilizer. By processing these "wastes", their quality and digestibility can be improved. Processing can also increase the protein content. A number of physical, biological, and chemical methods of treatment have been employed with the aim of increasing digestibility and voluntary consumption, thereby increasing the intake of digestible energy (DE) (Jayasuriya, 1989). For instance, using urea or ammonium bicarbonate can greatly enhance both the intake and digestibility of straw, and will improve the productive performance of animals (Liu, 1995). This approach (ammoniation) has received major attention as an appropriate system for developing countries. Further improvement in performance however, may be achieved by supplementing treated straw with fresh or dried forage (Owen and Jayasuriya, 1989). The use of physical and chemical methods to do this has not been ecologically and economically friendly (Ye *et al.*, 1999).

Most reports have indicated that bioconversion (using microorganisms) appears to be best suitable in confronting the situation (Beguin, 1990). Bioconversion can be defined as the improvement and conversion of biological materials into food and non-food products using microorganisms (bacteria and fungi). Most fungi (e.g. mushrooms) have been found to be very useful in recycling organic wastes, and also efficient in returning nutrients into the ecosystem. Fungi are good managers of agricultural wastes by converting them into various products. For this reason, mushrooms are largely used as bioprocessors. Aside this role played by mushrooms, they are also being cultivated for various purposes: food, religion, medicine, dyeing of fabrics, etc. They have gained a high recognition for their flavours, medicinal properties and hidden environmental importance. One of the edible mushrooms, *Pleurotus ostreatus* (oyster mushroom) has been cultivated on various lignocellulosic wastes and has been observed to improve upon the nutritive values of these 'wastes' (Adu, 2009; Alemawor *et al.*, 2009; Tasnim, 1988).

1.2. Problem Statement

The nature of the chemical composition of any feed item is a paramount factor that tells its value (Lund, 2002). The feed value of any feed item is its nutrient potent worthiness, both quantitatively and qualitatively required by an animal in order to maintain, or even keep it growing (Lund, 2002). Over the years, farmers have depended on agro-wastes and industrial by-products as sources of feed for their livestock**.** These can supply a substantial part of the maintenance needs of small ruminants (Jayasuriya, 1985). However, the use of these 'wastes' as unconventional feedstuff for livestock nutrition is largely limited by their high cell wall material content including lignin, cellulose and hemicellulose (Sobamiwa, 1993). According to some authors, cellulose and hemicellulose are poorly digested and utilized by monogastric animals such as pigs and poultry since they do not produce the appropriate enzymes needed for the complete breakdown of these polysaccharides (Fengler and Marquardt, 1988; Annison, 1990). Another limitation with the use of these 'wastes' as feed for livestock is the presence of anti-nutritional factors (ANFs) such as tannins, theobromines, phytates and saponins. According to McDonald *et al.* (1995), theobromine (an alkaloid) is known to be lethal to chicken, monogastric animals and young ruminants. A study by Pederson and Wang (1971) with high saponin alfalfa showed that saponin is bitter and astringent when consumed by humans. Also, dietary theobromines (Owusu-Domfeh *et al.,* 1970) and saponins (Clarke and Clarke, 1979) at certain levels and regardless of the source are deleterious to chick growth. Thus, pretreatment of agro-wastes is necessary to improve and maximize livestock utilization of agro-waste-based diets. Besides physical pretreatment methods such as size reduction, chemical treatment of 'wastes' such as the use of alkali- sodium hydroxide (NaOH) has been met with insignificant improvement of digestibility problems (Tuah, 1988). Because of the high cost of chemicals, and the laborious processes of using them, none of the chemical treatments known to remove considerable amounts of tannins and other anti-nutrients have been suggested to be economically

friendly (Ranjhan, 1997). However, the use of biological pretreatments involving microorganisms in fermentation methods to ameliorate nutritional values of fibrous materials for livestock is an area worth exploring.

1.3. Justification

High feed costs and competition by animals with humans for food items (cereals and legumes) suggest strongly that alternative sources of energy such as agro-wastes should be considered to be used to replace the uneconomical practice of feeding in the livestock industry. The abundance of crop residues and agro-industrial by-products make them cheap sources of nutrients for ruminants (Adebowale and Taiwo, 1996). These can be pretreated and used as feed.

Mushroom, a fungus, thrives well on most of these "wastes", by producing enzymes that are capable of degrading them to use the cellulose and lignin in them. This helps reduce the amount of these materials in them, thereby rendering them more digestible and utilizable by livestock.

1.4. Objectives

The main objective of this project is to compare the growth and bioconversion ability of *Pleurotus ostreatus* (oyster mushroom) on different agro-wastes (rice straw, groundnuts shells and a mixture of rice straw and groundnuts shells) as means of improving upon their nutritive values.

1.4.1 Specific objectives:

- To establish optimum fermentation period on each agro-waste.
- To monitor the proximate composition of the agro-wastes during fermentation.
- Analyze some mineral content during fermentation.

- To monitor the levels of fibre and tannin during fermentation.
- To evaluate the suitability of the treated agro-wastes for animal feed through *in vitro* digestibility studies.

1.5. Hypothesis

Pleurotus ostreatus (oyster mushroom) has the potential to improve upon the nutritive value of rice straw and groundnut shells to different degrees.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Agro-Industrial By-Products (AIBPs)

Huge volumes of lignocellulosic wastes are generated through agricultural practices, forestry and industrial processes, especially from agro-allied industries such as the breweries, paper and pulp, textile and timber industries. The wastes produced normally accumulate in the environment and cause pollution problems (Abu *et al.,* 2000). Most of the wastes are disposed off by burning, a practice considered as a major factor in global warming (Levine, 1996). However, the plant biomass regarded as "wastes" are biodegradable and can be converted into valuable products such as biofuels, chemicals, cheap energy sources for fermentation, improved animal feeds and human nutrients (Howard *et al.,* 2003).

The use of fungi for the conversion of lignocellulosic materials into protein-rich food and feed offers an alternative for developing unconventional source of proteins as feed. Yeasts and algal

proteins require sophisticated techniques and heavy inputs for their cultivation whereas the beauty of fungi (e.g. mushrooms) cultivation lies in its ability to grow on cheap lignocellulosic materials with minimum inputs and a high yield of valued food protein for direct human consumption (Vijay *et al*., 2007).

A wide range of diverse cellulosic substrates have been used for cultivation of *Pleurotus* species. According to Khanna and Garcha (1982), paddy straw is amongst the various cereal straws reported to be best substrate for the cultivation of oyster mushroom (*Pleurotus ostreatus)* whereas, next to paddy straw, wheat straw was proven to be the best substrate for the cultivation of *Pleurotus spp* (Bonatti *et al*., 2004). Sorghum straw was also effectively used to cultivate *P. sajor-caju* (Bahukhandi and Munjal, 1989). Similarly, Garcha *et al.* (1984) reported the use of pearl millet stalks in the cultivation of *P. sajor-caju.* Rye straw waste (Pal and Thapa, 1979), lawn grass (Yamashita *et al.,* 1983), banana waste (Bonatti *et al.,* 2004), maize straw (Bahukhandi and Munjal, 1989) and cocoa pod husk (Alemawor *et al*., 2009) were reported as suitable substrates for cultivations of different *Pleurotus spp*. Bhandari *et al*. (1991) successfully cultivated *P. sajor-caju* on straws of millets such as *Echinochloa frumentacea* and *Eleusine coracana,* and grasses such as *Heteropogon contortus* and *Andropogon purtuses*.

Most mushroom species possess the ability to degrade lignin, cellulose and hemicellulose and to produce fruiting bodies containing most of the essential amino acids, valuable vitamins, minerals and low energy carbohydrates. *Pleurotus spp.* especially, has the potential to convert cheap cellulosics into valuable protein at a low cost (Vijay *et al*., 2007). Mushrooms get nutrition from cellulose, hemicellulose and lignin, which are abundantly available in cereal straws. According to expert reports (Jandaik and Goyal, 1995; Royse, 2002), if only one-fourth (1/4) of the world's annual yield of cereal straws (2,325 million tonnes) was used to grow mushrooms, then about 377.8 million tonnes of fresh mushrooms could be produced, and such an amount would provide about 4.103 million people with 250 g of fresh mushroom on a daily basis.

Mushroom yield on most leguminous straws are low. Veena and Savalgi (1991) reported a low yield of mushrooms on groundnut haulms. They attributed the low yield to high moisture holding capacity and a high susceptibility to fungi and improper aeration. Also, Jadhav *et al.* (1996) reported lower yields on soybean straw and groundnut haulms. Leguminous straws in combination with cereal straws, however, produce good results. For instance, Anastazia *et al.* (1982) observed that legumes rich in nitrogen gave a higher yield in combination with paddy or wheat straw or corncobs. It has also been reported that groundnut haulms and soybean straw give good results when used in combination with cotton straw and/or pigeon pea stalks and leaves and/or wheat straw (Jadhav *et al.*, 1996). The table below shows the cellulose, hemicellulose and lignin content of some agro-wastes.

Table 2.1 Cellulose, hemicellulose and lignin Content of some agro-wastes (%DM)

Residue	Cellulose	Hemicellulose	Lignin
Rice straw	36	25	12
Soybean stalks	35	25	20
Wheat straw	39	36	10
Oats straw	41	11	11
Barley straw	44	27	7
Bagasse	41	20	20

Source: (Ali, 1986)

2.2. Major Challenges in Using Agro-Wastes As Livestock Feed

The major limitations of straw as an animal feed are low protein content (Vijay *et al.*, 2007) and low digestibility (Youn, 1975).

Almost all agricultural by-products or residues are highly fibrous due to the presence of a significant amount of complex macromolecular components such as lignin, cellulose,

hemicelluloses and pectins. Besides this limitation, the presence of anti-nutritional factors (ANFs) viz. tannins, saponins, theobromines, phytate and cyanogenic glycosides generally lower nutrient bioavailability. The presence and level of occurrence of these components however, depend on the type of agro-waste and the source (Meng, 2002).

It has been observed that the type of agro-waste often affects its digestibility value. An observation made by Tuah (1988) showed that peels of yam, plantain and cassava are more digestible than straws, corn cobs, coffee pulp and cocoa pod husk. The reason being that the peels are low in lignin, and with the exception of cassava, they have high crude protein contents compared to that of tropical grasses (Akinsoyinu and Adeloye, 1987).

Crop residues usually consist of the above-ground part of cereal plants after grain removal. They are potentially rich sources of energy because up to 80 percent of their dry matter consists of polysaccharides. However, they are not all well utilized as energy sources at present, since their digestibility is often low (Youn, 1975). They partly resist rumen microbial action so their digestion is far from complete. Due to their rigid structure and poor palatability, intake of crop residues is low. These constraints are mostly related to their specific cell wall structure and chemical composition, but there are also deficiencies of nutrients essential to ruminal micro-organisms, such as nitrogen, sulphur, phosphorus and cobalt (Meng, 2002).

2.3. Structure and Chemical Composition of Cell Wall

As parts of plants, crop residues contain five different tissue types, namely:

- vascular bundles containing phloem and xylem cells;
- parenchyma bundle sheaths surrounding the vascular tissue;
- sclerenchyma patches connecting the vascular bundles to the epidermis;
- mesophyll cells between the vascular bundles and epidermal layers; and
- a single layer of epidermal cells covered by a protective cuticle on the outside.

These tissues are digested to different degrees in the rumen. In general, the extent of tissue digestion by ruminal bacteria is in the order: mesophyll and phloem > epidermis and parenchyma sheath > sclerenchyma > lignified vascular tissue. Cells have two major components: contents and walls. The cell content fraction contains most of the organic acids, soluble carbohydrates, crude protein, fats and soluble ash. The cell wall fraction includes hemicellulose, cellulose, lignin, cutin and silica. In most crop residues, the cell wall fraction accounts for 60-80 percent of dry matter (Xiong, 1986).

2.3.1. Chemical Composition of Cell Walls

Cell walls of crop residues consist mainly of polysaccharides, protein and lignin.

Major polysaccharides in primary cell walls of many higher plants include cellulose, xyloglucan and pectic polysaccharides, while secondary cell walls contain mainly cellulose and xylans (Meng, 2002).

- **Cellulose**

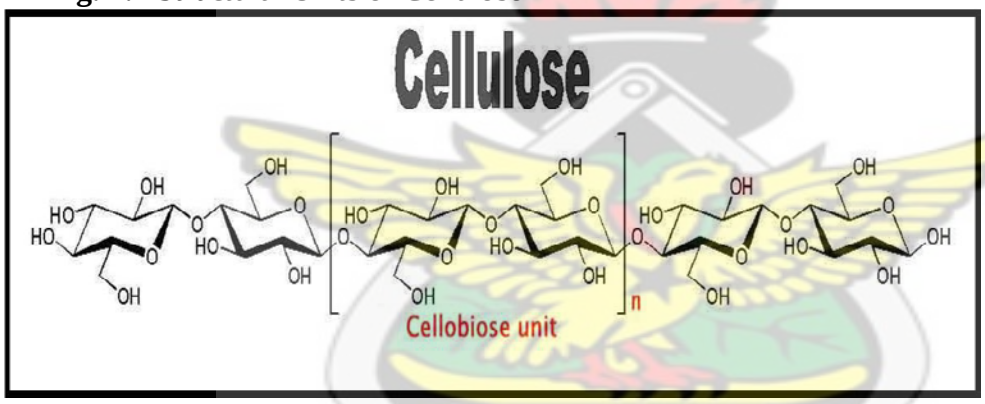
Cellulose constitutes on the average 30 to 50% of plant biomass and is the main carbon source delivered to soil with plant debris (Lynd *et al.*, 2002).

Most plant fibers contain cellulose, hemicellulose, and lignin. Cellulose is a highly ordered linear homopolymer of glucose linked by β-1,4-bonds. In all higher plants, cellulose in primary and secondary walls exists in the form of microfibrils. The crystallinity of cellulose microfibrils is highly variable depending on the source and age of the tissue. The crystallinity has been estimated to be 20-30 percent in primary walls, while in secondary walls it is 40-70 percent (Lam *et al*., 1990). Unlike hemicellulose, cellulose is resistant to hydrolysis. Cellulose fibers generally consist of a highly ordered crystalline structure of cellulose surrounded by a lignin seal, which becomes a physical barrier to easy hydrolysis. The difficulty in obtaining fast and complete

hydrolysis of the secondary hydroxyl linked polysaccharides is the inherently more resistant α-l, 4-glucan materials.

The easily hydrolysable portion of cellulose (amorphous region) is about 15% and the remainder, the resistant residue, is crystalline cellulose (Meng, 2002). Crystalline cellulose may be hydrolyzed by strong acid, but this also causes degradation of the glucose monomer. The strong crystalline structure and lignin barrier limits cellulose hydrolysis by either acids or enzymes. Acids are non-specific catalysts; they attack cellulose as well as lignin. On the other hand, enzymes (cellulases) are specific catalysts that convert cellulose into glucose with little byproduct. However, cellulase has no effect on lignin and, therefore, the cellulose is not accessible to the enzyme (Meng, 2002).

Fig. 2.1 Structural Units of Cellulose



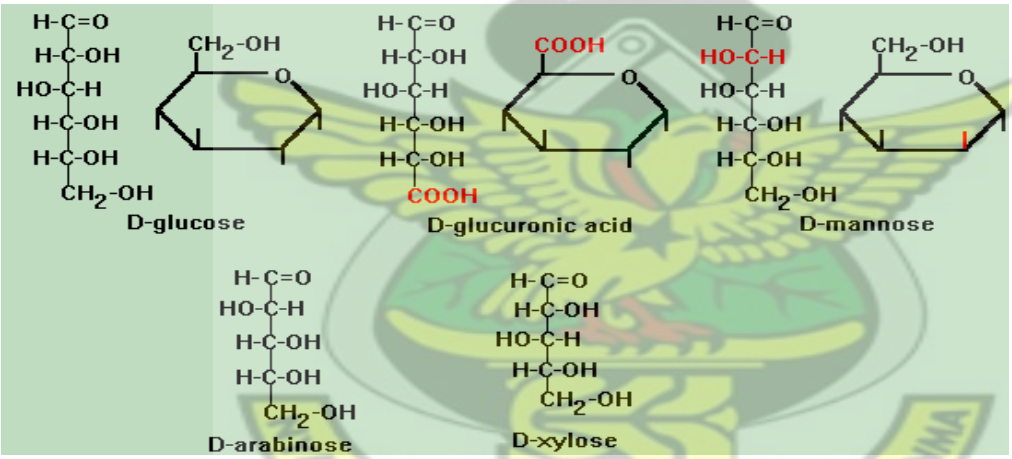
Source: Brownm (1983).

- **Hemicellulose**

Hemicellulose molecules are often polymers of pentoses, hexoses, and a number of sugar acids. Hydrolysis of hemicellulose to mono- and oligosaccharides can be accomplished with either acids or enzymes under moderate conditions (Lee *et al.*, 1979). D-xylan is the major hemi-cellulose found in woods and accounts for 20 - 35% of the total dry weight of hardwood and perennial plants (Haltrich *et al.*, 1996). The basic structure of xylan is a β- D-(1, 4)-linked xylopyranosyl residue with a few branch points (Kulkarni *et al.*, 1999). The major backbone

carries relatively short side chains of variable lengths. Due to the abundance and the structural heterogeneity of xylans, xylan-degrading enzymes are diverse (Lee *et al.*, 2003). Typical xylan-degrading enzymes are endo-β -xylanases (EC 3. 2. 1. 8) which attack the main chain of xylans, and β -xylosidases (EC 3. 2. 1. 37) which hydrolyze xylooligosaccharides into D-xylose. These two enzymes, also required for complete hydrolysis of native cellulose and biomass conversion, are produced by many bacteria and fungi. Microorganisms capable of efficiently degrading lignocelluloses, some cellulolytic microfungi including a wild strain of *Aspergillus niger* (ANL301) were isolated from decomposing wood-wastes in Nigeria (Nwodo-Chinedu *et al.*, 2005). Nwodo-Chinedu *et al.* (2007) noted that this micro-fungus grows effectively in mineral salt medium supplemented with sawdust or sugarcane as sole carbon sources.

Fig. 2.2 some monomers of hemicelluloses



Source: Brownm (1983).

- **Lignin**

Lignin is derived from the Latin word “lignum” which means wood (Boerjan, *et al.*, 2003). It is a complex chemical compound most commonly derived from wood, and is an integral part of the cell walls of plants. Lignin is a three-dimensional polymer of phenylpropane unit, and has several unusual properties (Lebo *et al.*, 2001). It is also seen as a natural, complex, heterogenous, phenylpropanoid polymer comprising 25-30% of plant biomass (Arora *et al.*, 2002). Lignin is

found in a complex with cellulosic and hemicellulosic polysaccharides, and this is often called lignocelluloses with lignin constituting between 26-29% (Raimbault, 1998). It is a large, cross-linked, racemic macromolecule with molecular mass in excess of 10,000 atomic mass unit (Davin and Lewis, 2005). It is relatively hydrophobic and aromatic in nature (Hatakka, 2001). The highest concentration of lignin is found in the middle lamella and in the corner regions of the plant cell wall structure, delaying the delignification of these areas (Blanchette, 1991). Different types of lignin have been described depending on the means of isolation (Davin and Lewis, 2005). Grass lignin is esterified by cinnamic acids, chiefly p-coumaric acid through hydroxyls on its monomers. In addition, ether-linked ferulic acids have been observed in lignin from maize stalks, wheat straw, rice straw and bagasse (Lam *et al.*, 1990).

Pectic polysaccharides are present in the primary cell walls of all seed bearing plants and are located particularly in the middle lamella. They are the major components of the primary cell walls of dicotyledons (e.g. legumes) but account for relatively less of the primary walls in monocotyledons (grasses). Three pectic polysaccharides have been structurally characterized from the primary walls of both monocotyledons and dicotyledons: rhamnogalacturonan I, rhamnogalacturonan II, and homogalacturonan (O'Neill *et al.*, 1990). Pectic polysaccharide concentration is quite low in grasses (monocotyledons), generally <10 to 40 g/kg DM, while fairly high in legumes (dicotyledons) ranging from 50 to 100 g/kg (Van Soest, 1994).

Proteins make up 2-10% of the primary cell wall of many dicotyledons and some monocotyledons. Cell wall proteins may also be involved in covalent bonding with polysaccharides. Glycoproteins seem to be invariably found in primary cell walls. Apparent covalent protein-lignin linkages have also been observed in wheat internodes (Iiyama *et al.*, 1993). Of the several types of structural proteins known, the best-characterized are the family of hydroxyproline-rich proteins (Meng, 2002). These glycoproteins with rod-like conformations are

components of the wall matrix in dicotyledons and in grass walls (e.g. maize pericarp). Other wall proteins, e.g. glycine-rich proteins, have been found in walls of herbaceous dicotyledons (Lamport, 1977).

Tannins are phenolic compounds synthesized by some plants as a defense. They may inhibit the activity of specific enzymes, such as cellulases. Since tannins are often insoluble, they can contaminate the crude lignin, resulting in higher analytical value. As a result of complexes with protein, tannins would depress its utilization, but may not affect cell wall carbohydrates (Meng, 2002).

2.4. Nutritive Value of Some Crop Residues

Nutritive value is generally determined by feed composition, intake and utilization efficiency of digested matter. Thus, the value of a feed depends on chemical composition, digestibility, intake and efficiency (Meng, 2002).

Table 2.2. Nutrient Content of Some Crop Residues (%DM)

Crop residue	**DM**	**CP**	**EE**	**CF**	**CW**	**Ca**	**P**
Wheat straw	91.6	3.1	1.3	44.7	73.0	0.28	0.03
Maize stovers	91.8	6.5	2.7	26.2	70.4	0.43	0.25
Rice straw	83.3	3.7	1.6	31.0	64.4	0.11	0.05

Sorghum stovers	95.2	3.9	1.3	35.6	74.8	0.35	0.21
Barley straw	88.4	5.5	3.2	38.2	80.1	0.06	0.07
Soybean straw	89.7	3.6	0.5	52.1	74.0	0.68	0.03
Oat straw	93.0	7.0	2.4	28.4	72.3	0.18	0.01
Millet straw	90.7	5.0	1.3	35.9	74.8	0.37	0.03
Peanut hay	90.0	12.0	2.7	24.6	88.8	0.13	0.01
Sweet potato vine	91.7	8.4	2.6	19.8	36.6	1.47	0.48

Source: (Anon, 2000).

KEY: DM = dry matter; CP = crude protein; EE = ether extract; CF = crude fibre; CW = cell wall or NDF; Ca = calcium; P= phosphorus.

