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ENRICHMENT OF CASSAVA (*MANIHOT ESCULENTA*) PEELS THROUGH *PLEUROTUS OSTREATUS* FERMENTATION FOR USE AS ANIMAL FEED

BY

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BY

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DECLARATION

I do declare that, except for references to other peoples work which have been duly cited, this work submitted as a thesis to the Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, for the degree of Master of Science in Biotechnology, is the result of my own investigation and has not been presented for any other degree.

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HEAD OF DEPARTMENT

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ABSTRACT

Livestock feeding is quite problematic in Ghana due to the high cost of feed ingredients. Cassava (*Manihot esculenta*) peel is a major by-product of the cassava processing industry and in Ghana where cassava is grown and tubers processed, the peel is largely underutilized as livestock feed. The application of biotechnology can help add value to agro-wastes for use as cheap sources of animal feed. Cassava peels were collected, dried and after pretreatment, the particle size was reduced to an average area of about 0.6cm^2, the peels were then composted and sterilized. The composted sterilized peels were then inoculated with the spawn of the oyster mushroom (*Pleurotus ostreatus*) and incubated at room temperature. Fermentation of cassava peels with the oyster mushroom over a seven week period significantly improved its nutritional value ($P<0.05$) when compared to the uninoculated peels. Six weeks of fermentation was found to be the optimum to produce the observed significant results. The protein content of the mushroom fermented peels more than doubled from 2.18% to 5.6% after fermentation, whereas cellulose, hemicellulose and lignin contents decreased significantly by 26.73%, 45.43% and 35.78% respectively. The fermented peels also had a significant increase in the calcium, potassium and phosphorous contents. The tannin content of the oyster mushroom fermented peels reduced drastically by 75.5% after six weeks of fermentation when compared with the uninoculated peels. *In vitro* digestibility studies revealed that, after the *Pleurotus* fermentation over a six week period, the peels were three times more digestible than the uninoculated peels. These results indicate the potential use of *Pleurotus ostreatus* for improving the feed value of cassava peels for the livestock industry in Ghana. The fermented peels can partially replace corn, the main ingredient in poultry feed formulation.

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important crops in Ghana and many tropical countries. Cassava species Manioc, *Manihot esculenta* Crantz or *Manihot utillisima* Pohl is believed to have originated from Brazil and was introduced into West Africa by the Portuguese (Silvestre, 1989).

Cassava roots play an important role in the African diet and they are processed using simple traditional methods into products such as "gari" and fufu, or lafun flour, some of which are fermented products (Odunfa, 1985). As a rough estimate, about 10 million tons of cassava are processed for gari annually in Nigeria alone (Okafor, 1992).

In the processing of fermented cassava products, the roots are normally peeled to rid them of two outer coverings: a thin brown outer covering, and a thicker leathery parenchymatous inner covering (Obadina *et al*., 2006). These peels are regarded as wastes and are usually discarded and allowed to rot. With hand peeling the peels can constitute 20-35% of the total weight of the tuber (Odunfa, 1985).

The wastes generated at present pose a disposal problem and would even be more problematic in the future with increased industrial production of cassava products such as in gari production, cassava flour and dried cassava fufu (Obadina *et al*, 2006). Since these peels could make up to 10% of the wet weight of the roots, they constitute an important potential resource if properly harnessed by a biotechnological system (Obadina *et al*, 2006).

Cassava peels continue to constitute waste in the cassava processing industry (Eustace and Dorothy, 2001). This is in spite of the potential of the use of the by-

product as an animal feedstuff. Considerable evidence has emerged for the possibility of using processed cassava peel as an energy source for pigs and poultry (Longe *et al*, 1983). Higher inclusion of the by-product in monogastric feed or formulation of diets with cassava peels, as sole energy source is limited because of its fibrous nature.

Fakolade (1997) and Arowora *et al* (1999) have reported the occurrence of high amounts of non-starch polysaccharides in cassava peels. Degradation of these carbohydrate compounds to simple sugars will further increase the energy value of cassava peels. Since the monogastric industry constitutes the largest consumer of commercial livestock feeds in Africa, it is imperative to find alternative feed sources to the expensive energy ingredients like the cereals (Arowora *et al*, 1999).

The use of fungi for the conversion of lignocelluloses into food and feed rich in protein offers an alternative for developing unconventional source of proteins as food or feed (Vijay *et al*, 2007). Yeast and algal proteins for example require sophisticated techniques and heavy inputs to produce whereas the beauty of mushroom cultivation lies in its ability to grow on cheap lignocellulose materials with minimum inputs and a high yield of valued food protein for direct human consumption and may also provide a rich source of animal feed (Vijay *et al*, 2007).