Table 2.2 above contains the nutrient content of some cereal crop residues. Crop residues also have a low mineral content, especially phosphorus, and are deficient in vitamins. Therefore, supplementation of crop residues before feeding is necessary, in addition to various treatments. Various crop residues have their own nutritional values and are used for different animal species. For instance, many years of research station feeding trials in the Gambia, using many types of crop residues in several combinations showed that weights of growing animals could at least be maintained, if not increased, over the dry season (Russo and Ceesay, 1986). Wheat straw and rice straw have high contents of cell walls, and are basically used for feeding ruminants. Millet straw and soybean straw in contrast, are fairly palatable (Zhou, 1994).

2.5. Factors Affecting the Nutritive Value of Crop Residues

A variety of factors have been identified that may influence the nutritive value of crop residues.

2.5.1. Plant Factors

The lignin fraction and associated phenolic compounds are factors most consistently associated

with the rigid structure of plants and limited accessibility. The association of lignin with cell wall polysaccharides is also believed to be responsible for resistance of plant cell walls to microbial digestion in the rumen. Table 2.3 shows the main chemical composition and *in vitro* DM digestibility of three major crop residues.

Table 2.3 Composition and *in vitro* DM digestibility of some major crop residues %DM

Residue	**DM**	**CP**	**NDF**	**NDS**	**ADF**	**CEL**	**HC**	**ADL**	**IVDMD**
Rice straw	90.6	4.7	67.2	32.8	46.3	33.8	20.9	5.2	42.2
Wheat straw	90.3	4.4	79.1	20.9	54.9	43.2	24.2	7.9	43.0
Maize stovers	96.1	9.3	71.2	28.8	38.2	32.9	32.5	4.6	49.1

Source: Xing (1995).

KEY: DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; NDS = neutral detergent soluble; ADF = acid detergent fibre; CEL = cellulose; HC = hemicellulose; ADL = acid detergent lignin; IVDMD = *in vitro* dry matter digestibility.

Wheat straw has higher lignin, and therefore lower DM digestibility, compared with maize stover. Although rice straw has a medium lignin content, its DM digestibility is rather low, which may be caused by its relatively high silica concentration (Xing, 1995).

Other plant factors include species, stage of maturity at harvest, cultivar, and proportions of leaf, sheath and stem. All these are believed to influence the nutritive value of crop residues. As plants mature, nutrient digestibility generally declines, linked to a decrease in the digestibility of cell wall components. Xing (1995) reported that, at an early growth stage, *in vitro* DM digestibility (IVDMD) of wheat straw is pretty high. The report added that as wheat matures, however, the IVDMD of straw progressively decreases. When the grain is completely mature at harvest, the straw has its lowest IVDMD value, resulting from decreased nitrogen content and increased lignifications.

2.5.2. Animal Factors

Little information is available about animal factors that influence the nutritive value of crop residues. Farmers have long known that different breeds and types of animals use crop residues with various efficiencies. Cattle, which retain fibrous matter in the rumen slightly longer than sheep or goats, presumably have an advantage with lower quality crop residues. For instance, (Kennedy, 1982) observed that cross-bred Brahman (*Bos indicus*) steers, when fed hay with 730 g/kg NDF, digested more NDF in the rumen and had longer ruminal retention time for lignin than did Hereford (*B. taurus*) steers.

2.5.3. Environmental Factors

Some environmental factors such as location, climate, soil fertility and soil type, influence the nutritive value of crop residues. Some studies reported that there can be significant differences in chemical composition and digestibility of crop residues grown on different soil types (Xing, 1995). The study noted that irrespective of crop cultivar, straw from wheat grown in the so-called tide soils (alluvial soils with diurnal variation in groundwater level) had considerably higher CP content and lower fibre (NDF, ADF and ADL) content than straw from drab soils (cinammon soils of forest origin). These could probably be the cause of digestibility differences.

2.6. Methods for Improving the Feeding Value of Crop Residues

Crop residues are the main agricultural by-products in Ghana. Since ancient times, Ghanaian farmers have traditionally fed crop residues to livestock. Most of these residues were untreated, and thus with low digestibility, low crude protein (CP) content and poor palatability, and so intake has been low. Untreated residues can barely satisfy maintenance requirements, and, as a result, livestock performance is poor. For the past years, scientists and technicians all over the world have studied and tested several methods for improving the feeding value of crop residues, and these are discussed below.

2.6.1. Traditional Processing and Feeding Methods

There is a Chinese farmers' proverb that says: *"chopping hay to one inch, fattening can be done without concentrate" (*Meng, 2002).

The ancient processing and feeding methods include particle reduction and reconstitution of roughage. Chopping and water soaking are popular practices for crop residue feeding. According to Xiong (1986) although they do not always result in consistent improvements in animal performance, they definitely result in reduced diet wastage and diet selection.

2.6.2. Physical Treatments

Numerous physical processing techniques to enhance the utilization of crop residues by livestock have been used, with varying degrees of successes. The most studied physical treatments for enhancing crop residue use by livestock are grinding and pelleting. Grinding, or fine chopping, decreases particle size, increases surface area and bulk density of both leaf and stem fractions, and hence raises rumen microbial accessibility or feed intake *(*Meng, 2002). Xiong (1986), recommended 6 mm for sheep and 12 mm for cattle as the appropriate screen sizes for hammer mills considering differences in intake between animal species and the energy expenditure for grinding.

Ground crop residues are often pelleted or cubed before feeding. Benefits derived from pelleting include a further increase in density, decreased dustiness and easier handling. However, DM digestibility of pelleted straws is depressed relative to the long or chopped forms, primarily due to faster passage rate. Pelleting usually augments straw intake due to quicker passage, which can offset the negative effect from decreased digestibility. Therefore, the net benefit of feeding pelleted crop residues in practice is increased energy intake and animal performance. Few studies

have been conducted to assess the feeding value of ground and pelleted crop residues in China. Fu *et al*. (1991) studied the response of lamb growth performance to ground and pelleted maize stover. Compared with coarse grinding of maize stover (through a 25-mm screen), processing with fine grinding (through an 8-mm screen) followed by pelleting increased feed intake by half and daily gain by 129%, and reduced the feed/gain ratio by 34.1%.

A novel method for processing crop residues using a kneading machine has been reported (Gao *et al.*, 1994). Fibrous crop residues are kneaded into threadlike fibres or hairs by a machine. Kneading extensively destroys the rigid structure and thus significantly increases voluntary intake. Unlike other physical processing such as grinding or pelleting, rubbing of crop residues produces long threadlike fibres (usually 8-12 cm long). However, compared to chopping, kneading requires higher energy expenditure.

Several studies have been conducted to compare kneading with traditional chopping. Sun *et al.* (1991) reported that dairy cows fed with scrubbed soybean residue had higher dietary DM intake and milk production than with untreated residue. A similar result with kneaded maize stover fed to dairy cows was reported by Zhao and Sun (1992).

Irradiation treatment of lignocellulosic materials to improve the utilization of cell wall polysaccharides dates back to the work of Lawton *et al.* (1951). They found that when basswood was irradiated with high velocity electrons, rumen bacteria fermentation was increased. Electron irradiation of straw can also increase polysaccharide digestibility by ruminal micro-organisms. Several studies on irradiation of crop residues for increasing their nutritive value have been conducted in China. Meng and Xiong (1990) treated wheat straw with a combination of α-rays from a cobalt-60 source and NH_3 (3% of DM) or NH_3 (1%) plus $Ca(OH)_2$ (5% of DM) at different moisture levels. They found that irradiation doses had a significant interaction with the

moisture level. In another study, Gu *et al.* (1988) found lower contents of fibrous fractions (NDF, ADF and lignin) and elevated *in vitro* dry matter digestibility (IVDMD) with irradiation of rice straw. Treatment of rice straw with a combination of electron irradiation and NaOH also resulted in a considerable higher glucose release (Lu and Xiong, 1991).

High pressure steaming involves placing crop residues in a closed steel tank, and saturated with high pressure steam. When the expected temperature (or pressure) and time are reached, a tank valve is suddenly opened allowing materials to enter a pressure-release tank through a specially designed tube. This high pressure steaming and explosion result in a brown straw with looser structure. This method significantly decreases straw CF (He *et al.*, 1989) and therefore increases the *in vitro* DM digestibility. Also, results from an *in situ* study by He *et al.* (1989) showed that NDF digestibility (48 hour incubation) of the treated wheat straw was increased by 68% in rumen-fistulated sheep and by 233% with caecum-fistulated pigs. Rumen volatile fatty acids (VFAs) concentration was also increased by 9.9% in sheep fed diets based on the high pressure steamed wheat straw, compared to untreated straw. Again, in lamb feeding trials by Hou *et al.* (1997), animals were fed equal amounts of mixed concentrate and wheat straw per day per animal. Lambs fed high pressure steamed straw ate more of it and gained faster than lambs with untreated straw.

One advantage of using high-pressure steam method is that it does not require reagents and thus minimizes potential environmental pollution. Also, in relation to other physical treatments, it is more effective in improving crop residue nutritive value. However, it implies high investment for equipment and a steam generator.

There is also the so-called "salting" method, in which chopped straw is soaked in a dilute salt

solution before feeding. Although this method has not been scientifically tested, many farmers in northeast and north China practice it, considering it effective.

2.6.3. Chemical Treatment

One major breakthrough made to improve the digestibility and nutritive value of crop residues is chemical treatment to remove encrusting substances such as cellulose, hemicellulose and lignin. Chemicals [sodium hydroxide (NaOH), ammonia (NH_3), ammonium hydroxide (NH_4OH) and calcium hydroxide [$Ca(OH)_2$] are being routinely used for this method of treatment (Meng, 2002).

2.6.3.1 Sodium Hydroxide Treatment

Efforts have been made to increase the feed value of cereal straws by chemical treatment, as well as nutrient supplementation. Alkalization and ammoniation are the main chemical methods. The digestibility of various crop straws can be increased by treating with NaOH or NH_4OH, but the low protein still requires nitrogen supplementation. Sodium hydroxide treatment of crop residues has been investigated and used in some areas. The treatment with NaOH (Xiong, 1986) results in increases in crop residue palatability and digestibility, and in animal performance. According to Sun (1985), steers fed rations based on NaOH-treated wheat straw gained 20 percent faster than did the control group when concentrate was half of total ration. Ye *et al.* (1999) also reported that dairy cows fed NaOH-treated rice straw diets ingested 86.4 percent more straw and produced 7.9 percent more milk per day than those on untreated-straw diets. Campling *et al.* (1972); Oh *et al.* (1971) have also observed that additions of urea, molasses, branched-chain fatty acids, sulfur, and other minerals have met with varying successes in the improvement of residues.

Although NaOH treatment works effectively in improving the nutritive value of crop residues, NaOH is expensive, corrosive and its use may result in significant excretion of sodium ions in animal excreta. Long-term accumulation of sodium may lead to soil fertility problems and environmental pollution. Thus, application of NaOH treatment of crop residues is not popular with most farmers (Ye *et al.,* 1999).

2.6.3.2. Ammoniation

Studies have shown a decreased NDF content, but little change in ADF and ADL contents of crop residues due to ammoniation (Wu, 1996). Meng and Xiong (1993) found that lambs fed ammoniated wheat straw had increased dietary intake, body weight gain and better concentrate conversion efficiency compared with animals fed untreated wheat straw. Ammoniation has been widely applied in many parts of the world due to its low level of environment pollution, lower cost and ease of application. Although ammoniation has practical advantages, it improves digestibility less than does alkalization.

Other treatments involve the use of limestone ($Ca(OH)_2$) to treat crop residues. Calcium hydroxide is generally less effective in treating crop residues than other alkaline sources, such as NaOH or NH_3 (Meng, 2002).

Some Scientists are studying combinations of the two treatments [i.e. $Ca(OH)_2$ with urea or alkalis]. Mao and Feng (1991) observed that combining $Ca(OH)_2$ with urea or other alkalis showed that rice and wheat straw treatment increased the CP content by 3.5 times and *in situ* DM digestibility by 69.8%. In a related study by Feng (1996) dairy heifers fed such treated rice straw showed significant increases in dietary DM intake, weight gains and feed conversion as compared with those fed the untreated straw. Also, Cao *et al.* (2000) reported significant improvements in the nutritive value of wheat and rice straws as a result of combination treatment

with urea, calcium hydroxide and common salt.

2.7. Biological Approach

This technique generally involves the application of biological agents to improve the nutritive value of crop residues and feedstuffs. The use of specific whole microorganisms in bioconversion technologies or fermentation processes is one of the major types of biological approaches that can be considered in the livestock feed industry (Iyayi and Aderolu, 2004). According to Motarjemi (2002), this method of bioconversion of organic substances by microorganisms is one of the oldest applied biotechnologies and has been used in food processing and preservation for over 6000 years. Microbial processes are normally characterized by multi-step processes including pre-treatment (i.e. size reduction and pasteurization) of the raw material (substrate) prior to inoculation, an incubation step which allows the bioprocess to take place, product recovery stage, and/or a post-bioprocessing stage to enhance storability (Grethein and Converse, 1991). Fermentation processes may have advantages over chemical methods in upgrading raw organic materials into enriched or value-added products such as feed material for livestock because little capital investment is needed to establish and maintain them. In addition, fermentation enhances the nutrient content of foods and feedstuffs through the microbial biosynthesis of vitamins, essential amino acids and proteins, improves fibre digestibility, enhances micronutrient bioavailability and helps in degrading anti-nutritional factors (Achinewhu *et al.*, 1998). Another advantage is that fermentation improves the safety and shelf-life of the final product following the production of some antimicrobial factors such as organic acids (Mbongo and Antai, 1994).

Two main fermentation processes include submerged or liquid-substrate fermentation such as that which is applied in the brewery industries, and solid-state fermentation such as that which is

used in the production of mushrooms.

2.7.1. Solid-State Fermentation (SSF)

Moo-Young *et al. (*1983); Cannel and Moo-Young (1980), described this process as the growth or cultivation of microorganisms on moist solid organic materials in the complete or almost complete absence of free-flowing water or liquid. The water which is an important component for microbial activities is present in an absorbed state, or it usually forms complexes with the solid matrix or the substrate (Cannel and Moo-Young, 1980). Because the amount of water available in this process is not enough, fungi are the predominant types of microorganisms most commonly used (Pandey *et al*., 2000; Zheng and Shetty, 2000). As these microbes in SSF grow under conditions closer to their natural habitats, they become more capable of producing enzymes such as proteases, cellulases, lignases, xylanases, pectinases and amylases (Jecu, 2000). The enzymes and metabolites (including flavour and anti-microbial factors) produced modify the composition of the solid substrate or medium. The process also reduces or eliminates the anti-nutritive components in the fermented products (Nout and Rombouts, 1990).

2.7.2. Use of Mushroom-Substrate Residues

Crop residues have been used as substrates to grow mushrooms. The substrate residue after mushroom harvest can be used to feed animals. The most commonly used crop residues are cottonseed hulls, wheat straw, rice straw and maize stover. The residues usually have higher crude protein (CP) and lower crude fibre (CF) contents compared with the original substrate. Adu (2009) reported an increase of 5.6% in CP at the end of week six (6), while CF declined by 34.32% at the end of the 8th week when cassava peels was fermented with *P. ostreatus*. Alemawor *et al*. (2009) also reported 32.28% and 39.93% increases in CP for control and Mn-amended cocoa pod husk (CPH) respectively at the end of the 6th week, while CF declined

significantly at weeks 5, 6 and 7, with averages recording 8.28% and 17.08% for the control and Mn-amended substrates respectively. Yang *et al*. (1986) also reported that after the 2nd, 3rd and 4th harvest of mushrooms, the CP content of the residual substrate increased by 32.5, 44.2 and 60.9 percent, while its CF content reduced by 42.4, 48.1 and 50.4 percent, respectively. When the substrate residue was included in growing pig diets at a level of 5 percent (replacing half of the wheat bran), there was no significant difference in average daily gain and feed conversion (Liu *et al.*, 1998). However, growth performance of pigs decreased with increased substrate residue inclusion (Zhou, 1991). The only benefit from inclusion of the substrate residues at a low rate in pig diet is the decreased consumption of concentrate or feed cost per unit of body weight gain (Lu *et al.*, 1995).

2.7.3. Enzymatic Treatment

Fungi produce various enzymes that hydrolyze the raw materials and change their texture, taste and aroma. Enzymes secreted by fungi could hydrolyse lipids, polysacchrides and protein (Nout and Rombouts, 1990). The use of enzymes to attack the lignocellulose structure of crop residues for enhancing their feeding value has been attractive. Crude enzyme products, with cellulolytic and hemicellulolytic capabilities, are usually added to fibrous feeds in attempt to improve their digestibility. Wang (1998) observed that treatment of maize stover with an enzyme product, prepared from *Trichoderma viride*, reduced the contents of some cell wall components and enhanced the ruminal digestibility in sheep. Commercial cellulase products were also added to diets to increase the supply of readily available carbohydrate. When the enzyme products were included at 0.1% to 0.2% in the diet of pigs, cattle and geese, animal performance was considerably improved. Chen *et al*. (1986) also reported the use of crude enzyme products prepared from *Trichoderma viride* as feed additives for growing rabbits. In eight growth trials,

rabbits fed on a diet with addition of the cellulolytic enzymes gained 17.5% to 39.3% faster than the control. The difference was consistent and highly significant ($P < 0.05$).

2.8. Biodegradation of Lignocellulose in Crop Residues

Of all the three components of lignocellulose, lignin is the most stubborn to degradation whereas cellulose, because of its highly ordered crystalline structure, is more resistant to hydrolysis than hemicellulose. Much of the cellulose in nature is bound physico-chemically with lignin. Thus the highly resistant lignin, which protects cellulose, must be degraded by chemical or biological means first before the cellulose can be utilized. According to Call and Mùcke (1997) biological treatments involving enzymes or whole microorganisms are preferred to chemical methods since they are specific biocatalysts and can operate under much milder reaction conditions. More interesting is the fact that biological treatment does not produce undesirable products and are also environmentally friendly.

Lignocellulose-degrading fungi are divided into three major groups. These groupings depend on the type or morphology of rot they cause in the material (substrate). Thus we have white-rot, brown-rot and soft-rot fungi (Steffen, 2003). Of these groups, the white-rot fungi are the most rapid and extensive lignin degraders (Akin *et al*., 1995; Hatakka, 2001). The white-rot group of fungi is heterogeneous by nature, classified in the division Basidiomycota. They are obligate aerobes deriving their nourishment from the biological combustion of wood or lignocellulosic materials using molecular oxygen as a terminal electron acceptor (Kirk and Cullen, 1998). Different white-rot fungi vary considerably in the relative rates at which they attack lignin and carbohydrates in lignocellulose or woody tissues. The white-rot type exhibit two gross patterns of decay: a) selective decay, where lignin and hemicellulose are degraded significantly more than cellulose, and b) non-selective (i.e. simultaneous) decay, where equal amounts of all components of lignocellulose are degraded (Blanchette, 1995; Hatakka, 2001). Some examples of white-rot

fungi are *Planerocheate chrysosporium, Phellinus nigrolimitatus, Ceriporiopsis subvermispora, Phlebia radiate, Formes fomentarius, Pleurotus ostreatus,* etc. Peng (1998) treated wheat straw with *Pleurotus ostreatus* for 30 days. The results showed that the treatment decreased the neutral detergent fibre (NDF) from 71.4% (control) to 67.9%. Lateef *et al*. (2008), studied the effect of solid-state fermentations of some agro-wastes, namely cocoa pod husk (CPH), cassava peel (CP), and palm kernel cake (PKC) with the fungal strain *Rhizopus stolonifer* LAU 07. Results showed that the nutritional qualities and antioxidant activities of all the investigated solid substrates were enhanced by fungal fermentation. The protein contents of the substrates increased by 94.8%, 55.4% and 33.3%, while the crude fibre contents decreased by 7.2%, 8.6% and 44.5% in CPH, CP, and PKC, respectively. The cyanide content of cassava peel was also reduced by 90.6%. In another study, solid-state fermentation of aspen (*Populus tremuloides*) wood with *Merulius tremellosus* for 8 weeks removed 52% of the lignin and increased the cellulase digestibility to 53% from 18% (Ian, 1985).

Brown-rot fungi mainly degrade cellulose and hemicellulose, leaving the lignin more or less intact as a brown layer (and hence the name brown-rot). The residual lignin is chemically modified and the brown-rot attacks result in only a limited decrease in lignin content (Eriksson *et al*., 1990). The presence of lignin stimulates cellulose degradation by the brown-rot fungi, although lignin is degraded to a lesser extent (Blanchette, 1995; Hatakka, 2001). An example of brown-rot fungi is *G. trabeum.*

The soft-rotters, being the minority wood-decaying fungi, mostly belong to the Ascomycetes. They are commonly found in hardwoods where they soften the wood surface layers (Daniel and Nilsson, 1998). They predominate in excessively wet or dry environments. They tolerate better, wider ranges of temperatures, pH, and oxygen limitation than white-rot or brown-rot fungi

(Blanchette, 1995). The soft-rot fungi degrade cellulose and hemicellulose, but only little lignin (Rayner and Boddy, 1988).

2.9. Some Anti-Nutritive Factors (ANFs) in Agro-residues and Effects of Microbial Treatment

Trees and shrub foliage and agro-industrial by-products are of importance in animal production because they do not compete with human food and can provide significant protein supplements, especially in the dry season. But, these feed resources are generally rich in anti-nutritional factors (ANFs), particularly tannins (Makkar, 2003). Tannins are polyphenolic substances with various molecular weights and a variable complexity. These are chemically not well-defined substances but rather a group of substances with the ability to bind proteins in aqueous solution. According to Kamalak *et al.* (2004); Seresinhe and Iben (2003), tannins give anti-nutritional effects and this limit their use as feed ingredients. Their multiple phenolic hydroxyl groups lead to the formation of complexes primarily with proteins and to a lesser extent with metal ions, amino acids and polysaccharides (Makkar, 2003). Tannins are tentatively classified into two classes: hydrolysable and condensed tannins. Several factors contribute to the anti-nutritional effects of condensed tannins. First, the binding of the polyphenolic compounds to cell wall polyssacharides reduces their digestibility in ruminants (Schofield *et al.*, 2001; Kumar and D'Mello, 1995). Furthermore, soluble tannins form strong complexes with proteins which do not dissociate at physiological pH. Consequently, a variety of digestive enzymes, including cellulase and α-amylase, are inhibited (Kandra, 2004; Maitra and Ray, 2003). In addition, condensed tannins impart astringent taste and depress feed intake and use by animals (Brooker *et al.*, 1994), leading to growth depression. Tannins also decrease the attachment of microbes to feed particles (Makkar *et al.*, 1989). McAllister *et al.* (1994) have also shown that *Lotus corniculatus* condensed tannins caused a considerable detachment of *Fibrobacter succinogenes* S85 from colonized filter paper after a 30

minutes exposure. This could also be due to increase in the growth of microbes, which did not bind to tannins and were left active in the system. Waghorn *et al*. (1994); Stienezen *et al.* (1996) also suggest lower *in vivo* rate of feed digestion in the presence of condensed tannins. Ruminants, in general, have the ability to tolerate much higher concentrations of ANFs as compared to non-ruminants. Table 2.4 gives some ANFs in some feeds.

Table 2.4 Some Anti-Nutritional Factors in Agro-industrial by-product Feeds

By-products feed	**Anti-nutritional factor**
Soybean meal	Haemagglutinins, Goitrin, Protease inhibitors, Saponins
Sesame meal	Mineral binders
Beet pulp	Saponins
Guar meal	Protease inhibitors
Peanut meal	Aflatoxin, goitrogen, Protease inhibitors, saponins
Linseed meal	Cyanogens, Anti-B6

Cotton seed cake	Gossypol
Castor seed meal	Ricin, Haemagglutinin
Rape seed and Mustard	Thioglucoside, Goitrin, isothiocyanate

Source: (Bhatti & Khan, 1996)

A number of technologies and methods have also been developed to detoxify or at-least minimize the effect of these toxins or anti-nutritional factors in animal feeds as shown in Table 2.5.

Table 2.5 Natural inhibitors in feedstuffs

Feedstuff	**Inhibitor(s) toxins**	**Deactivation process**
Cottonseed meal	Gossypol Cyclopropene fatty acids	Adding iron salts; rupturing pigment gland
Soybean meal	Trypsin inhibitors an unidentified factor	Heat; autoclaving
Linseed meal	Crystalline water soluble substance	Water treatment
Raw fish	Thiaminase	Heat
Lucerne meal	Saponins: pectin methyl esterase	Limit amount of feed

Rapeseed	Isothiocyanate Thyroactive materials	-
Groundnut meal	Aflatoxin	Treatment with ammonia or ammonium hydroxide

Source: Benerjee (1993)

Table 2.6 also shows the level of Anti-Nutritional Factors in some fruits.

Table 2.6 Level of Anti-Nutritional Factors in 16 wild fruits (%).

Fruits	**Oxalate**	**Phytate**	**Saponin**	**Tannin**
Adansonia digitata (Baobab)	9.5 ± 0.42	0.69 ± 0.15d	10.51 ± 0.11	2.22 ± 0.32
Balanite aegyptiaca (Desert date)	14.50 ± 2.08a	1.90 ± 0.27	16.01± 0.02e	7.40 ± 0.14g
Borassus aethiopum (Toddy palm)	11.30 ± 1.70	0.65 ± 0.18d	7.04 ± 0.05	3.18 ± 0.30
Nuclea latifolia (African fan peach)	2.22 ± 0.42b	0.95 ± 0.19	9.01 ± 0.01	2.80 ± 0.12
Detarium macrocarpum (Tallow tree)	13.50 ± 2.16	2.13 ± 0.97	12.10 ± 0.05	3.54 ± 0.28
Diospyros mespiliformis (Monkey guava)	12.20± 1.70	0.92 ± 0.08	4.04 ± 0.10	2.61 ± 0.16
Haematostaphis barteri (blood plum)	6.30 ± 1.91	3.30 ± 0.10c	5.03 ± 0.15	2.13 ± 0.81
Hyphaena thebaica (Egyptian doum palm)	13.50 ± 5.73	1.18 ± 0.05	8.25 ± 0.31	6.39 ± 0.51g
Parkia biglobosa (Locust bean)	11.10 ± 3.52	2.13 ± 0.51	12.23 ± 0.46	0.93 ± 0.11h

Vitex doniana (Black plum)	10.10 ± 2.12	0.75 ± 0.16	6.14 ± 0.32	4.83 ± 0.15
Vittaleria paradoxum (Shea nut)	7.02 ± 1.20	0.92 ± 0.14	1.50 ± 0.10f	3.83 ± 0.32
Zizyphus mauritiana (Indian jijube)	15.50 ± 1.50a	1.57 ± 0.33	7.13 ± 0.21	2.42 ± 0.04
Borassus aethiopum (young shout)	02.20 ± 0.07b	0.72 ± 0.03	11.08 ± 0.02	5.90 ± 0.13
Phoenix dactylifera (Date)	6.90 ± 0.91	0.52 ± 0.03d	2.04 ± 0.01f	0.93 ± 0.21h
Sclerocarya birrea (African plum)	4.90 ± 1.70	3.56 ± 0.54c	7.35 ± 0.10	2.04 ± 0.30
Zizyphus spina-chrit *(Chinese date)*	16.20 ± 2.12a	0.88 ± 0.28	6.02 ± 0.03	5.28 ± 0.09

Source: Umaru *et al.* (2007)

Results are mean of three (3) determinations ± SD.

a= significantly higher compared with other fruits under oxalate column ($P < 0.05$)
b=significantly lower compared with other fruits under oxalate column ($P < 0.05$)
c=significantly higher compared with other fruits under phytate column ($P < 0.05$)
d=significantly lower compared with other fruits under phytate column ($P < 0.05$)
e=significantly higher compared with other fruits under saponin column ($P < 0.05$)
f=significantly lower compared with other fruits under saponin column ($P < 0.05$)
g=significantly higher compared with other fruits under tannin column ($P < 0.05$)
h=significantly lower compared with other fruits under tannin column ($P < 0.05$)

Information on the tannin content of feed ingredients is essential as is the identification of simple inexpensive methods for removing them (Adamafio *et al.*, 2004). The treatment of crop residues with *Pleurotus ostreatus* fermentation is an effective way of reducing the tannin content and upgrading polysaccharide digestion.

2.10 *In vitro* dry matter enzymatic digestibility (IVDMED)

How valuable a feed is, is dependent on its digestibility and level of utilization to an animal. The evaluation of the quality of feed is therefore important for the prediction of an animal's

performance. The energy value of feeds can be determined by enzymatic methods which do not require rumen fluid (Tatli and Cerci, 2006). The study uses enzymes to act on feed outside the body of the animal in test tubes to evaluate the extent to which livestock can utilize the feed. This method is more cost effective compared to *in vivo* measurements which are expensive and laborious as far as the procedures are concerned (Tatli and Cerci, 2006). The *in vitro* digestibility study helps ascertain the ease of digestion of the feed by the enzymes system possessed by the organism outside of its body. This is used to make comparison with the *in vivo* digestion. Its error margin is lower than any chemical method (Brown *et al*., 2002).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Sources of substrates

Rice straw was obtained from a farm at Tamale in the Northern region of Ghana. Groundnut shells were obtained from a local groundnut processing centre in Tamale market. *Pleurotus ostreatus* spawn was purchased from ROB-ART enterprise (a mushroom producing enterprise) at Kenyase, Kumasi. All relevant equipment including Soxhlet and Kjeldhal apparatuses, muffle furnace, desiccators, flasks, as well analytical reagents and chemicals were obtained from the laboratory of the Biochemistry and Biotechnology Department, KNUST. Aluminium trays were purchased from the market.

3.2. METHODS

3.2.1. Substrate Pre-treatment

The substrates were first cleaned and sorted by removing (hand-picking) foreign matter and other residues that had mixed with them. They were then solar-dried to a moisture content of 10%. Rice straw was chopped into lengths of 1 to 4cm, while the groundnut shells were pounded (using mortar and pestle) to an average size of 0.6 cm^2.

3.2.2. Experiments

Three different substrates were experimented under the same conditions:

1. Solid-State Fermentation (SSF) of rice straw (RS).
2. SSF of groundnut shells (GS).
3. SSF of 50% RS + 50% GS.

3.2.3. *Pleurotus ostreatus* SSF of substrates

3.2.3.1. Preparation of substrates and pasteurization

Fifteen kilograms (15 kg) of each substrate was soaked in a concrete basin with clean tap water for five hours (5 hrs) to ensure that the residues absorbed enough water. The soaking was also to ensure the washing off of soil particles. After draining the water from the substrates, they were allowed to stand for three hours (3 hrs). Two hundred and fifty grams (250 g) portions of each of the substrates were weighed and transferred into cleaned, labelled aluminium trays measuring 15cm × 11cm × 4cm. The open end of the aluminium trays were covered with polyethylene films and aluminium foil. The trays and their contents were then steam-pasteurized for four (4 hrs) in a 200-L metal barrel. After pasteurization, the trays were taken immediately to a previously disinfected inoculation room. There were 40 trays for each substrate; 20 trays from each sample served as control (i.e. pasteurized but not inoculated with *Pleurotus ostreatus*). The 20 other trays were labelled as *Pleurotus ostreatus*-inoculated.

3.2.3.2. Inoculation and incubation of substrates

The trays were allowed to cool to room temperature before inoculation was done. Inoculation was done by carefully lifting the foil covering each tray and quickly broadcasting one gram (1 g) portion of *P. ostreatus* spawn grain onto substrates using a disinfected inoculation spoon. Additionally, a sterilized inoculation pin was used to evenly distribute the spawn onto the substrates, quickly covered and held firmly with rubber band and cellotape. The trays were then arranged in shelves in an incubation room so that fermentation could commence.

3.2.3. Sampling and substrate analysis

There was an initial analysis of each of the substrates to determine their nutrient compositions before pasteurization and fermentation. These samples were labelled "RAW SAMPLES" (i.e. not pasteurized, not *Pleurotus ostreatus*-inoculated). Experiments were carried out in triplicate at weekly intervals and the period of fermentation lasted for five weeks. The sampled substrates were solar-dried for three (3) days and then milled for their compositional analyses.

3.2.4. Experimental design

The Completely Randomized Design (CRD) was used to determine the effect of fermentation on both the proximate composition and the individual fibre components of the substrates.

3.2.5. Analyses of samples

Proximate analysis was by AOAC (1990) standard procedures.

3.2.5.1. Description of analytical protocols

3.2.5.1.1. Moisture determination

Two grams (2 g) each of the raw, uninoculated` and inoculated samples were transferred into

different pre-weighed crucibles, and placed in an oven thermostatically controlled at 105^{O}C for five hours (5 hrs). The crucibles were removed and placed in a dessicator to cool. Their weights were taken, and the procedure repeated until a constant weight was attained. The moisture content was calculated by difference and expressed as a percentage of the initial weight of the sample.

3.2.5.1.2. Ash determination

Two grams (2 g) of each of the raw and treated samples were transferred into previously weighed and dried crucibles and placed into a muffle furnace at 600^{o}C for two hours (2 hrs). The samples were removed from the furnace to a dessicator, cooled and weighed immediately. The amount of ash was determined by the calculation in percentage.

3.2.5.1.3. Crude Protein (CP) Determination

Sample digestion

Two grams (2 g) each of the samples were put in a dried Kjeldhal digestion flask and a catalyst (half tablet of selenium) and a few anti-bumping agents were added. To these, 25 ml of conc. H_2SO_4 were added and the contents shaken gently. The flasks were then heated gently on digestion burner until frothing stopped, while observing the formation of a clear solution. The solution was allowed to cool to room temperature and then transferred into a 100 ml volumetric flask and topped up with distilled water to the mark.

Distillation of digested samples

A volumetric flask (200 ml) containing 25 ml of boric acid plus two (2) drops of mixed indicator was placed under the stem of the condenser in a manner that the tip of the condenser was immersed in the boric acid solution. Through a funnel on the stem jacket, an aliquot (10 ml) of

the digested samples plus 18 ml of 40%NaOH were poured into the decomposition chamber of the distillation apparatus. The distillation was continued till the pink boric acid solution changed to yellowish-green. This was maintained for at least five (5) minutes when almost all the nitrogen would have been distilled and collected in the receiving flask.

Titration and calculation

The distillates of the digested samples were collected and titrated using 0.1N HCl. The percent crude protein was obtained by multiplying the percent total nitrogen by a factor (6.25).

3.2.5.1.4. Crude Fibre (CF) Determination

Defatted samples from tumbles used in fat extraction were transferred into a 750-ml Erlenmeyer flask plus 0.5 grams of asbestos and 200 ml of pre-heated 1.25% H_2SO_4 solution. The flask with its content was connected to the condenser and boiled on a hot plate for 30 minutes. The flask was swirled intermittently to keep particles away from its walls. After the 30 minutes it was removed and the content filtered through linen cloth in funnel and washed with boiling water until no traces of acidity remained. The residue was washed back into the flask with 200 ml boiling 1.25% NaOH solution and the flask reconnected to the condenser. This stayed on the hot plate for another 30mins. The content was filtered and washed with boiling water and 15% alcohol. The residue was then transferred into a Gooch / ashing crucible, dried at 105^{o}C for two hours (2 hrs), cooled in a desiccator and weighed. The dried residue was ashed in a furnace for 30 minutes at 600^{O}C. It was cooled in a desiccator and re-weighed.

3.2.5.1.5. Neutral Detergent Fibre (NDF) Determination

One gram (1 g) of dried sample was transferred into a 750-ml Erlenmeyer flask. To this, 100 ml of neutral detergent solution (NDS) plus 0.5g of sodium sulfite and two drops of n-octanol were added. The flask was connected to a condenser, heated to boiling and refluxed for one (1) hr

from onset of boiling. After this, the content in the flask was filtered and washed three (3) times with boiling water, and then two (2) times with cold acetone. The residue was transferred into a crucible, dried for eight (8 hrs) at 105^{O}C, cooled and weighed.

3.2.5.1.6. Acid Detergent Fibre (ADF) Determination

One gram (1 g) of dried sample was transferred into a 750-ml Erlenmeyer flask. To this, 100 ml of acid detergent solution (ADS) plus some drops of n-octanol were added. The flask was connected to a condenser, heated to boiling and refluxed for one (1 hr) from onset of boiling. After this, the content in the flask was filtered and washed three (3) times with boiling water, and then two (2) times with cold acetone. The residue was transferred into a crucible, dried for eight (8 hrs) at 105^{O}C, cooled and weighed.

3.2.5.1.7. Acid Detergent lignin (ADL) Determination

The ADF crucible was placed in a beaker (50-ml volume) and placed on a tray. The residue in the crucible was covered with 25 ml of 72% H_2SO_4 (at room temperature) and stirred gently with a glass rod to a smooth paste. Refilling with 72% H_2SO_4 and stirring were done three (3) times at hourly intervals to extract the acid. The residue was then washed with boiling water three (3) times or until there were no traces of acidity (tested with litmus paper). Crucible containing residue was dried at 105^{O}C for eight (8 hrs), cooled and weighed. Ashing of the residue in a muffle furnace at 600^{0}C for two (2 hrs) then followed. The samples were then cooled and weighed, and percentage lignin determined (Appendix IIB).

3.2.5.1.8. Hemicelluloses and cellulose determination

These were calculated using values obtained from NDF, ADF and lignin (Appendix IIB).

3.2.5.1.9. Measurement of total sugar and *in vitro* dry matter enzymatic digestibility (IVDMED)

To two (2 g) of the sample was added 150 ml of 1M $NaHPO_4$ buffer of pH 6.8 in the presence of 0.4 ml 1.3U.ml^{-1} α-amylase and incubated at a temperature range of 36 to 39^{0}C for four (4 hrs). At the end of the incubation period, 5 ml of 1% (w/v) NaOH was added to the samples and the suspensions filtered through Whatman No. 1 filter paper. The amount of reducing sugars present in each filtrate was determined using a refractometer (ABBE 60). A drop of the filtrate was put on the glass surface of the refractometer and the sugar level determined in degree brix. Controls were without the enzyme.

3.2.5.1.10. Determination of Phenolics and Tannins using Folin-Ciocalteu Method

Total phenolics and tannins of both raw and fermented samples were determined using the Folin-Ciocalteu Method according to Makkar *et al*. (1993) with modifications.

3.2.5.1.10.1 Preparation of sample extracts (supernatant)

To 200 mg of the samples in centrifuge tubes was added 20 ml 70% aqueous acetone of pH 3-3.5. The samples were allowed to stand at room temperature for about 20 minutes while vortexing gently at various intervals. The tubes were then centrifuged for 10 minutes at 3000 rpm at 4^{0}C using the refrigerated centrifuge. The supernatant (sample extract) containing the total polyphenols was collected, the volume noted and kept on ice until the analysis was completed.

3.2.5.1.10.2 Determination of Total Phenolics

To an aliquot (0.02, 0.04, 0.06, 0.1ml) of the supernatants in test tubes was added distilled water and the volume topped to 2 ml. One (1ml) Folin-Ciocalteu reagent (1N) and 10 ml 20% Na_2CO_3 solution were added and the tubes vortexed and incubated for 40 minutes. The absorbance of

each of the solutions was read at 725 nm against a blank using the UV-Visible Spectrophotometer. The amount of total phenols was then measured as tannic acid equivalent from a calibration curve plotted using the corresponding absorbance values of standard tannic acid solutions prepared by serial dilutions of stock tannic acid solution (0.1mg/ml) and the value expressed on dry matter basis (x%).

3.2.5.1.10.3. Determination of Non-Tannins

To 500 mg polyvinyl polypyrrolidone (PVPP) in centrifuge tubes was added five (5 ml) of the supernatant plus 5 ml distilled water. The tubes were kept at 40^0C for 15 minutes and vortexed, and then centrifuged at 3000 rpm for 10 minutes at 40^0C and the supernatant collected. The phenolic content of the supernatant was measured and the content of the non-tannin phenols (y %) expressed on dry matter basis. The difference, (x-y) is the percentage of tannins (tannic acid equivalent) on dry matter basis.

3.2.5.1.11. Determination of minerals

Two grams (2 g) of the ground sample was weighed into a crucible and ashed at 600^0C for 30 minutes. The sample was cooled and 2 ml of conc. HCl added to dissolve the ash. The content was emptied into a 50 ml volumetric flask and topped up to the mark with distilled water.

Potassium was measured using flame photometer (JENWAY PFP 7), while measurement of phosphorus and calcium were done using spectrophotometer (OPTIMA SP-300).

3.3. Analysis of data

The results are presented as the mean standard values of triplicates each. A one-way analysis of variance (ANOVA) and the Least Significant Difference (LSD) were carried out. Significance was accepted at $P < 0.05$. All the data were also subjected to ANOVA and the significant mean

differences were tested by the Duncan's New Multiple Range Test.

CHAPTER FOUR

4.0. RESULTS AND DISCUSSION

Plant biomass regarded as “wastes” are biodegradable and can be converted into valuable animal feeds (Howard *et al.,* 2003). In this study, the determined proximate composition, mineral and tannin contents for the substrates used are presented in Table 4.1.

Table 4.1 Chemical composition of substrates used for the study

Parameter (%)	RS	GS	RSGS
Dry Matter	93.23±0.10	94.33±0.01	93.70±0.20

Crude Protein	2.85±0.44	5.32±0.25	5.03±0.44
Crude Fibre	33.35±0.06	65.46±0.57	49.01±0.22
Ether Extract	1.05±0.03	2.28±0.03	1.74±0.08
Ash	14.83±0.19	4.13±0.05	9.23±0.23
NFE[1]	47.93±0.45	22.87±0.71	34.98±0.67
NDF[2]	66.20±0.23	77.85±0.34	71.25±0.13
ADF[3]	48.10±0.18	60.00±0.18	54.89±0.20
Hemicellulose	18.09±0.06	17.86±0.19	16.30±0.25
Lignin	12.41±0.07	18.52±0.51	15.46±0.10
Cellulose	35.69±0.24	41.48±0.34	39.43±0.16
Tannins	0.78	0.96	0.84
Potassium (mg/g)	0.29	0.10	0.15
Phosphorous (mg/g)	0.32	0.11	0.22
Calcium (mg/g)	0.70	0.22	0.46

[1]NFE=Nitrogen Free Extract; [2]NDF=Neutral Detergent Fiber; [3]ADF=Acid Detergent Fiber;
RS= rice straw; GS= groundnut shell; RSGS=50% rice straw + 50% groundnut shell

4.1. Crude protein (CP)

Vijay *et al*. (2007) stated that the major limitations of straw as an animal feed are low protein content and poor digestibility. However, *Pleurotus* species have the potential to convert these straws into valuable protein at a low cost (Vijay *et al*., 2007). Lateef *et al*. (2008) studied the effect of solid-state fermentation of some agro-wastes with the fungus *Rhizopus stolonifer* and

the protein contents of the substrates increased significantly by up to 94.8%. Yang *et al*. (1986) also reported an increase of CP content up to 60.9 % after the harvest of mushrooms from the residual substrate.

In this study using rice straw (RS), groundnut shell (GS) and a mixture of rice straw and groundnut shell (RSGS), the raw samples registered initial values of 2.85, 5.32 and 5.03 percent respectively. The crude protein (CP) content of substrates increased significantly ($P < 0.05$) with fermentation time following treatment with *Pleurotus ostreatus* (Figs. 4.1-4.3). RS had an improvement in protein level of 132.98% at the end of the 4th week and declined in the 5th week slightly; RSGS also had an improvement of 61.43% at the end of the 4th week and declined in the 5th week slightly, while GS substrate showed a 65.41% improvement by the end of the 5th week (Figs. 4.1-4.3). The higher initial crude protein value for GS might be due to the fact that it is a leguminous crop residue, since legumes have high protein levels. For rice straw, there was no significant difference ($P > 0.05$) observed between weeks 4 and 5. A similar observation was made for the mixture (RSGS) during weeks 3, 4 and 5; there was no significant difference ($P > 0.05$). Thus, for RS and RSGS, the optimum fermentation period was achieved within four (4) weeks when there was complete colonization of the substrates. GS recorded the highest improvement at week 5 (Fig.4.2). Optimum fermentation period occurs after complete colonization of the substrates by the organism (Adu, 2009; Alemawor *et al*., 2009). The increase in crude protein may be due to the addition of fungal protein or the bioconversion of carbohydrates in the colonized substrates into mycelia protein or single cell protein (SCP) by the growing fungus during the fermentation process (Iyayi, 2004). It may also be partly due to the secretion of some extracellular enzymes such as cellulases and amylases by the fungus in an attempt to use cellulose and starch as sources of carbon (Raimbault, 1998; Oboh *et al*., 2002). The decrease in crude protein at week 5 for rice straw and the mixture is as a result of the utilization of nutrients by the mycelia during fruiting (Rypacek, 1966). This was also observed

by Alemawor *et al.* (2009) and Brimpong *et al.* (2009) that beyond the optimum fermentation period crude protein level declined.