Chang (1980) defined mushroom as a fungus with a distinctive fruiting body which can be either epigeous or hypogenous and large enough to be seen with the naked eye and be picked by the hand. The history of mushrooms in development goes far back in time to 400 BC when Hippocrates first wrote about their medicinal value (Bosompem, 2009). Mushrooms belong to the kingdom fungi and phylum basidiomycota.

They lack the photosynthetic pigment called chlorophyll and for that matter cannot utilize the solar energy or similar sources of energy to manufacture their own food by the process of photosynthesis (Oei, 1991). However, mushrooms extract carbohydrates, proteins and other nutrients from rich medium of decaying organic matter to become one of the most rewarding food sources on earth (Bosompem, 2009). Mushroom forming fungi are therefore amongst nature's most powerful decomposers, secreting strong extracellular enzymes due to their aggressive growth and biomass production (Adenipekun, 2009). They are primary decomposers and saprophytic fungi, which grow easily in clusters on decomposing lignocellulosic, capable of utilizing a wide range of substrate material, with high biological efficiency and good mycelia growth rate (Achio, 2009). They therefore undergo extracellular digestion. They have the capability to produce a wide range of enzymes such as laccases, cellulases and hemicellulases that are capable of breaking down complex substrates namely; lignin, cellulose and hemicellulose respectively into simpler soluble substances and absorb them for their growth and development (Oei, 1991). According to Omoanghe and Nerud (1999), white rot fungi produce extracellular lignin-modifying enzymes, the best characterized of which are laccases, lignin peroxidases, and manganese peroxidases.

Sharma *et al* (1999) reported that the degradation of all complex lignocellulosic waste by micro-organisms largely depends upon the synergistic action of extracellular enzymes like cellulases and hemicellulases.

On various substrates containing lignin and cellulose, mushroom grows very well by converting these substrates into digestible protein-rich materials and may even be used as animal feed (Oei, 1991). Some of the arrays of substrates with such qualities are; sawdust, rice bran, rice straw, corncobs, cocoa pod husk, sugar cane leaves,

grasses, rice hull, leaves of water hyacinths, etc. These agricultural wastes can be used for the cultivation of mushroom and apart from their low costs; the practice also reduces the incidence of environmental pollution (Oei, 1991).

The oyster mushrooms (*Pleurotus ostreatus*) are by far the easiest and least expensive mushrooms to grow (Oei, 1991) and they exhibit a higher degree of adaptability, aggressiveness and productivity.

A wide range of diverse cellulosic substrates are used for cultivation of *Pleurotus species*. Amongst various cereal straws, paddy straw was reported to be the best substrate for the cultivation of oyster mushroom (Garcha *et al*., 1984), whereas, next to the paddy straw, wheat straw proved 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 utility of pearl millet stalks in the cultivation of *P. sajor-caju*. Rice straw waste, lawn grass, maize cobs, banana wastes (Bonatti *et al*, 2004) and maize straw (Bahukhandi and Munjal, 1989) were reported as suitable substrates for cultivation of different *Pleurotus spp*. Cultivation of *Pleurotus ostreatus* mushroom is based on usage of lignocellulose materials. These materials are used as substrates for spawning and the growth of the mushrooms. Sawdust, parings, tree bark, cassava peels, seed husks and other lignocellulose materials can also be used (Ivanka *et al*, 2004).

About 900 000 tons of the mushrooms of *Pleurotus* family and 2300 000 tons of spent substrata are produced all over the world each year (Chang and Miles, 1993). Spent substrata can be used as fertilizer.

Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter (Howard *et al*, 2003). Lignocellulose consists of lignin, hemicellulose and cellulose (Howard *et al*, 2003). The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value (Malherbe and Cloete, 2003). Large amounts of lignocellulosic "waste" are generated through forestry and agricultural practices, paper-pulp industries, timber industries and many agro-industries and they pose an environmental pollution problem (Howard *et al*, 2003). Sadly, much of the lignocellulose waste is often disposed of by biomass burning, which is not restricted to developing countries alone, but is considered a global phenomenon (Levine, 1996).

However, the huge amounts of residual plant biomass considered as "waste" can potentially be converted into various different value-added products including biofuels, chemicals, and cheap energy sources for fermentation, improved animal feeds and human nutrients (Howard *et al*, 2003).

Lignocellulytic enzymes also have significant potential applications in various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper, and agriculture (Howard *et al*, 2003).