In general, the observed increases in crude protein content are indications of the positive effects that *Pleurotus ostreatus* and other fungi species have on cheap lignocellulosics and low-grade agro-wastes, transforming them into protein-rich products at low cost (Belewu and Banjo, 1999; Miszkiewicz *et al.*, 2004; Vijay *et al.*, 2007; Belewu and Babalola, 2009).

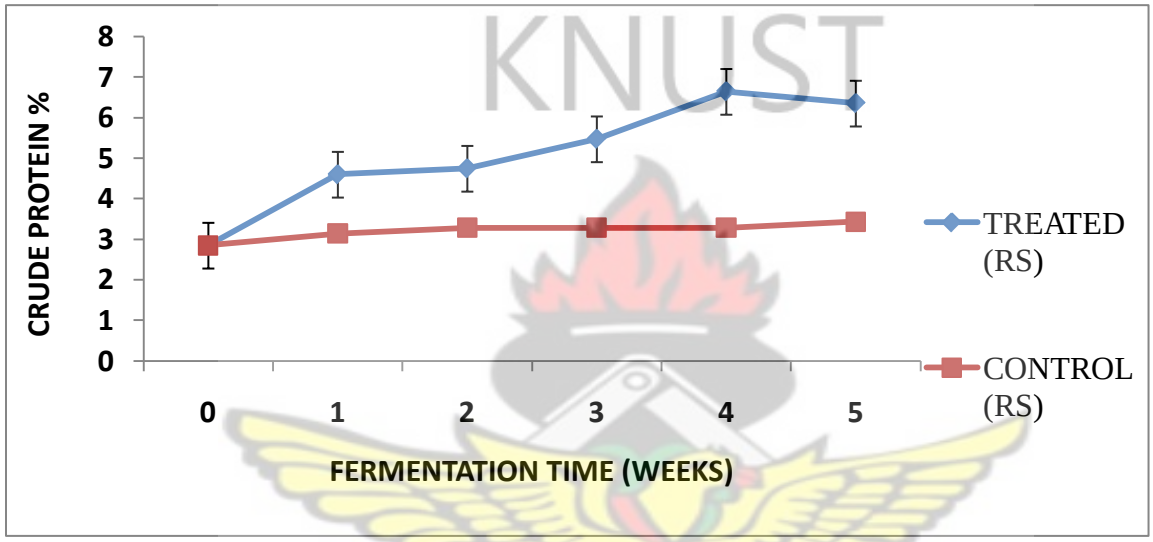


Figure 4.1 Effect of fermentation time on **crude protein** content of Rice Straw (RS) substrate

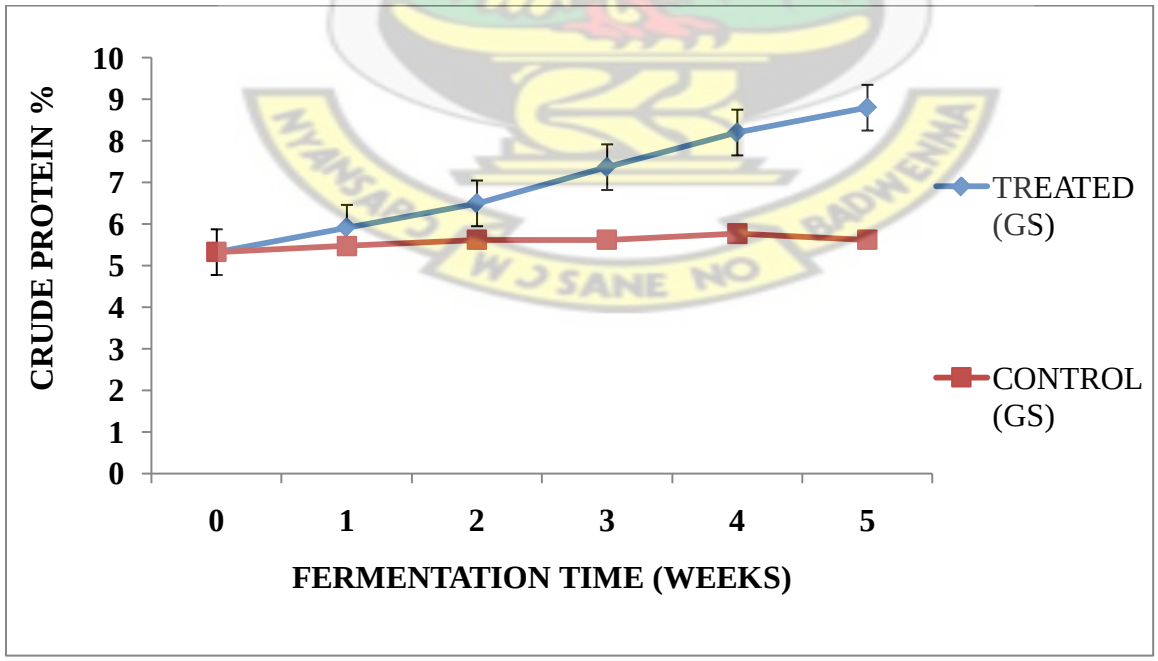


Figure 4.2 Effect of fermentation time on **crude protein** content of Groundnut Shell (GS) substrate

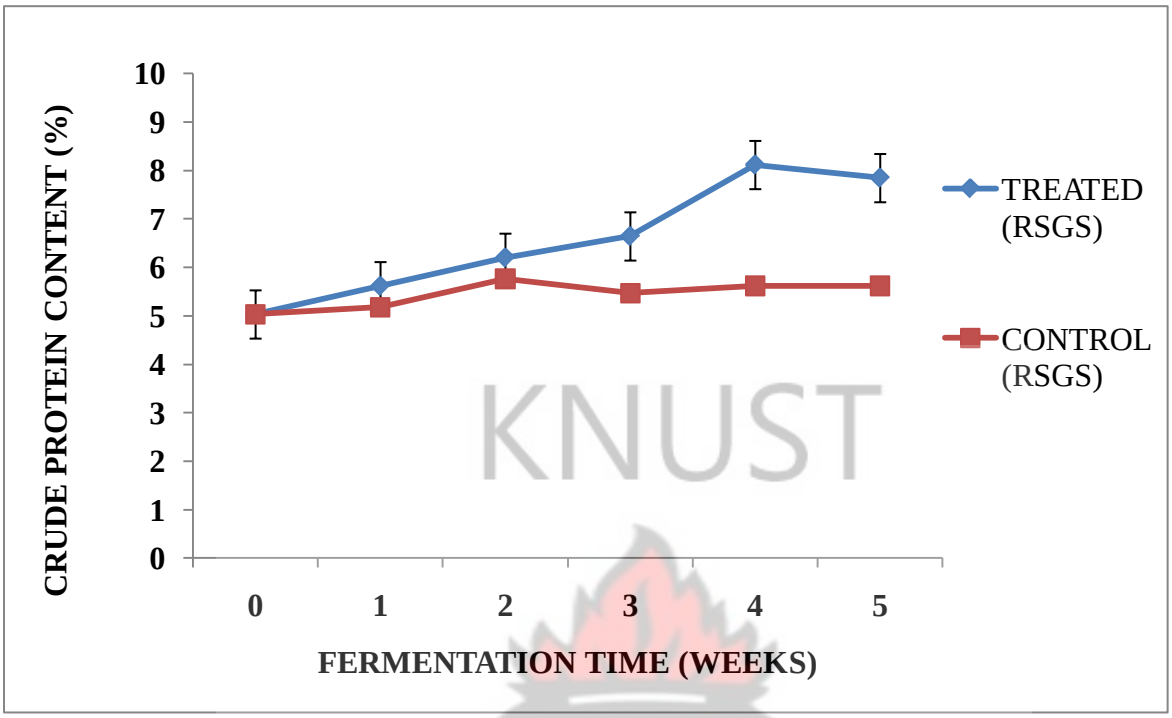


Figure 4.3 Effect of fermentation time on **crude protein** content of 50%Rice Straw + 50% Groundnut Shell (RSGS) substrate

4.2. Ash

Ash constitutes on the average 12 to17% of plant biomass (Koji *et al.*, 1982; Sallam, 2005). Figures 4.4 - 4.6 indicate the effects of fermentation time on ash for the various substrates. This reflects the mineral worth of the substrates. The ash content of all the *P. ostreatus* treated samples increased significantly ($P < 0.05$). The initial values in the substrates were 14.83, 4.12 and 9.23 percent for RS, GS and RSGS respectively (Table 4.1). At the end of the optimum fermentation period the values increased by 59.47%, 71.84% and 60.13% for RS, GS and RSGS respectively (Fig. 4.4 - 4.6). These increments could be attributed to the fact that the mycelia of the fungus had enriched the mineral content of the substrates (Bano *et al.*, 1986). Similar results were also reported by other workers (Asmah, 1999; Alemawor *et al.*, 2009) who found various

levels of increases in ash during *Pleurotus ostreatus* fermentation of corncobs and cocoa pod husk (CPH) respectively. There were no significant differences ($P > 0.05$) observed among the control substrates. This proves that the *P. ostreatus* had a positive influence on the substrates by causing significant changes in the inoculated substrates.

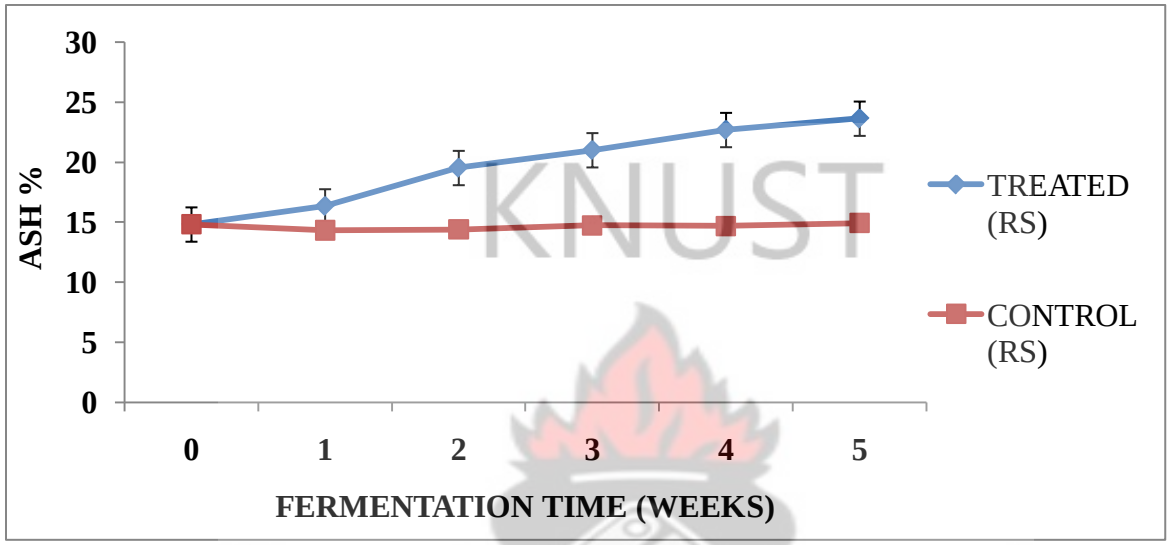


Figure 4.4 Effect of fermentation time on **ash** content of Rice Straw (RS) substrate

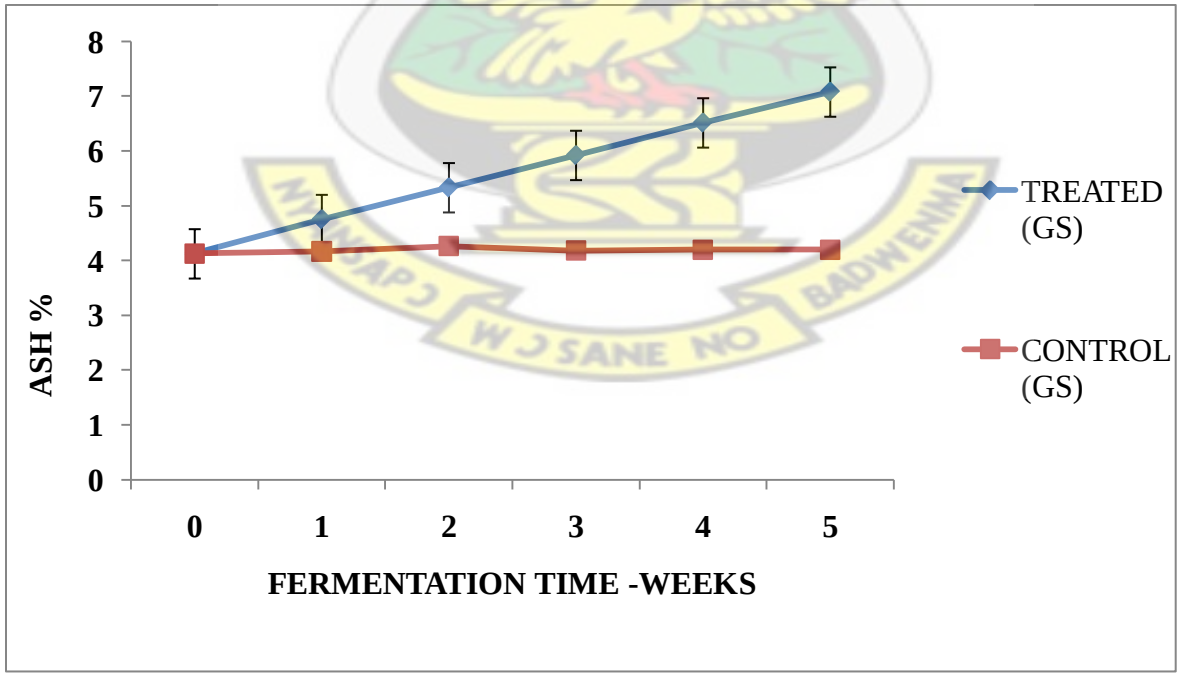


Figure 4.5 Effect of fermentation time on **ash** content of Groundnut Shell (GS) substrate

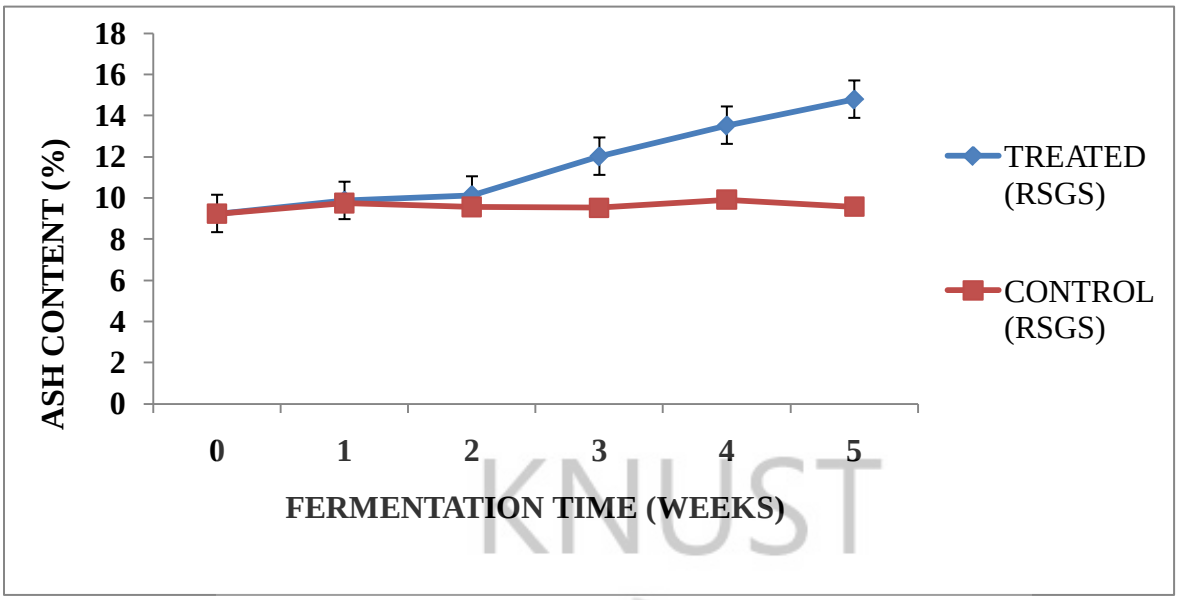


Figure 4.6 Effect of fermentation time on **ash** content of 50%Rice Straw + 50% Groundnut Shell (RSGS) substrate

4.3. Crude fibre

Crude fibre constitutes on the average 30 to 36% of plant biomass (Koji *et al.*, 1982; Anon 2000; Sallam 2005). Lateef *et al*. (2008) studied the effect of the fungal strain *Rhizopus stolonifer* LAU 07 on cocoa pod husk (CPH), cassava peel (CP), and palm kernel cake (PKC) and observed that the crude fibre contents decreased by 7.2%, 8.6% and 44.5% in CPH, CP, and PKC, respectively after the fermentation. Yang *et al*. (1986) also reported that after the 2nd, 3rd and 4th harvest of mushrooms, crude fibre content reduced by 42.4%, 48.1% and 50.4% respectively.

In this research, the crude fibre (CF) content of the *Pleurotus ostreatus* treated samples decreased significantly ($P < 0.05$) from 33.35% to 20.32% for RS, 65.46% to 37.80% for GS and 49.01% to 29.56% for RSGS respectively at the end of the optimum fermentation period (Figs. 4.7 – 4.9). The decline in crude fibre levels is supported by Kutlu *et al*. (2000) and Alemawor *et al*. (2009) who reported a reduction in crude fibre levels in the substrates they fermented with *P. ostreatus*. The reduction in the crude fibre content could probably be due to the action of the

enzymes secreted by the fungus, as suggested by Miszkiewics *et al*. (2004). During biodegradation the enzymes from the fungus break down polysaccharides into less complex structures (Aderemi and Nworgu, 2007).

Thus the treatment of the substrates with the *P. ostreatus* has improved their nutritional value, suggesting that they can be useful livestock feedstuffs since their complex components have been broken down by the fungus.

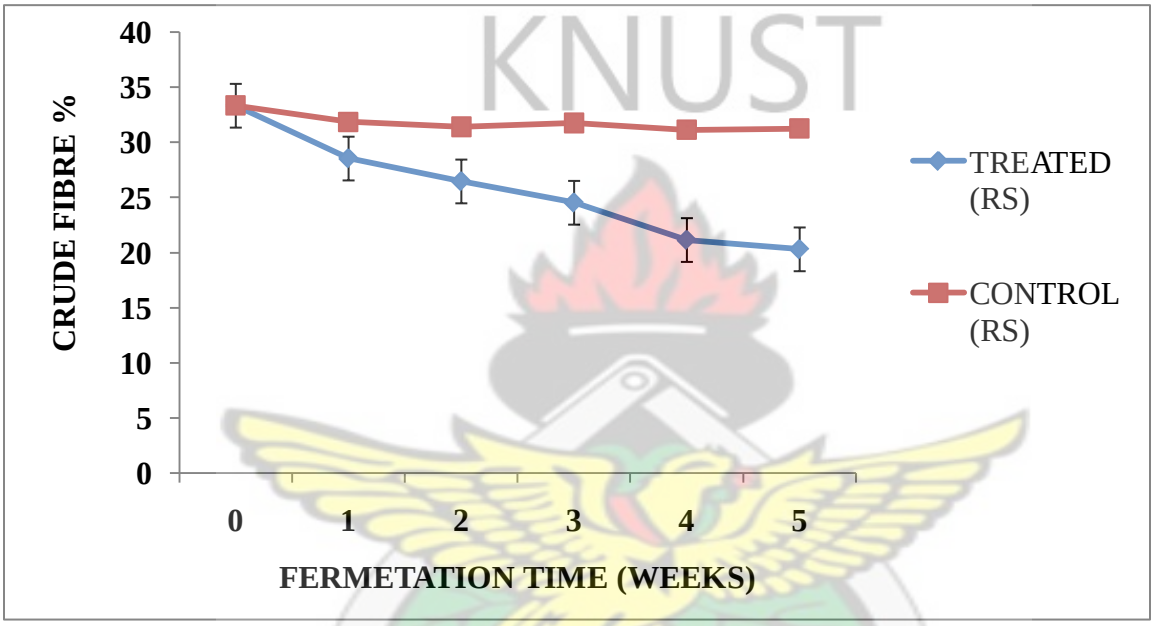


Figure 4.7 Effect of fermentation time on **crude fibre** content of Rice Straw (RS) substrate

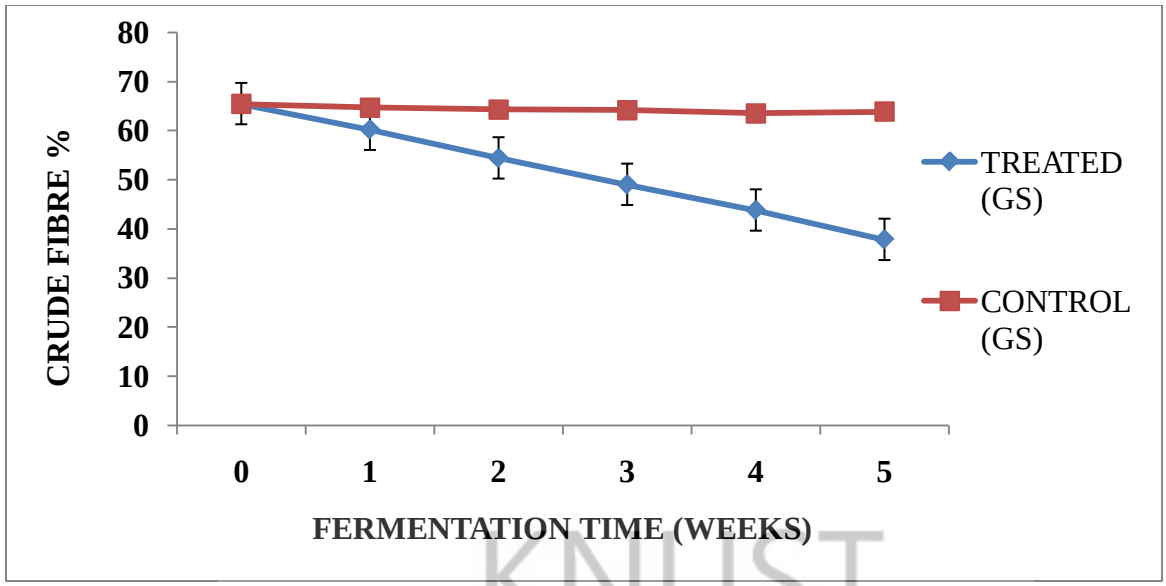


Figure 4.8 Effect of fermentation time on **crude fibre** content of Groundnut Shell (GS) substrate

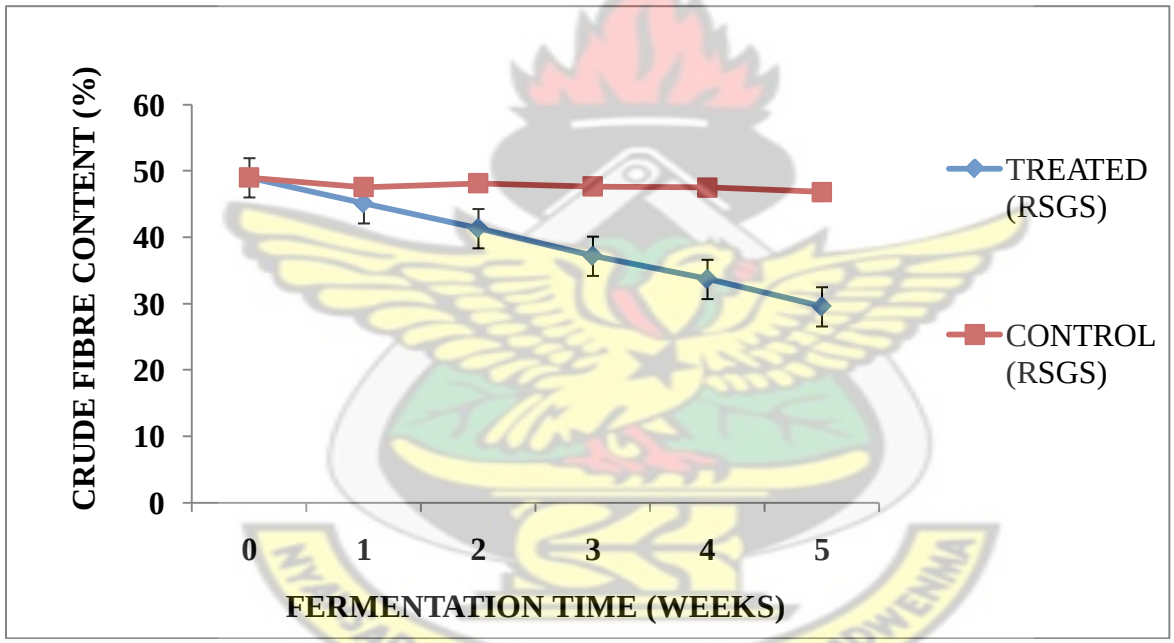


Figure 4.9 Effect of fermentation time on **crude fibre** content of 50% Rice Straw + 50% Groundnut Shell (RSGS) substrate

4.4. Fungi degradation of lignocellulose

The cell wall fraction includes hemicellulose, cellulose, lignin, cutin and silica. In most crop residues, the cell wall fraction accounts for 60-80% of dry matter (Xiong, 1986). Cellulose constitutes on the average 30 to 50% of plant biomass (Koji *et al*. 1982; Xing 1995; Lynd *et al.*,

2002; Sallam 2005), whiles lignin forms 4.5 to 13.7% of plant biomass (Koji *et al.*, 1982; Xing 1995; Sallam 2005). As microbes in solid-state fermentation grow under conditions closer to their natural habitats, they become more capable of producing enzymes such as proteases, cellulases, lignases, xylanases, pectinases and amylases (Jecu, 2000).