The problem statement

Cereals have remained the major energy source in livestock diets with maize being the most common and the major ingredient contributing about 60-80% (Damisa *et al*., 2007). Inadequate supply and prohibitive prices of these cereal grains particularly corn and consequent increase in the cost of livestock products have led animal nutritionists to search for cheaper alternative sources of energy to feed livestock (Adebowale, 1981). The need to find alternative and cheaper ingredients to replace the expensive ones is inevitable since cost of feed accounts for 70-80% of the cost of poultry production in Ghana. Feed cost alone according to Sastry and Thomas (1976) accounts for 67.2% of the total pig production cost. It is therefore pertinent to scout for cheaper and readily available sources for compounding swine rations. In recent times, cassava or tapioca meal has been extensively used as a replacement ingredient to corn (Damisa *et al*., 2007).

Two important biological wastes that may cause damage to the environment are generated during the traditional processing of cassava storage roots for 'gari' production in Africa, namely, the cassava peels and the liquid squeezed out of the fermented parenchyma mash (Oboh, 2006). Cassava peels derived from gari processing are normally discarded as wastes and allowed to rot in the open, thus resulting in health hazards (Oboh, 2006).

As a rough estimate, about 10 million tons of cassavas are processed into gari annually in Nigeria alone (Oboh, 2006). Since these peels could make up to 10% of the net weight of the roots, they constitute an important potential resource for animal feeds if properly processed by a bio-system (Antai and Mbongo, 1994).

About 60% of the cassava produced all over the world is used for human consumption (Obadina *et al*, 2006). Cassava peels are the major by-product of the cassava processing industry and these peels are largely underutilized as livestock feed in Ghana (Annan, 1998). The waste peels are found to contain 42.6% carbohydrate, 1.6% protein, 12.1% ether extract, 5.0% total ash and 22.5% crude fibre (Obadina *et al*, 2006).

With the advent of biotechnology approaches, there are opportunities for economic utilization of agro industrial residues such as cassava peel waste.

Justification of the problem statement

Feed represents a major portion of the over all production cost in the poultry and livestock industry in the country. A major constraint in the poultry industry in Ghana is the availability of feed ingredients all the year round at economic prices. This problem is further compounded by the fact that most of these ingredients are imported at high foreign exchange costs. Therefore, alternative sources of energy for animal feeds which are nutritionally adequate and cheap must be found locally to reduce the cost.

One source of great potential which is increasingly being used for animal feed is cassava which can completely replace maize and other cereals in livestock and poultry feed-formulation. Cassava peels contain high amount of non-starch polysaccharides, mostly non- digestible carbohydrates such as cellulose and hemicelluloses (Oke, 1992).

These are poorly digested and bio-utilized by livestock. The digestibility of cassava peels as feed for both ruminants and non-ruminants tends to decrease with crude

fibre content (Fakolade, 1997). *Pleurotus ostreatus* is known to reduce this high fibre content by degrading the hemicelluloses, cellulose and lignin components of the peels and also improve the low protein content of the peels as animal feed (Hadar *et al*., 1992).

Thus through the fermentation process, the two major limitations associated with the use of cassava peels as animal feed are catered for and also environmental pollution by agro-wastes such as cassava peels is minimized.

Objectives of project

The main objective of this project is the use of *Pleurotus ostreatus* as a useful biocatalyst for fermentation of cassava peels to improve its nutritional value.

Specific objectives

- To determine the optimum duration of the fermentation process
- To monitor the proximate composition during the fermentation process
- To determine the level of degradation of the individual fibre components in the substrate during the fermentation by *Pleurotus ostreatus*
- To evaluate the suitability of the processed peels for animal feed through *in vitro* digestibility studies.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Cassava

2.1.1Origin, Distribution and Production

The plant *Maniholt esculenta* (Crantz) is a short-lived perennial shrub which is grown almost anywhere between the latitudes 30N and 30S, an area which encompasses some of the poorest countries of the world (Bokanga, 1993). It is well adapted to areas that experience a long dry season and uncertain rainfall, thus qualifying as the crop of choice for drought-prone areas (Cock, 1985). Cassava is a major root crop in the tropics and its roots are significant source of calories for more than 500 million people worldwide (Cock, 1985). It is one of the cheapest sources of calories for human consumption, contributing about 40% of food calories in Africa (IITA, 1990).