In this study, the lignin content and other fibre fractions such as, cellulose and hemicellulose progressively decreased with fermentation time (Figs.4.10-4.18). Generally there were significant decreases in the levels of lignocellulose fractions in all the substrates. However, there were no significant changes ($P > 0.05$) observed in their respective controls. This is an indication that the *P. ostreatus* did break down these fibre fractions as carbon source for its growth. Vijay *et al*. (2007) stated that most mushroom species possess the ability to degrade lignin, cellulose and hemicellulose. Solid-state fermentation of aspen (*Populus tremuloides*) wood with *Merulius tremellosus* for eight (8) weeks removed 52% of the lignin (Ian, 1985). Rolz *et al*. (1986) reported that fungi (particularly white-rots) have the enzymatic potential to use lignocellulose component as sources of carbon and energy. This results in biomass breakdown and lignin removal, accompanied by the removal of polysaccharides. Thus the results obtained are in agreement with those of other workers.

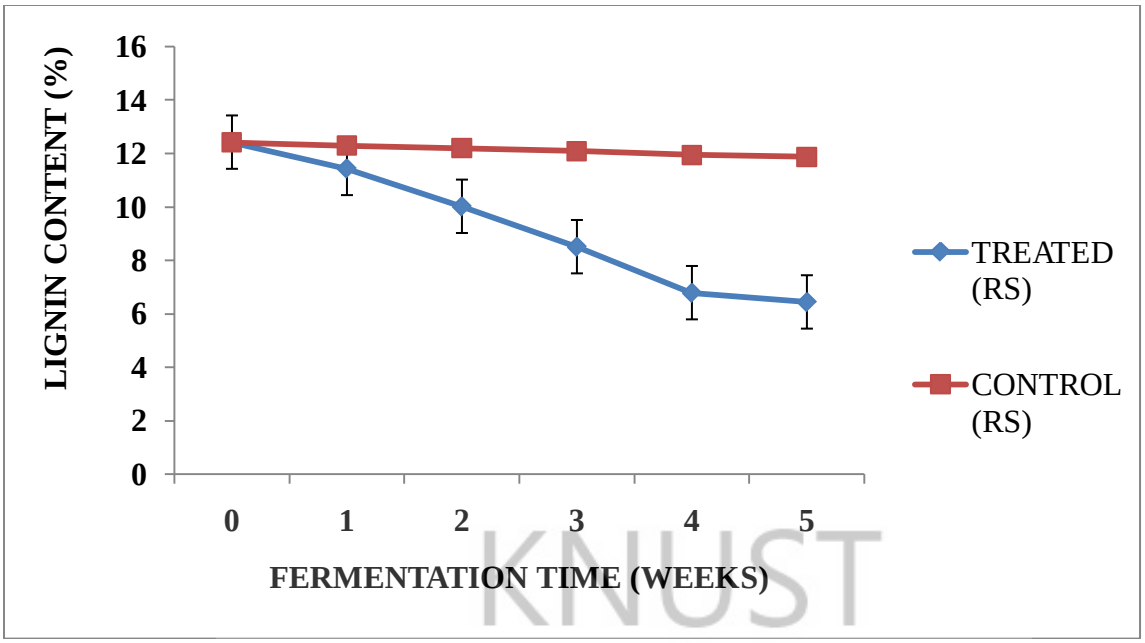


Figure 4.10 Effect of fermentation time on **lignin** content of Rice Straw (RS) substrate

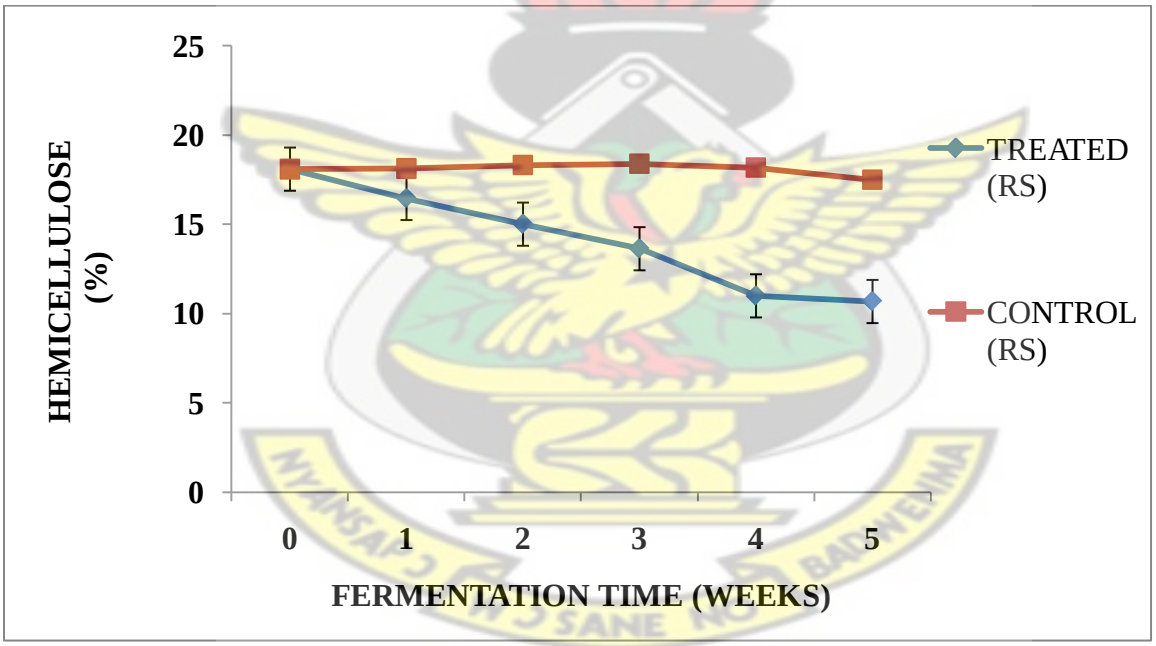


Figure 4.11 Effect of fermentation time on **hemicellulose** content of Rice Straw (RS) substrate

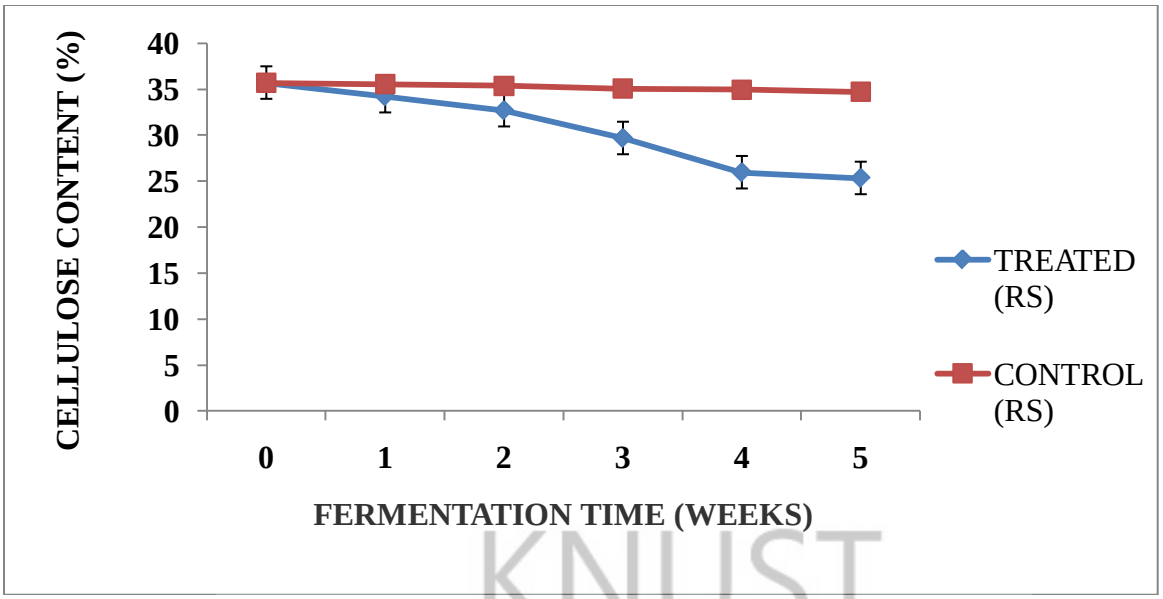


Figure 4.12 Effect of fermentation time on **cellulose** content of Rice Straw (RS) substrate

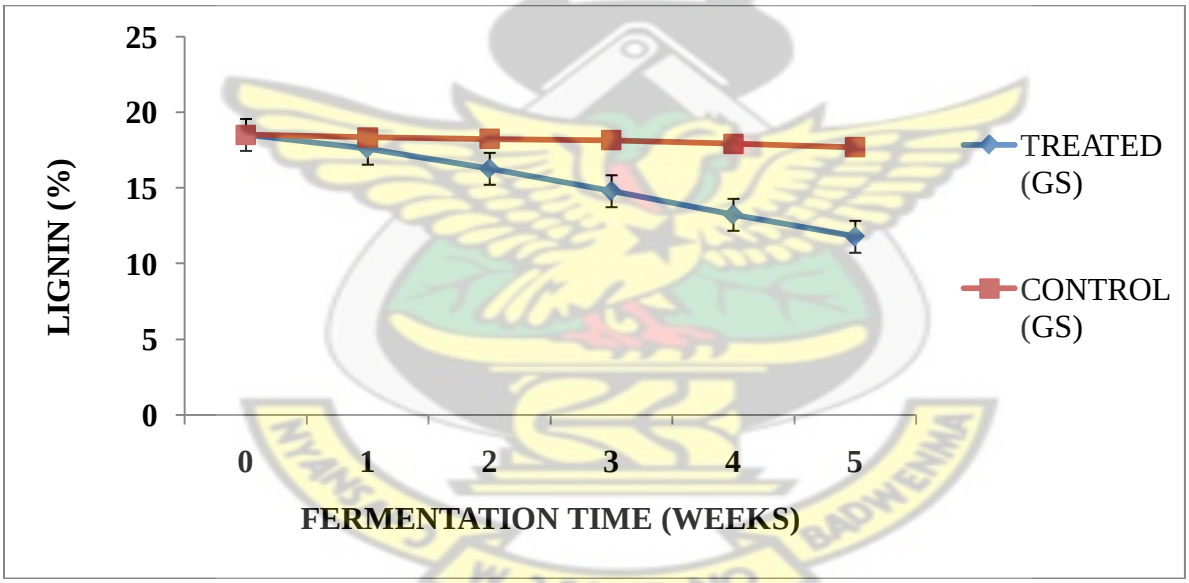


Figure 4.13 Effect of fermentation time on **lignin** content of Groundnut Shell (GS) substrate

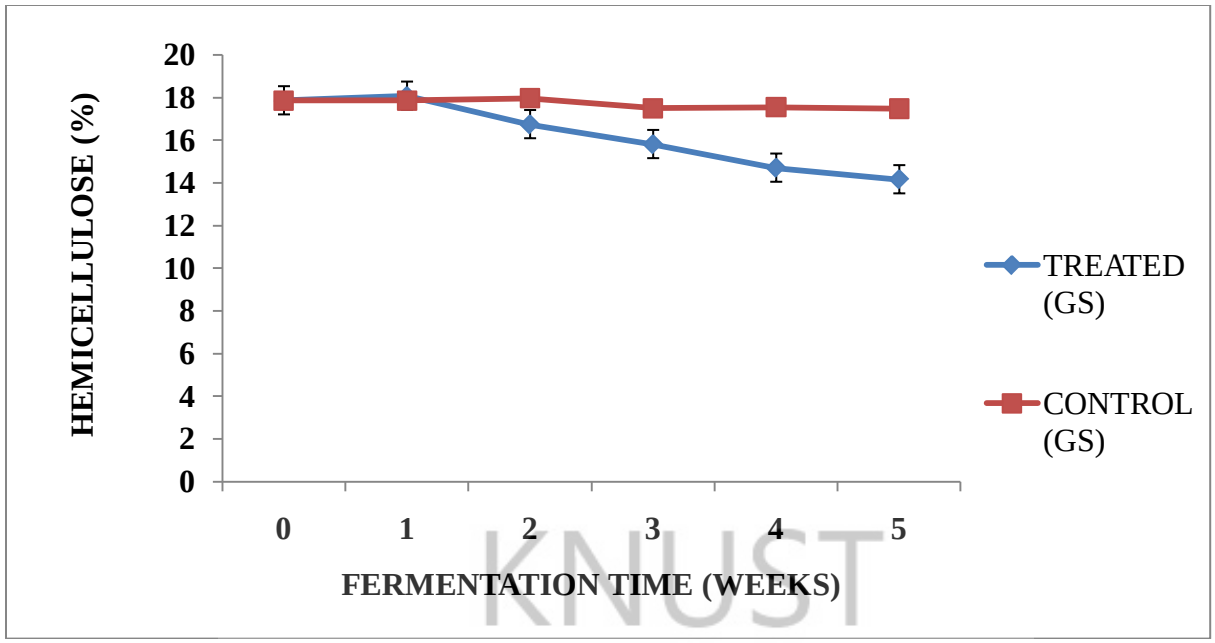


Figure 4.14 Effect of fermentation time on **hemicellulose** content of Groundnut Shell (GS) substrate

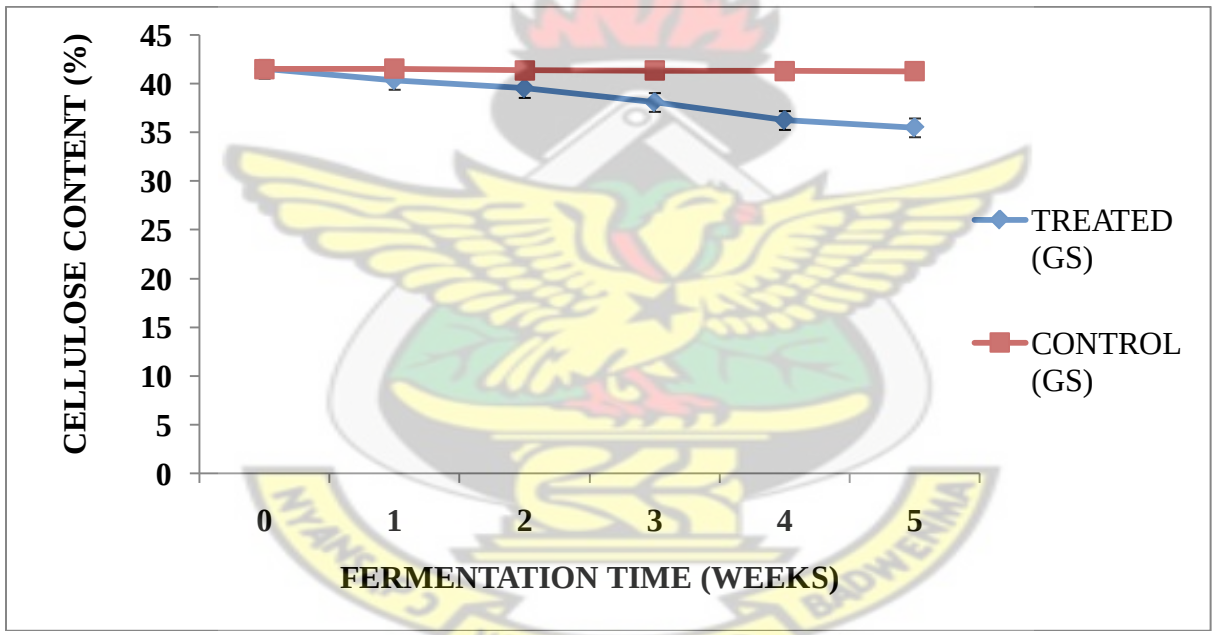


Figure 4.15 Effect of fermentation time on **cellulose** content of Groundnut Shell (GS) substrate

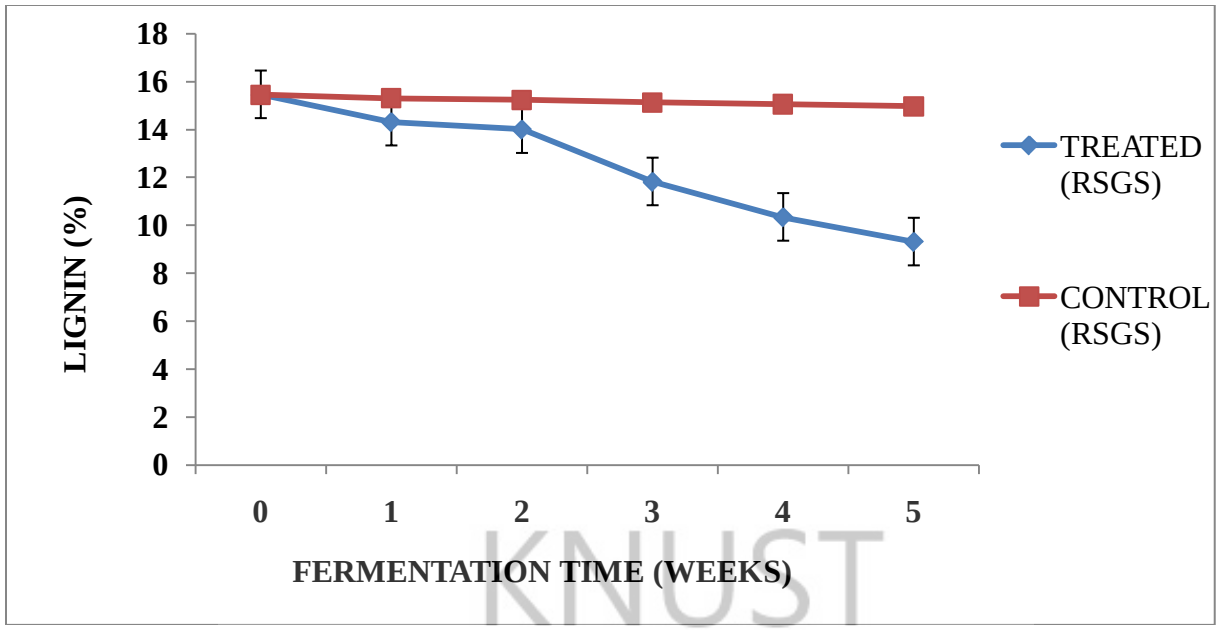


Figure 4.16 Effect of fermentation time on **lignin** content of 50%Rice Straw + 50% Groundnut Shell (RSGS) substrate

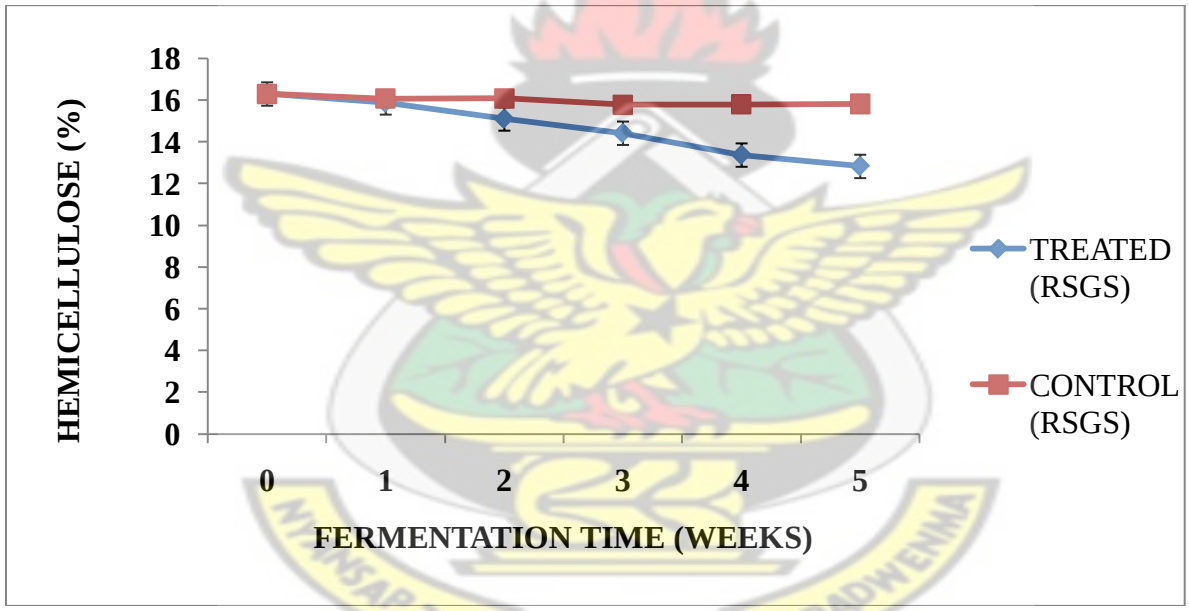


Figure 4.17 Effect of fermentation time on **hemicellulose** content of 50%Rice Straw + 50% Groundnut Shell (RSGS) substrate