It is estimated that 2 million tonnes of cassava crop will be produced over the next ten years, yielding about 480,000 tonnes of cassava starch and about 640,000 tonnes of cassava peels (Adu-Amankwa, 2006). The cassava starch revenue is projected at about US$96 million annually and with the expressed interest in cassava starch, the technical problem is the utilization of the cassava peel and cassava meal by-products (Adu-Amankwa, 2006).

About 46% of that amount was produced in Africa, 33% in Asia and 21% in Latin America (Bokanga, 1993). The major producing countries are Nigeria, Brazil, Zaire, Thailand, and Indonesia, which together produce more than two-thirds of the world production (Bokanga, 1993). About 20% of the Asian production (mainly from Thailand) is exported primarily to Western Europe, where it is used as cheap

carbohydrate source in animal feed (Bokanga, 1993).The remainder of the world production is used as food and, to a limited extent, as feed (Bokanga, 1993). The table below gives a summary of the leading producers of cassava in tropical Africa.

Table 2.1 Leading producers of cassava in tropical Africa

COUNTRY	CASSAVA AREA (000ha)	WORLD CASSAVA AREA* (%)	PRODUCTION* (000 tonnes)	WORD PRODUCTION (%)	YIELD (kg/ha)
Nigeria	1700	10.8	20,000	13.0	11765
Zaire	2388	15.2	18,277	11.9	7631
Tanzania	604	3.9	6,266	4.1	10370
Mozambique	972	6.2	3,690	2.4	3796
Ghana	535	3.4	3,600	2.3	6733
Uganda	380	2.4	3,350	2.2	8816
Madagascar	343	2.2	2,290	1.5	6676
Angola	500	3.2	1,850	1.2	3700
Cote D'Ivore	257	1.6	1,435	0.9	5578
Cameroon	92	0.6	1,230	0.8	13370
Benin	120	0.8	889	0.6	7410
Central African Rep.	167	1.1	520	0.3	3114

Source: FAO Production Year Book 1991

*Figures quoted were projected estimates for 1989.

Table 2.2 Selected data on cassava production, consumption and exports in Ghana

1.Growth Rate for Area Planted 1977/79-1987/89	 4.5%
2.Production Growth Rate 1967/69-1977/79 1977/79-1987/89	 6.6% 2.7%
3. Yield (1987-1990)	7.8mt/ha
4.Achievable Yield (mt/ha)*	28.0
5. Consumption: 1980 1985 1990	145.2kg/head/yr 146.3kg/head/yr 148.0kg/head/yr
6.Exports of Cassava Products Cassava Dough/Flour (mt) 1988 1989 Cassava Chips (mt) 1988	 1.7 6.9 9.5

Source: Ministry of Food and Agriculture (Ghana) 1991

*Indicate yields that have achieved isolated cases due to more effective extension and other logistic support

2.2 Lignocellulosic materials

Lignocelluloses are the most abundant materials present on earth, comprising 50% of all biomass with an estimated annual production of 5×10^{10} tonnes (Goldstein, 1981).

According to Han and Smith, (1978), the most abundant renewable biomass on earth consists of cellulose, with between 5 and 15 tons per person being synthesized

annually by photosynthesis. Much of the cellulose in nature is bound physico-chemically with lignin.

Because lignin is highly resistant, it protects cellulose against attack by most microbes, and it must be degraded by chemical or biological means before the cellulose can be utilized (Salvagi and Kaulkarnis, 2001).

The use of the polysaccharides in the lignocellulosic complex is limited due to their high lignin content (Hadar *et al*, 1992). It has also been estimated that about one-half the total production of plant residues from agriculture and industrial processes remains unused and burdens the environment (Zadrazil and Grabbe, 1983).

Chang (1989) noted that all agricultural production from plant crops generated enormous waste, because little of each crop was actually used; typically 80-90% of the total biomass of agricultural production is discarded as waste and this is because only part of the organic matter synthesized through photosynthesis every year is directly edible in the form of fruits, vegetables and food grains and assumes various forms, such as inedible sugarcane bagasse and corn cobs (Savalgi and Kaulkarnis, 2001).

The handling and disposal of these lignocellusic residues are often problematic due to their chemical structure and decomposition properties (Philippoussis *et al*, 2001).

Lignocellulosic biomass is not only a renewable resource but also the most abundant source of organic components in high amount on the earth (Taniguchi *et al*, 2005). To date, intensive research and development studies on the effective utilization of lignocellulosic materials have been carried out (Taniguchi *et al*, 2005).