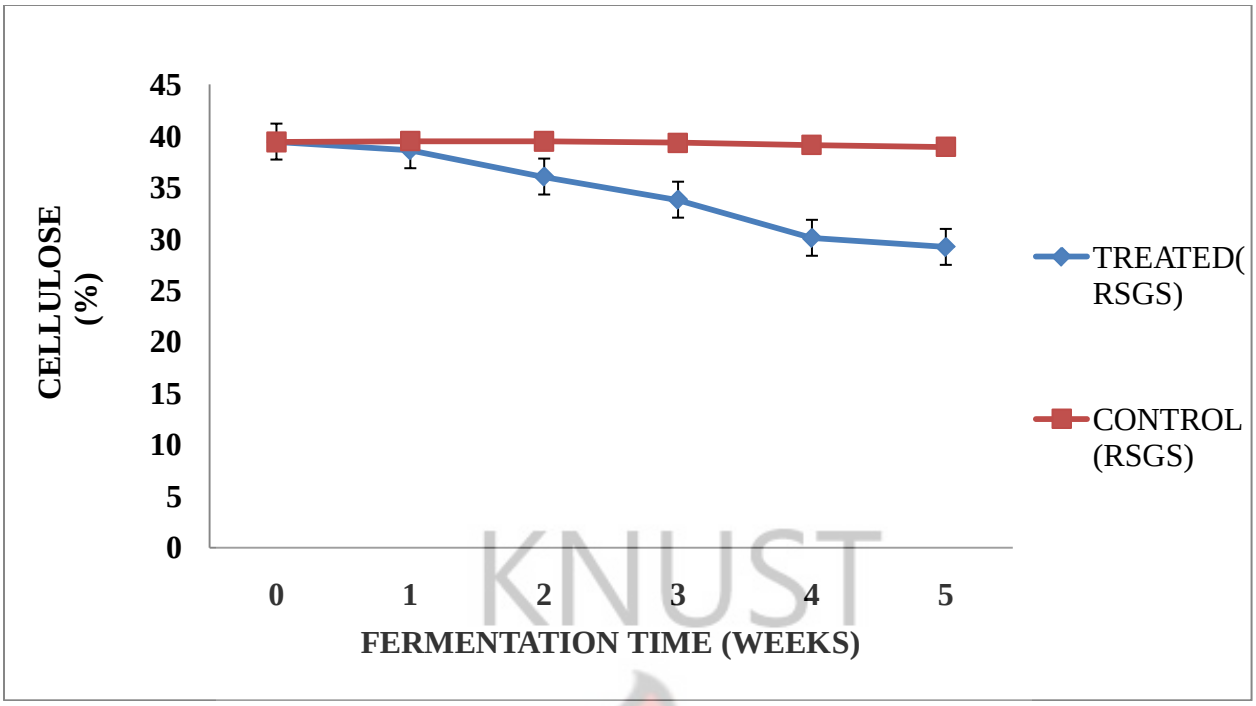


Figure 4.18 Effect of fermentation time on **cellulose** content of 50%Rice Straw + 50% Groundnut Shell (RSGS) substrate

Table 4.2 Changes in fibre levels of substrates following *P. ostreatus* fermentation for five (5) weeks

Parameter **%**	**ADF**	**Hemicellulose**	**Lignin**	**Cellulose**
Raw RS	48.10±0.18	18.09±0.06	12.41±0.0	35.69±0.24
Treated RS	31.75±0.11	10.68±0.06	6.44±0.06	25.32±0.15
% decline	**33.99**	**41.02**	**48.12**	**29.06**
Raw GS	60.00±0.18	17.86±0.19	18.52±0.51	41.48±0.34
Treated GS	47.26±1.02	14.16±1.14	11.78±0.09	35.48±0.97
% decline	**21.23**	**20.73**	**36.39**	**14.46**

Raw RSGS	54.89±0.20	16.30±0.25	15.46±0.10	39.43±0.16
Treated RSGS	38.51±0.09	12.83±0.13	9.31±0.06	29.20±0.15
% decline	**29.84**	**21.30**	**39.78**	**25.94**

In general, the hemicellulose content of all the substrates decreased significantly ($P < 0.05$) with fermentation time. However, no significant differences occurred between values at weeks 4 and 5 for rice straw (RS) and groundnut shell (GS). Hemicellulose level reduced by 41.02%, 20.73% and 21.30% for rice straw (RS), groundnut shell (GS) and the mixture (RSGS) respectively (Table 4.2). These reductions may be due to the activities of hemicellulolytic enzymes secreted by *P. ostreatus* on the substrates. *P. ostreatus* species have the ability to produce enzymes that are capable of breaking down a variety of β-(1,4) linked glucan substrates as well as glycosides (Highley, 1976). The results are in agreement with previous studies on various agro-wastes which reported 20-45% reduction in hemicellulose (Adu, 2009; Alemawor *et al*., 2009; Brimpong *et al*., 2009).

A similar trend was observed for cellulose degradation. Generally the reductions were significantly different ($P < 0.05$) for all the substrates with the exception of GS which did not show significant changes between weeks 4 and 5 (Fig.4.14). The percentage declines are shown in Table 4.2. The reduction in levels of cellulose might be due to the activities of extracellular fungal hydrolases (collectively known as cellulases) that degrade cellulose materials (Datta and Chakravarty, 2001). The significant reduction in the cellulose content of the substrates implies that the fermented feed items can be hydrolysed by animals better than in their raw

(unfermented) states. It also means that more crop residues which would rather be classified as "wastes" can now be turned into nutritious animal feed when fermented with *P. ostreatus* for a certain length of time depending on the type of residue.

The levels of lignin of treated samples declined significantly ($P < 0.05$) with fermentation time. Rice straw (RS), groundnut shell (GS) and the mixture (RSGS) recorded 48.12%, 36.39% and 39.78% reductions respectively at the end of the optimum fermentation period (Table 4.2). The decline in the lignin content of the treated materials were also found to be significantly different from their respective controls ($P < 0.05$). According to Brimpong *et al*. (2009), *P. ostreatus* treatment decreased lignin content of corn cobs by 42.3% at the end of the optimum fermentation period. According to Argyropoulos and Menachem (1997), lignin impedes the biological degradation of cellulose and hemicelluloses. Therefore the extent of lignin degradation observed suggests the availability of cellulose and hemicelluloses for the fungus to easily break down and subsequently utilize**.** Extracellular enzymes produced by the *P. ostreatus* oxidise both the aromatic rings and the aliphatic side chains of lignin to produce low-molecular weight products that can easily be absorbed by the fungus (Lo *et al*., 2001). Thus, *P. ostreatus* fermentation of substrates for a period of at least 4 weeks will help enhance the digestibility and subsequent utilization of these agro-residues.

4.5. Total sugar and *in vitro* dry matter enzymatic digestibility (IVDMED) of substrates

The *in vitro* digestibility study helps ascertain the ease of digestion of the feed by the enzymes system possessed by the organism outside of its body. Figures 4.19 - 4.21 show the changes in sugar levels of the various substrates. It was observed that the sugar levels in each substrate more than doubled after the *P. ostreatus* fermentation.

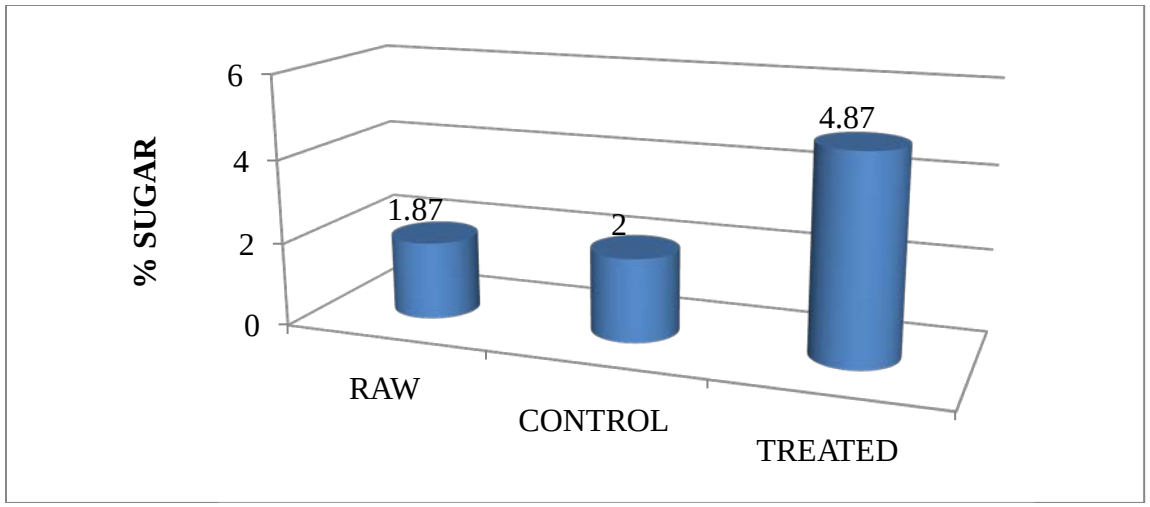


Figure 4.19 Changes in soluble **sugar content** following fermentation of RS with *P. ostreatus*

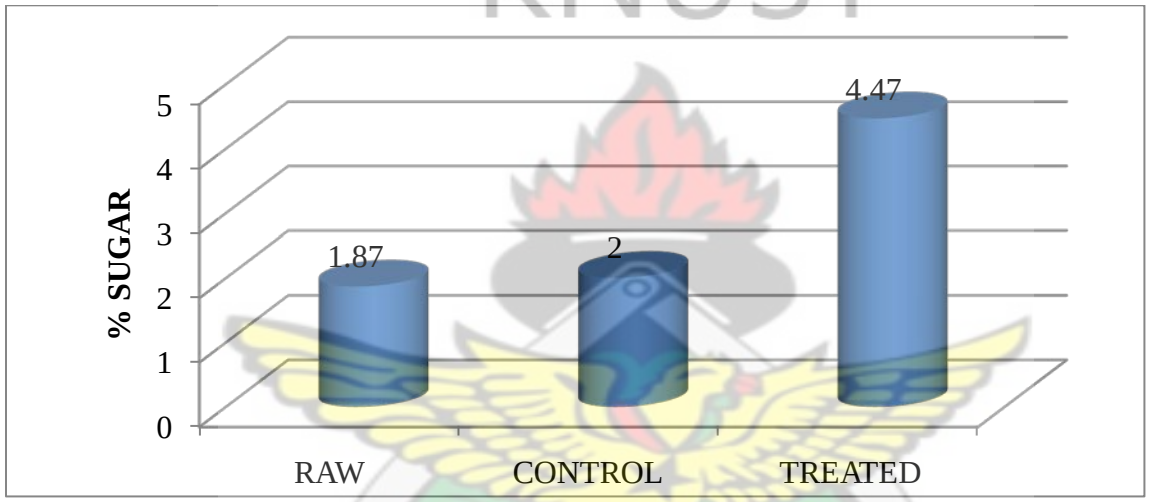


Figure 4.20 Changes in soluble **sugar content** following fermentation of GS with *P. ostreatus*

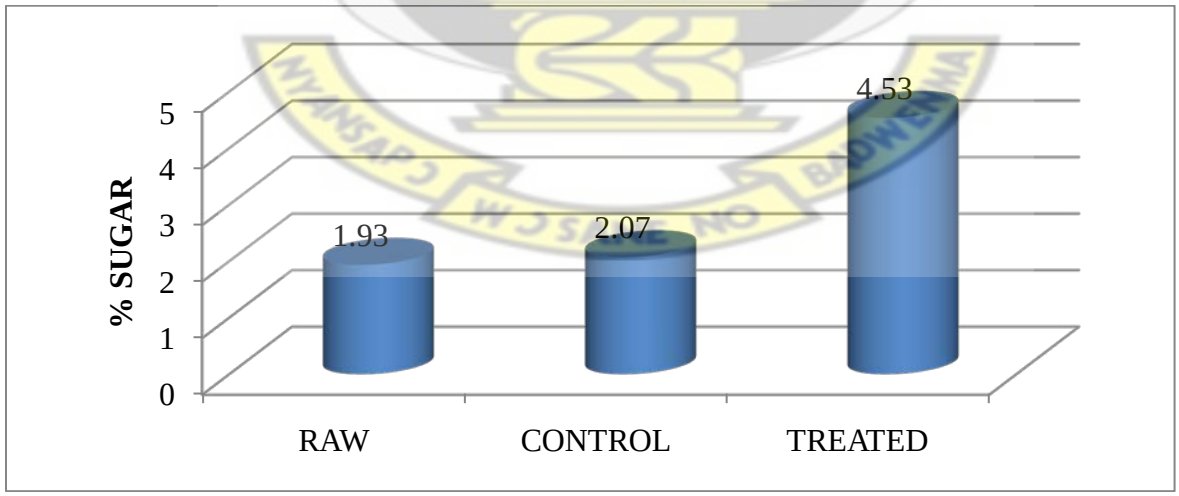


Figure 4.21 Changes in soluble **sugar content** following fermentation of RSGS with *P. ostreatus*

Crude enzyme products, with cellulolytic and hemicellulolytic capabilities, are usually added to fibrous feeds in attempt to improve their digestibility. Wang (1998) observed that treatment of maize stover with an enzyme product, prepared from *Trichoderma viride*, reduced the contents of some cell wall components and enhanced the ruminal digestibility in sheep. Commercial cellulase products were also added to diets to increase the supply of readily available carbohydrates. When the enzyme products were included at 0.1% to 0.2% in the diet of pigs, cattle and geese, animal performance was considerably improved. In another study, solid-state fermentation of aspen (*Populus tremuloides*) wood with *Merulius tremellosus* for 8 weeks increased the cellulose digestibility from 18% to 53% (Ian, 1985).

The *in vitro* dry matter enzymatic digestibility (IVDMED) analyses were done at the 4th week for rice straw (RS) and the mixture (RSGS), and 5th week for groundnut shell (GS), (their optimum fermentation periods). *P. ostreatus* fermentation increased significantly ($P < 0.05$) the IVDMED of the three fermented substrates as compared to their respective controls (Figures 4.22, 4.23, 4.24). Modification or degradation of polysaccharides and lignin by the fungus mainly contributed to the observed increase in IVDMED values for the substrates. When the RS samples were *in vitro* treated with α-amylase, the results showed a sugar level of 4.93%, 5.07% and 12.13% respectively for the raw, control and fermented samples (Fig. 4.22). This implies that the fermented sample became 139.3% more digestible compared to the control sample at the end of the 4th week. Results for groundnut shell (GS) also showed that the fermented sample was 120.3% more digestible compared to the control at the end of the 5th week (Fig. 4.23). A similar trend was observed for RSGS (128.6%). This shows that the enzyme (α- amylase) degraded the polysaccharides to release more sugars in the media; thus the high levels of sugar in the enzyme-treated samples, and more especially in the *Pleurotus*-fermented samples.

Comparing Figures 4.19 and 4.22; 4.20 and 4.23, and 4.21 and 4.24 shows that when the

fermented samples were treated with the enzyme (α- amylase), more sugars were released. For instance, fermented RS sample not treated with the enzyme (α- amylase) produced 4.87% sugar compared to the fermented RS sample treated with the enzyme (α- amylase) – 12.13%. This is an increase of 149%. Similar observations are made for GS and RSGS with increases of 140% and 148.7% respectively.

The extra sugars are seen to be coming from the action of the commercial enzyme (α- amylase) which further saccharified the samples to release more sugars. Alemawor *et al.* (2009) reported a positive effect of the action of commercial enzymes on cocoa pod husk when he combined various enzymes to determine total sugars in the husk.

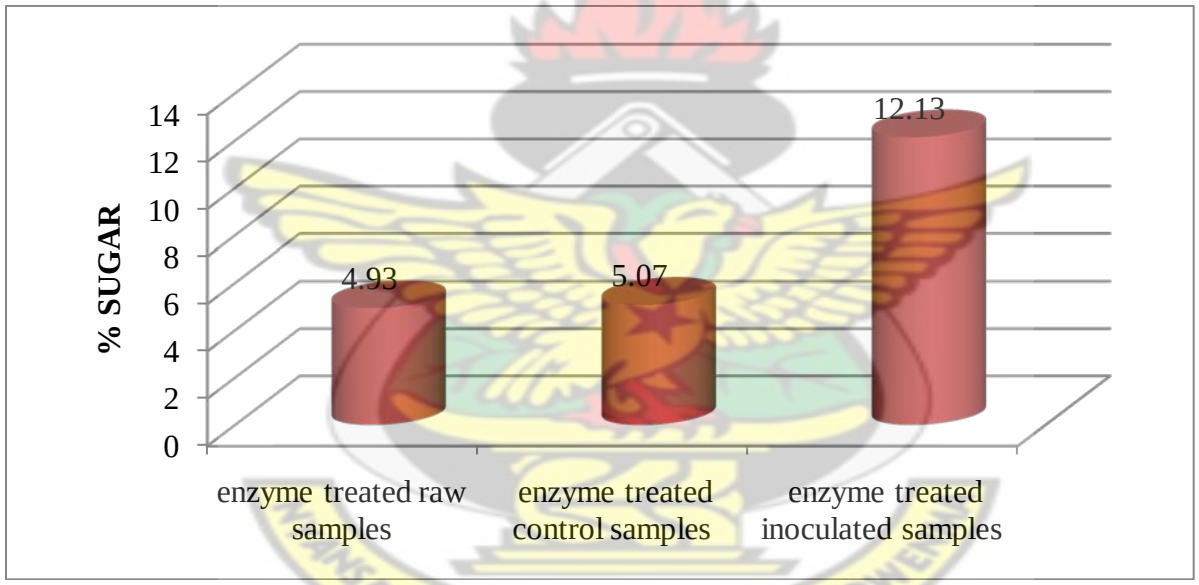


Figure 4.22 Changes in soluble **sugar content** of RS after treatment with α-amylase

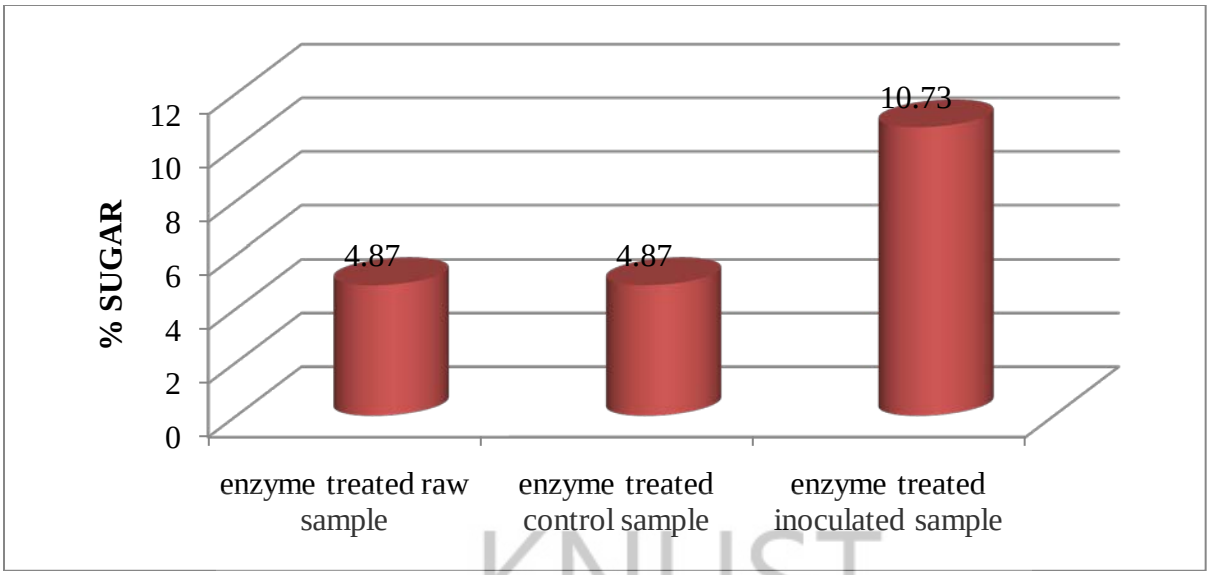


Figure 4.23 Changes in soluble **sugar content** of GS after treatment with α-amylase

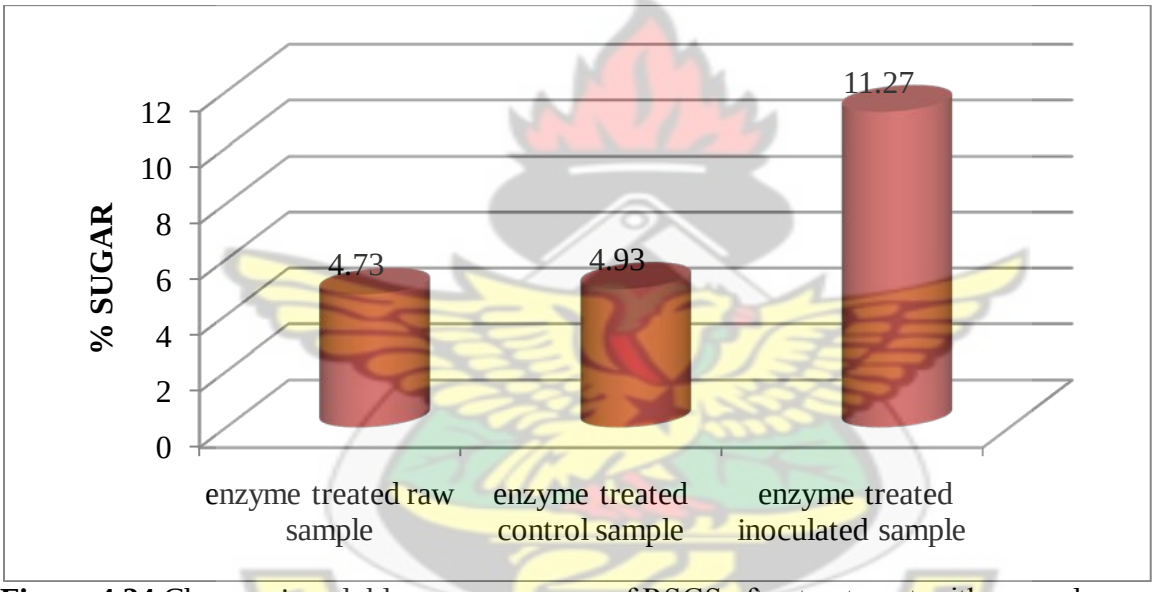


Figure 4.24 Changes in soluble **sugar content** of RSGS after treatment with α-amylase

4.6. Changes in mineral content of substrates fermented with *P. ostreatus*

The results in Figures 4.25 - 4.27 show the changes in mineral content following fermentation of the various substrates with *P. ostreatus*. The mineral analyses were done at 4th week for rice straw (RS) and the mixture (RSGS), and 5th week for groundnut shell (GS) (their optimum fermentation periods). Minerals are important in animal nutrition, hence the need to analyze the mineral content of the samples upon fermentation. Mineral contents of substrates increased after

fermentation with *P. ostreatus*. The results show that RSGS recorded the highest increase in phosphorus and potassium (145.83% and 74.64%) respectively (Figs. 4.25, 4.27), while GS recorded the least improvement in calcium and potassium (Figs. 4.25, 4.26). RS had the least improvement in phosphorus (56.04%), but recorded the highest in calcium (88.7%). The higher mineral content of the treated samples agrees with the report of Adu (2009) who reported 168.3% increase in calcium, 340.23% increase in potassium and 46.3% increase in phosphorus when cassava peels were fermented with *P. ostreatus*. Similar observations were made in studies conducted by Jacqueline and Broerse (1996) and Belewu and Babalola (2009). The increases in mineral content may be as a result of the increases in the ash levels of the substrates as the fermentation time progressed, since ash represents the mineral worth of the substrates, even though the percentage increases in the ash did not correspond to that of the minerals. The increases observed are partly due to mineral contribution from the spawn grain by *P. ostreatus* and the fact that fungi accumulate minerals from the environment. Since these minerals are required for growth, there is no doubt that their enhancement by the *P. ostreatus* will go a long way to tremendously improve the health of animals when they feed on the fermented materials.

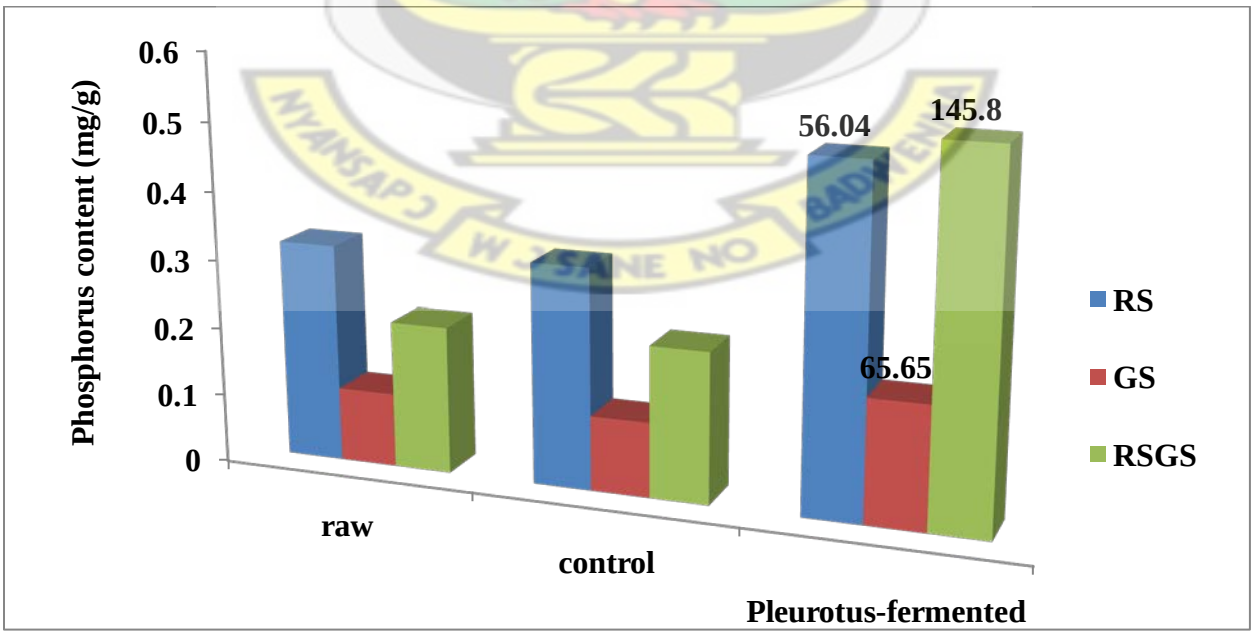


Figure 4.25 Changes in **phosphorus** content of substrates following fermentation with *P. ostreatus* (numbers on bars indicate % increase over controls)

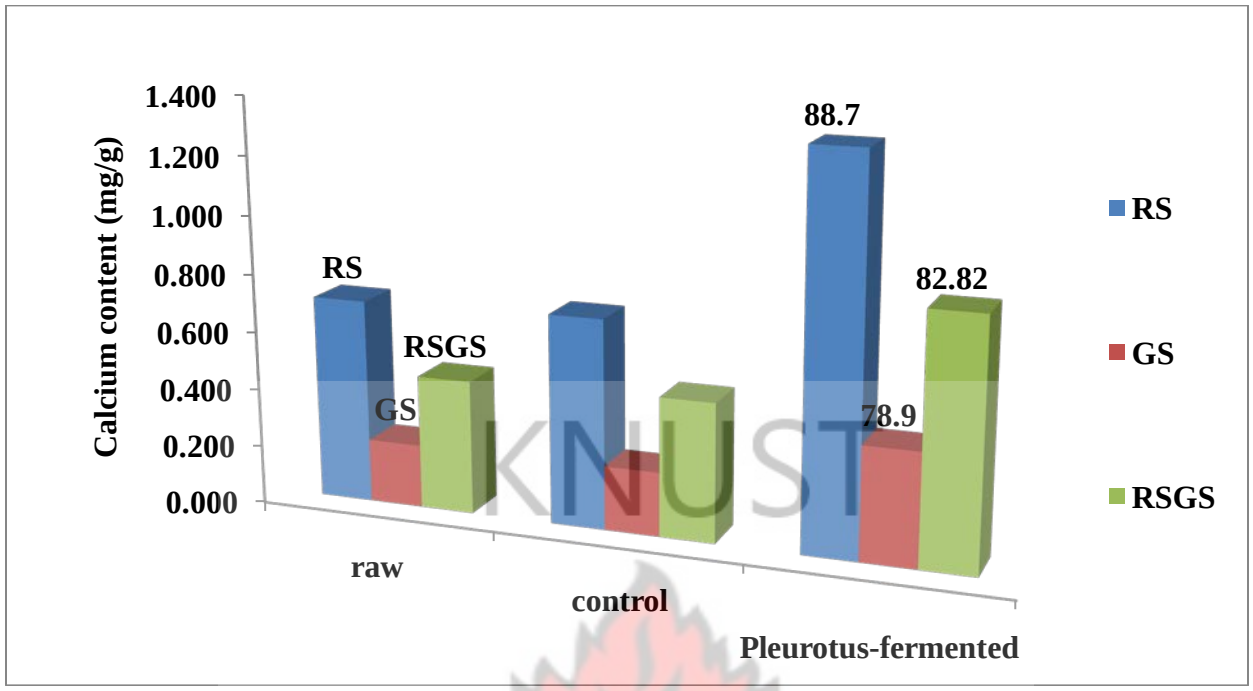


Figure 4.26 Changes in **calcium** content of substrates following fermentation with *P. ostreatus* (numbers on bars indicate % increase over controls)

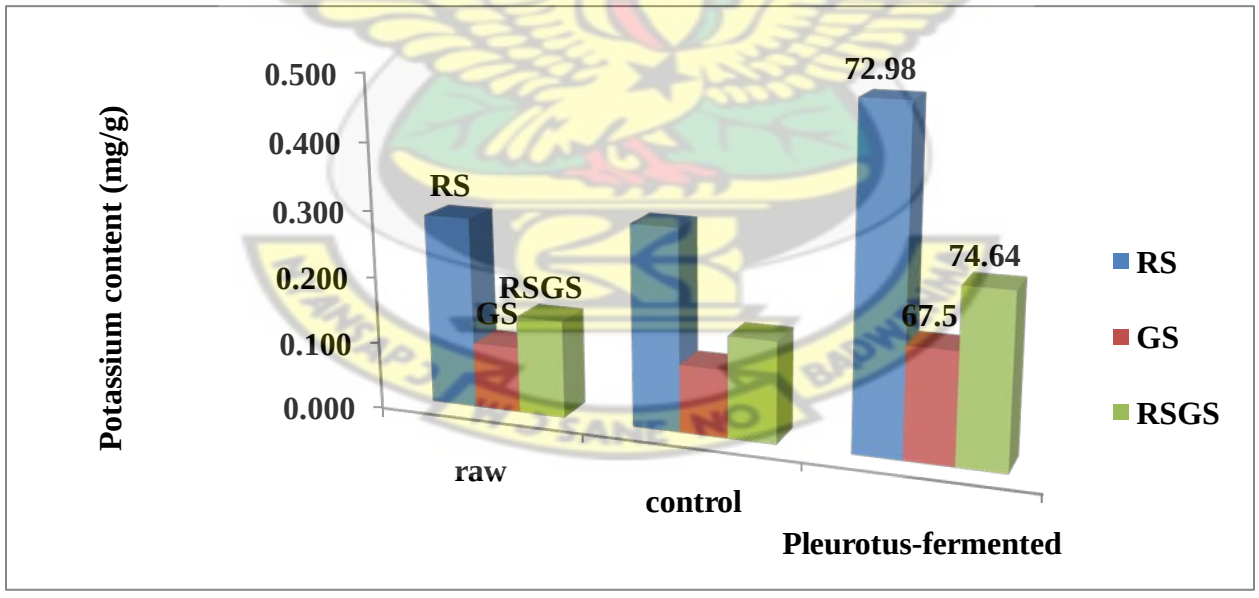


Figure 4.27 Changes in **potassium** content of substrates following fermentation with *P. ostreatus* (numbers on bars indicate % increase over controls)

4.7. Changes in tannin levels of substrates following fermentation with *P. ostreatus*

Agro-residues are generally rich in anti-nutritional factors (ANFs), particularly tannins (Makkar, 2003). According to Seresinhe and Iben (2003) and Kamalak *et al. (*2004), tannins give anti-nutritional effects and this limits their use as feed ingredients. The enzymes and metabolites (including flavour and anti-microbial factors) produced by microbes modify the composition of the solid substrate or medium. The process also reduces or eliminates the anti-nutritive components in the fermented products (Nout and Rombouts, 1990). The current study shows that the tannin levels for RS, RSGS, and GS after their optimum fermentation with *P. ostreatus* decreased significantly ($P < 0.05$) compared to their respective controls (Fig. 4.28). Modification of the substrates by the fungus mainly contributed to the observed decrease in tannin content for the samples. Tannin contents for RS, GS and RSGS were found to decrease by 76.9%, 75% and 73.8% respectively (Fig. 4.28). This agrees with reports of Adu (2009) and Alemawor *et al.* (2009) who observed similar trends after treating cassava peels and cocoa pod husk respectively with *P. ostreatus* for a period of time. Tannins are known to impart astringent taste and depress feed intake and use by animals (Brooker *et al.,* 1994), leading to growth depression. They also reduce digestibility in ruminants (Schofield *et al.,* 2001). Tannins also bind to proteins and thus, not making them readily available in the diet (Kamalak 2004). Therefore, by these results *P. ostreatus* fermentation enhances the feed value (more protein available) of the agro-wastes as potential feed source.

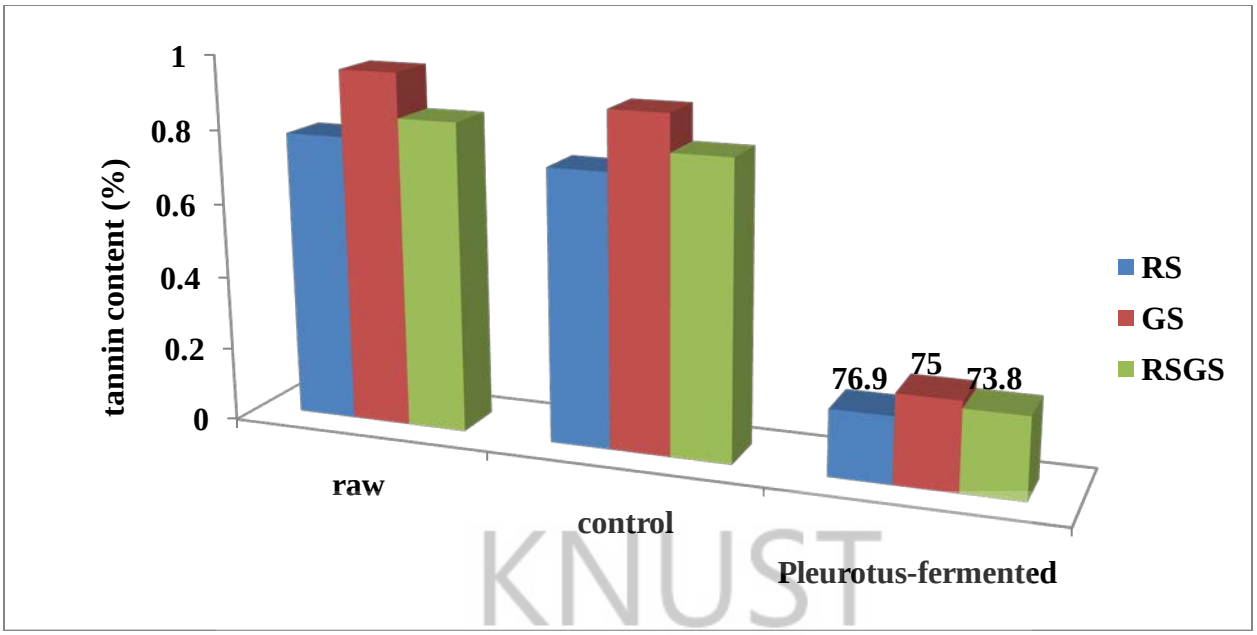


Figure 4.28 Changes in **tannin** content of substrates following fermentation with *P. ostreatus* (numbers on bars indicate % decrease over controls)

CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

Pleurotus ostreatus solid state fermentation of the substrates (RS, GS and RSGS) enhanced the biomass protein, mineral contents and IVDMED, while significantly reducing the levels of fibre *fractions and tannins.* Among the three substrate types studied, the bioconversion ability of the *P. ostreatus* on rice straw was the best in terms of overall improvement in the nutritive value, while the groundnut shell gave the least improvement. The compositional improvement obtained is dependent on the enzymatic activities of the tested fungus hence fermentation of waste agricultural residues with *Pleurotus ostreatus* could help in the production of novel feedstuff without compromising the quality for livestock production.

5.2. RECOMMENDATIONS

- Significant protein enhancement in all the three substrates may not be enough reason to conclude that *Pleurotus ostreatus* fermentation of agricultural residues will meet the dietary requirements of livestock. Therefore there will be the need to further investigate the various amino acid levels in the fermented substrates. This may be comprehensively conclusive.
- The use of drum to pasteurize the substrates is time consuming and hectic. Determining the right amount of temperature for adequate pasteurization is difficult. Therefore a more efficient method could be adopted and used. For instance the use of ash to pasteurize substrates should be investigated further to ascertain its effectiveness.
- Further work such as feeding trial using the fermented substrates as supplementary diets for animals, especially monogastrics should be carried out to ascertain the impact on performance of the animal.
- Finally it is strongly suggested that mushroom production should be integrated into the programmes of the Ministries of Agriculture and that of Science and Environment, as a

way of encouraging farmers and the general populace as a whole to upgrade as well as manage 'wastes'.

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7.0 APPENDICES

APPENDIX I: COMPOSITION OF REAGENTS AND STANDARD SOLUTIONS

*****NEUTRAL DETERGENT SOLUTION (NDS) -1L**

Dissolve 30 g sodium lauryl sulfate (sodium dodecyl sulphate) in about 300ml of distilled water, and add10 ml ethylene glycol or ethylene glycol monoethyl ether (2-ethoxy ethanol). Separately put 18.61 g disodium dihydrogen ethylene diamine tetraacetic dehydrate (EDTA-disodium salt) and 6.81g sodium borate decahydrate (Borax) in about 300 ml of distilled water in a beaker and heat until it dissolves. Also dissolve 4.56 g disodium hydrogen phosphate anhydrous (Na_2HPO_4) in a beaker. Add all the solutions together and top with distilled water to the 1 L mark. Manage the pH to 6.9 – 7.1 using NaOH or HCl.

*****ACID DETERGENT SOLUTION (ADS) -1L**

Pour about 500 ml of distilled water into a volumetric, and gently add 27.84 ml of concentrated H_2SO_4 into the flask. Add 20 g cetyl trimethylammonium bromide (CTAB) to the flask containing the acid solution, and top it up to the 1L mark.

*****SODIUM CARBONATE SOLUTION (20% Na_2CO_3 SOLUTION)**

Weigh 20 g of the carbonate and dissolve in about 80 ml of distilled water. Top it up to 100 ml mark.

*****FOLIN-CIOCALTEU REAGENT (1N)**

Dilute Folin-Ciocalteu reagent (2N) with an equal amount of distilled water. Keep in a brown bottle and store in a refrigerator at 4^0C.

APPENDIX II: CALIBRATION CURVES AND FORMULAE

Appendix II-A: STANDARD CURVES

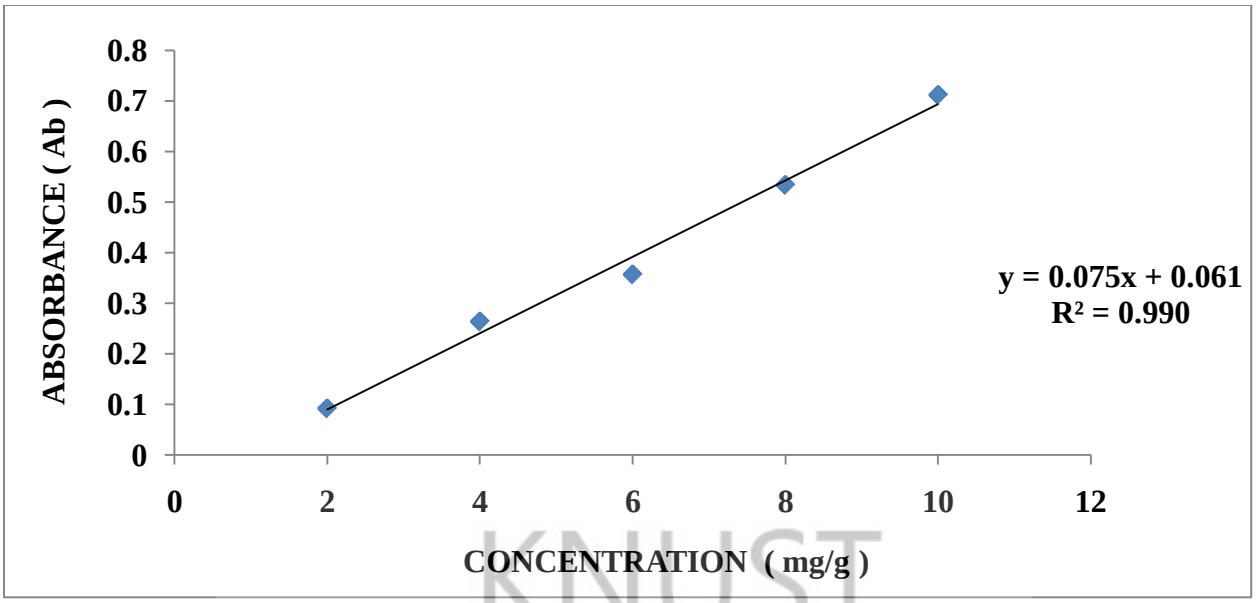


Figure A1: Calibration curve for PHOSPHORUS

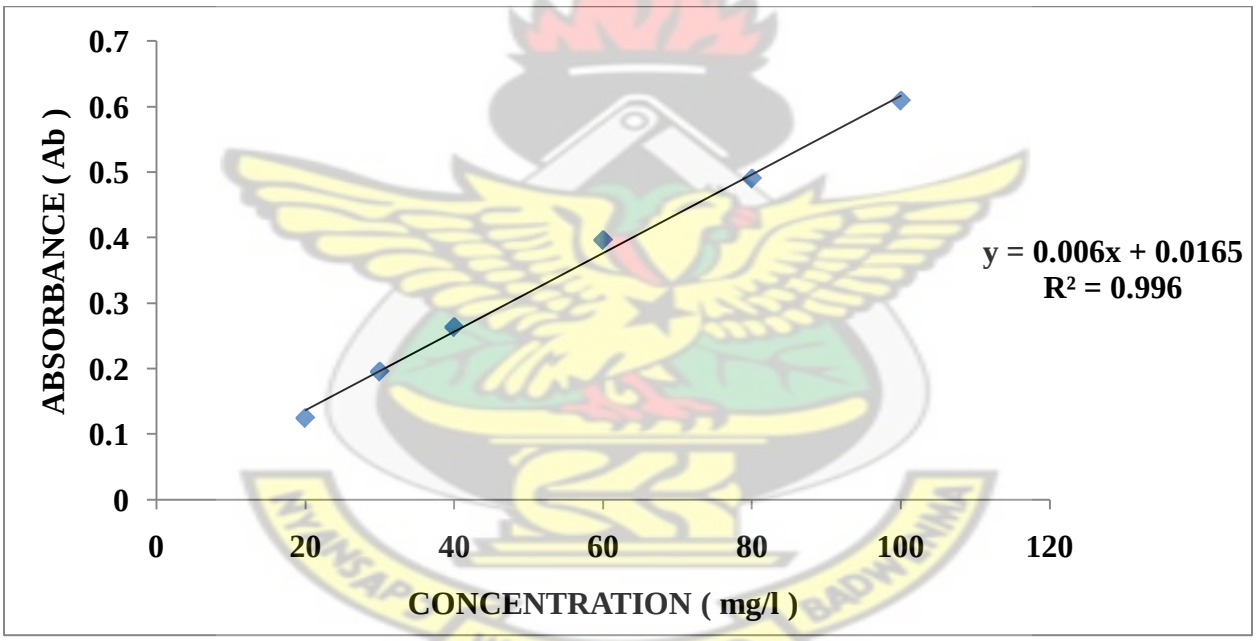


Figure A2: Calibration curve for CALCIUM

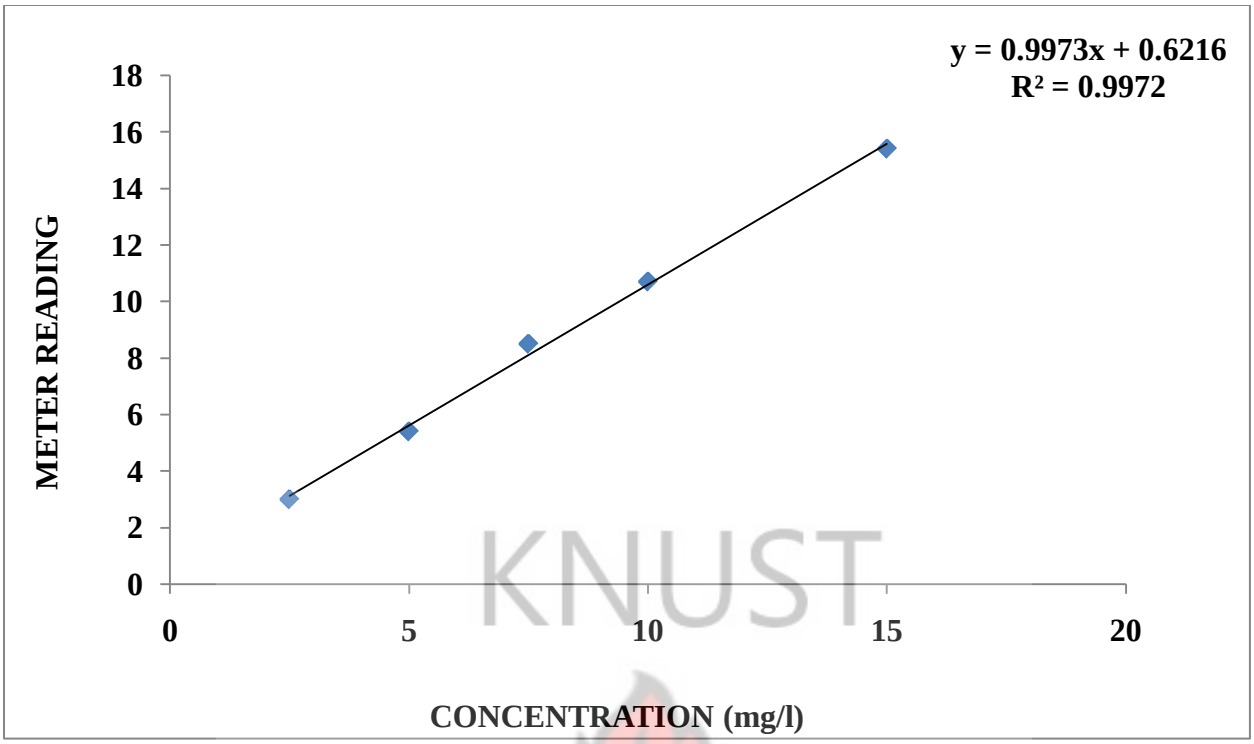
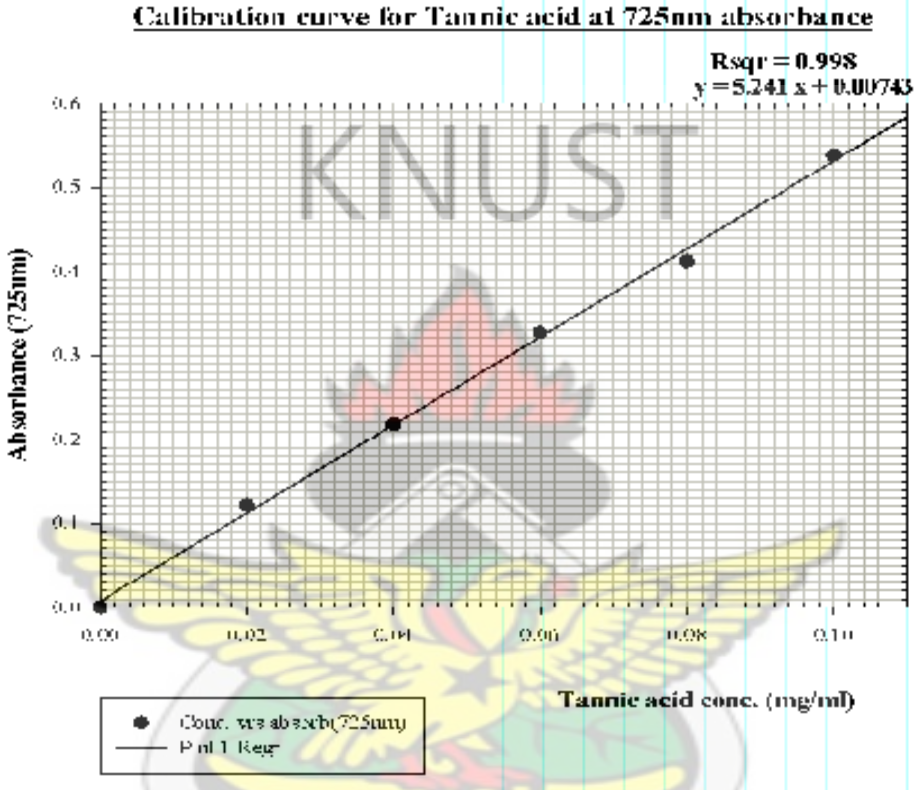


Figure A3: Calibration curve for POTASSIUM



Conc.	Absorb.
0.000	0.000
0.020	0.122
0.040	0.218
0.060	0.327
0.080	0.412
0.100	0.538

APPENDIX II-B: FORMULAE

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PROXIMATE ANALYSIS

1. **MOISTURE**

Weight of crucible = W_1

Weight of crucible + wet sample = W_2

Weight of crucible + dry sample = W_3

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

2. **CRUDE FAT (ETHER EXTRACT)**

Weight of flask =X

Weight of flask fat =Y

Weight of fat = Y-X

$$\% \text{ Fat} = \frac{(Y-X)*100}{S \text{ (weight of sample)}}$$

3. **CRUDE FIBRE**

Weight of crucible + dry sample (from oven) =X

Weight of crucible + ash (from furnace) = Y

Weight of fibre = X-Y

$$\% \text{ Fibre} = \frac{(X-Y) * 100}{M \text{ (weight of sample taken for fat)}}$$

4. **ASH**

Weight of crucible …….x

Weight of crucible + dry sample (before ashing)…….Y

Weight of crucible + dry sample (after ashing)…….Z

Weight of sample (S)……. (Y-X)

Weight of ash …… (Z-X)

$$\%ASH = \frac{(Z-X) * 100}{(Y-X)}$$

5. CRUDE PROTEIN

$$\% \text{ TOTAL NITROGEN} = \frac{100 * (V_A - V_B) * N_A * 0.01401 * 100}{W * 10}$$

Where:

V_A= Volume of standard acid used in the titration

V_B= Volume of standard acid in blank (0.15)

N_A= Normality of acid (HCl)

W = Weight in grams of sample

VAN SOEST FIBRE ANALYSIS

1. NEUTRAL DETERGENT FIBRE-NDF%

Weight of dry crucible + dry NDF (N_1)

Weight of dry crucible (N_2)

Weight of dry sample (S)

$$\% \text{ NDF} = \frac{(N_1 - N_2)}{(S)} * 100$$

2. ACID DETERGENT FIBRE-ADF%

Weight of dry crucible + dry ADF (A_1)

Weight of dry crucible (A_2)

Weight of dry sample (S)

$$\% \text{ ADF} = \frac{(A_1 - A_2)}{(S)} * 100$$

3. ACID DETERGENT LIGNIN-ADL

Weight of dry residue after treating the ADF with 72% H_2SO_4 (L_1)

Weight of ash after igniting L_1 in a furnace (L_2)

Weight of Sample (S)

$$\% \text{ ADL} = \frac{(L_1 - L_2)}{S} * 100$$

4. Hemicellulose = NDF-ADF

5. CELLULOSE = ADF-ADL

APPENDIX III-ANOVA TABLES

APPENDIX III-A: RICE STRAW ANOVA

APPENDIX III-A1: ASH-TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	184.178	5	36.8356	656.09	0.0000
Within groups	0.673733	12	0.0561444		
Total (Corr.)	184.852	17			

APPENDIX III-A2: ASH - CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.855044	5	0.171009	3.39	0.0386
Within groups	0.6052	12	0.0504333		
Total (Corr.)	1.46024	17			

APPENDIX III-A3: CRUDE PROTEIN -TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	28.5861	5	5.71722	38.72	0.0000
Within groups	1.772	12	0.147667		
Total (Corr.)	30.3581	17			

APPENDIX III-A4: CRUDE PROTEIN- CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.604311	5	0.120862	0.57	0.7186
Within groups	2.52307	12	0.210256		
Total (Corr.)	3.12738	17			

APPENDIX III-A5: ETHER EXTRACT-TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.537228	5	0.107446	201.46	0.0000
Within groups	0.0064	12	0.000533333		
Total (Corr.)	0.543628	17			

APPENDIX III-A6: ETHER EXTRACT -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.0122944	5	0.00245889	4.43	0.0162
Within groups	0.00666667	12	0.000555556		

Total (Corr.)	0.0189611	17			

APPENDIX III-A7: MOISTURE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	79.8862	5	15.9772	2326.78	0.0000
Within groups	0.0824	12	0.00686667		
Total (Corr.)	79.9686	17			

APPENDIX III-A8: MOISTURE- CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.134894	5	0.0269789	3.12	0.0495
Within groups	0.103867	12	0.00865556		
Total (Corr.)	0.238761	17			

APPENDIX III-A9: DRY MATTER -TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	79.8862	5	15.9772	2326.78	0.0000
Within groups	0.0824	12	0.00686667		
Total (Corr.)	79.9686	17			

APPENDIX III-A10: DRY MATTER -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.115644	5	0.0231289	2.29	0.1114
Within groups	0.121267	12	0.0101056		
Total (Corr.)	0.236911	17			

APPENDIX III-A11: NITROGEN FREE EXTRACT -TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	5.69538	5	1.13908	7.99	0.0016
Within groups	1.71	12	0.1425		
Total (Corr.)	7.40538	17			

APPENDIX III-A12: NITROGEN FREE EXTRACT- CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	8.38123	5	1.67625	6.87	0.0030
Within groups	2.92633	12	0.243861		
Total (Corr.)	11.3076	17			

APPENDIX III-A13: NDF -TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1417.27	5	283.453	6795.63	0.0000
Within groups	0.500533	12	0.0417111		
Total (Corr.)	1417.77	17			

APPENDIX III-A14: NDF -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4.67224	5	0.934449	3.14	0.0484
Within groups	3.5712	12	0.2976		
Total (Corr.)	8.24344	17			

APPENDIX III-A15: ADF- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	686.829	5	137.366	2190.65	0.0000
Within groups	0.752467	12	0.0627056		
Total (Corr.)	687.582	17			

APPENDIX III-A16: ADF -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4.98083	5	0.996166	1.81	0.1862
Within groups	6.6206	12	0.551717		
Total (Corr.)	11.6014	17			

APPENDIX III-A17: HEMICELLULOSE -TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	131.624	5	26.3248	220.19	0.0000
Within groups	1.43467	12	0.119556		
Total (Corr.)	133.059	17			

APPENDIX III-A18: HEMICELLULOSE -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1.49836	5	0.299672	1.54	0.2496
Within groups	2.33433	12	0.194528		
Total (Corr.)	3.83269	17			

APPENDIX III-A19: CELLULOSE -TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*

Between groups	281.398	5	56.2797	776.09	0.0000
Within groups	0.8702	12	0.0725167		
Total (Corr.)	282.269	17			

APPENDIX III-A20: CELLULOSE -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2.08409	5	0.416819	0.90	0.5142
Within groups	5.58407	12	0.465339		
Total (Corr.)	7.66816	17			

APPENDIX III-A21: LIGNIN -TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	89.6925	5	17.9385	2992.52	0.0000
Within groups	0.0719333	12	0.00599444		
Total (Corr.)	89.7644	17			

APPENDIX III-A22: LIGNIN -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	89.6925	5	17.9385	2992.52	0.0000
Within groups	0.0719333	12	0.00599444		
Total (Corr.)	89.7644	17			

APPENDIX III-B: GROUNDNUT SHELL ANOVA

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APPENDIX III-B1: ASH-TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	18.2728	5	3.65456	496.47	0.0000
Within groups	0.0883333	12	0.00736111		
Total (Corr.)	18.3611	17			

APPENDIX III-B2: ASH -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.0305333	5	0.00610667	3.84	0.0260
Within groups	0.0190667	12	0.00158889		
Total (Corr.)	0.0496	17			

APPENDIX III-B3: CRUDE FIBRE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1597.19	5	319.438	2500.93	0.0000
Within groups	1.53273	12	0.127728		
Total (Corr.)	1598.72	17			

APPENDIX III-B4: CRUDE FIBRE -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	6.68763	5	1.33753	11.22	0.0003
Within groups	1.43007	12	0.119172		
Total (Corr.)	8.11769	17			

APPENDIX III-B5: CRUDE PROTEIN- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	27.161	5	5.4322	66.20	0.0000
Within groups	0.984667	12	0.0820556		

Total (Corr.)	28.1456	17			

APPENDIX III-B6: CRUDE PROTEIN-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.348117	5	0.0696233	1.29	0.3312
Within groups	0.648333	12	0.0540278		
Total (Corr.)	0.99645	17			

APPENDIX III-B7: ETHER EXTRACT-TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1.22478	5	0.244957	393.68	0.0000
Within groups	0.00746667	12	0.000622222		
Total (Corr.)	1.23225	17			

APPENDIX III-B8: ETHER EXTRACT -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.213428	5	0.0426856	33.85	0.0000
Within groups	0.0151333	12	0.00126111		
Total (Corr.)	0.228561	17			

APPENDIX III-B9: MOISTURE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	66.3047	5	13.2609	845.24	0.0000
Within groups	0.188267	12	0.0156889		
Total (Corr.)	66.493	17			

APPENDIX III-B10: MOISTURE -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.1712	5	0.03424	1.18	0.3749
Within groups	0.3486	12	0.02905		
Total (Corr.)	0.5198	17			

APPENDIX III-B11: DRY MATTER- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	55.5718	4	13.8929	2037.09	0.0000
Within groups	0.0682	10	0.00682		
Total (Corr.)	55.64	14			

APPENDIX III-B12: DRY MATTER -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.17225	5	0.03445	1.23	0.3552
Within groups	0.337	12	0.0280833		
Total (Corr.)	0.50925	17			

APPENDIX III-B13: NITROGEN FREE EXTRACT- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	991.363	5	198.273	882.19	0.0000
Within groups	2.697	12	0.22475		
Total (Corr.)	994.06	17			

APPENDIX III-B14: NITROGEN FREE EXTRACT-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*

Between groups	4.98969	5	0.997939	4.72	0.0129
Within groups	2.53773	12	0.211478		
Total (Corr.)	7.52743	17			

APPENDIX III-B15: NEUTRAL DETERGENT FIBRE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	643.583	5	128.717	3629.23	0.0000
Within groups	0.4256	12	0.0354667		
Total (Corr.)	644.009	17			

APPENDIX III-B16: NEUTRAL DETERGENT FIBRE -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	5.10698	5	1.0214	1.96	0.1576
Within groups	6.25493	12	0.521244		
Total (Corr.)	11.3619	17			

APPENDIX III-B17: ACID DETERGENT FIBRE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	367.854	5	73.5708	357.78	0.0000
Within groups	2.4676	12	0.205633		
Total (Corr.)	370.322	17			

APPENDIX III-B18: ACID DETERGENT FIBRE -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2.40118	5	0.480236	2.03	0.1462

Within groups	2.83867	12	0.236556		
Total (Corr.)	5.23984	17			

APPENDIX III-B19: HEMICELLULOSE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	39.3674	5	7.87349	29.49	0.0000
Within groups	3.20407	12	0.267006		
Total (Corr.)	42.5715	17			

APPENDIX III-B20: HEMICELLULOSE -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.691294	5	0.138259	1.00	0.4565
Within groups	1.65347	12	0.137789		
Total (Corr.)	2.34476	17			

APPENDIX III-B21: CELLULOSE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	83.3567	5	16.6713	62.59	0.0000
Within groups	3.1964	12	0.266367		
Total (Corr.)	86.5531	17			

APPENDIX III-B22: CELLULOSE -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.1802	5	0.03604	0.26	0.9289
Within groups	1.6932	12	0.1411		

Total (Corr.)	1.8734	17			

APPENDIX III-B23: LIGNIN- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	100.411	5	20.0821	273.47	0.0000
Within groups	0.8812	12	0.0734333		
Total (Corr.)	101.292	17			

APPENDIX III-B24: LIGNIN -CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1.34878	5	0.269756	0.64	0.6755
Within groups	5.07553	12	0.422961		
Total (Corr.)	6.42431	17			

APPENDIX III-C: RICE STRAW + GROUNDNUT SHELL ANOVA

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APPENDIX III-C1: ASH- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	74.3814	5	14.8763	191.20	0.0000
Within groups	0.933667	12	0.0778056		
Total (Corr.)	75.3151	17			

APPENDIX III-C2: ASH-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.795094	5	0.159019	7.70	0.0019

Within groups	0.247867	12	0.0206556		
Total (Corr.)	1.04296	17			

APPENDIX III-C3: CRUDE FIBRE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	788.84	5	157.768	2028.30	0.0000
Within groups	0.9334	12	0.0777833		
Total (Corr.)	789.773	17			

APPENDIX III-C4: CRUDE FIBRE-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	8.16613	5	1.63323	10.93	0.0004
Within groups	1.79347	12	0.149456		
Total (Corr.)	9.9596	17			

APPENDIX III-C5: CRUDE PROTEIN- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	22.2954	5	4.45908	40.57	0.0000
Within groups	1.3188	12	0.1099		
Total (Corr.)	23.6142	17			

APPENDIX III-C6: CRUDE PROTEIN-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1.20709	5	0.241419	3.24	0.0443
Within groups	0.894733	12	0.0745611		
Total (Corr.)	2.10183	17			

APPENDIX III-C7: ETHER EXTRACT- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	3.19691	5	0.639382	360.78	0.0000
Within groups	0.0212667	12	0.00177222		
Total (Corr.)	3.21818	17			

APPENDIX III-C8**:** ETHER EXTRACT-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.0691778	5	0.0138356	10.12	0.0006
Within groups	0.0164	12	0.00136667		
Total (Corr.)	0.0855778	17			

APPENDIX III-C9: MOISTURE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	47.4852	5	9.49705	892.21	0.0000
Within groups	0.127733	12	0.0106444		
Total (Corr.)	47.613	17			

APPENDIX III-C10**:** MOISTURE-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.0375833	5	0.00751667	0.23	0.9438
Within groups	0.398067	12	0.0331722		
Total (Corr.)	0.43565	17			

APPENDIX III-C11: DRY MATTER- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	47.4852	5	9.49705	892.21	0.0000
Within groups	0.127733	12	0.0106444		
Total (Corr.)	47.613	17			

APPENDIX III-C12: DRY MATTER-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.0375833	5	0.00751667	0.23	0.9438
Within groups	0.398067	12	0.0331722		
Total (Corr.)	0.43565	17			

APPENDIX III-C13: NITROGEN FREE EXTRACT- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	292.285	5	58.457	135.77	0.0000
Within groups	5.16687	12	0.430572		
Total (Corr.)	297.452	17			

APPENDIX III-C14: NITROGEN FREE EXTRACT-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4.32287	5	0.864573	4.22	0.0191
Within groups	2.45833	12	0.204861		
Total (Corr.)	6.7812	17			

APPENDIX III-C15: NEUTRAL DETERGENT FIBRE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	979.403	5	195.881	3392.85	0.0000
Within groups	0.6928	12	0.0577333		
Total (Corr.)	980.096	17			

APPENDIX III-C16**:** NEUTRAL DETERGENT FIBRE-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	3.86396	5	0.772792	1.90	0.1672
Within groups	4.86813	12	0.405678		
Total (Corr.)	8.73209	17			

APPENDIX III-C17: ACID DETERGENT FIBRE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	670.853	5	134.171	6103.29	0.0000
Within groups	0.2638	12	0.0219833		
Total (Corr.)	671.117	17			

APPENDIX III-C18**:** ACID DETERGENT FIBRE-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2.28678	5	0.457356	1.72	0.2049
Within groups	3.1936	12	0.266133		
Total (Corr.)	5.48038	17			

APPENDIX III-C19**:** HEMICELLULOSE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*

Between groups	28.2592	5	5.65184	73.12	0.0000
Within groups	0.9276	12	0.0773		
Total (Corr.)	29.1868	17			

APPENDIX III-C20: HEMICELLULOSE-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.671161	5	0.134232	0.58	0.7119
Within groups	2.7552	12	0.2296		
Total (Corr.)	3.42636	17			

APPENDIX III-C21**:** CELLULOSE- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	274.462	5	54.8924	1879.88	0.0000
Within groups	0.3504	12	0.0292		
Total (Corr.)	274.812	17			

APPENDIX III-C22: CELLULOSE-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.798894	5	0.159779	0.81	0.5668
Within groups	2.37853	12	0.198211		
Total (Corr.)	3.17743	17			

APPENDIX III-C23**:** LIGNIN- TREATED

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	88.7884	5	17.7577	2927.09	0.0000

Within groups	0.0728	12	0.00606667		
Total (Corr.)	88.8612	17			

APPENDIX III-C24: LIGNIN-CONTROL

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	0.468044	5	0.0936089	2.68	0.0751
Within groups	0.419133	12	0.0349278		
Total (Corr.)	0.887178	17			

APPENDIX IV: LIST OF PLATES

Plate A1: RICE STRAW- WK5

Plate A2: RICE STRAW -WK 3

Plate A3: MIXTURE -WK 4

Plate A4: GROUNDNUT SHELL -WK 5

Plate A5: GROUNDNUT SHELL-WK 4