However, various problems associated with the practical utilization of these materials have not yet been solved (Taniguchi *et al*, 2005). One of the key problems hindering the effective utilization of this renewable resource as raw material for chemical reactions and feeds is the low susceptibility of lignocellulose to hydrolysis, which is attributable to the crystalline structure of cellulose fibrils surrounded by hemicellulose and the presence of the lignin seal which prevents penetration by degrading enzymes (Chahal *et al*, 1999).

Table 2.3 Types of lignocellulosic materials and their current uses

Lignocellulosic material	Residues	Competing Use
Grain harvesting Wheat, rice, oats, barley and corn	Straw, cobs, stalks and husks	Animal feed, burnt as fuel, compost, soil conditioner
Processed grains, corn, wheat, rice, and soybean	Waste water and bran	Animal feed
Fruits and Vegetables	Seeds, peels, husks, rejected fruits and juice	Animal and fish feed, some seeds for oil extraction
Sugar cane and other sugar products	Bagasse	Burnt as fuel Animal feed, fertilizer, burnt fuel
Oils and oil seeds, plant nuts,Cotton seeds, Olives, Soybean etc	Shells, husks, lint, fibre, sludge, presscake, waste water	Animal feed, fertilizer, burnt fuel
Animal waste	Manure, other waste	Animal feed, fertilizer, burnt fuel
Forestry- Paper and Pulp Harvesting of logs	Wood residuals, barks, leaves.Etc	Soil conditioners, burnt
Saw and plywood waste	Wood chips, wood shavings, saw dust	Pulp and paper industries, chip and fibre board

Pulp and paper mills	Fibre waste, sulphite liquor	Reused in pulp and board
Lignocellulose waste from communities	Old newspapers, paper, old boards, disused furniture	Small percentage recycled, others burnt
Grass	Utilized grass	Burnt

Source: Howard *et al* 2003

Table 2. 4 Lignocellulose contents of common agricultural residues and wastes

Lignocellulose material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shell	25-30	25-30	30-40
Corn cobs	45	35	15
Paper	85-99	0	0-15
Rice straw	30	50	15
Wheat straw	32.1	24	18
Sorted refuse	60	20	20
Leaves	15-20	80-85	0
Cotton seed hairs	80-95	5-20	0
Newspaper	40-55	25-40	18-30
Waste paper from chemical pulp	60-70	10-20	5-10
Primary waste water solids	8-15	NA	24-29
Fresh bagasse	33.4	30	18.9
Swine waste	1.6-4.7	28	N.A
Solid cattle manure	25	1.4-3.3	2.7-5.7
Coastal Bermuda grass	45	31.4	6.4
Switch grass	32	40	12.0
Orchard grass	25-40	25-50	4.7

SOURCE: Betts *et al* 1991

2.3 Nature of lignocellulosic materials

2.3.1 Chemical composition

The chemical composition of plants differ considerably and is influenced by genetic and environmental factors (Breen and Singleton, 1999). Cellulose, hemicellulose, and lignin are the main constituents of lignocellulosic materials (Deobald and Crawford, 1997). Apart from these primary polymers, plants comprise other structural polymers (e.g. waxes, proteins) (Leonowicz *et al*., 1999). The exact formulation of lignocelluloses is not known because it can be formulated from the breakdown products of various chemical treatments (Brownm, 1983). In addition, it varies from source to source both in terms of its chemical constituent and their relative ratios (Brownm, 1983).

2.3.2 Tertiary architecture of lignocellulose

The tertiary architecture of lignocellulose structures is directed by a variety of covalent and non-covalent linkages between the various constituents (Eriksson, 1995). Cellulose is complexed with hemicellulose, lignin and other components, which complicates their hydrolysis – hence the comparison with reinforced concrete (Leonowicz *et al*.,1999; Tomme *et al*., 1995). Cellulose microfibrils are stabilized by intra- and intermolecular hydrogen bonds and surrounded by hemicellulosic polysaccharides (mannans and xylans) linked to cellulose by covalent and hydrogen bonds (Heredia *et al*., 1995). These covalent bonds are extremely resistant to chemical and biological hydrolysis (Leonowicz *et al*., 1999). On the other hand, amorphous regions within the cellulose crystalline structure have a heterogeneous composition characterized by a variety of different bonds (Eriksson, 1995).

2.4 Structure and properties of lignocellulosic components

2.4.1Cellulose

It was first isolated in 1834 by the French chemist Anselme Payen (1795–1871), who earlier had isolated the first enzyme. While studying different types of wood, Payen obtained a substance that he knew was not starch (glucose or sugar in its stored form), but which still could be broken down into its basic units of glucose just as starch can. He named this new substance "cellulose" because he had obtained it from the cell walls of plants (Brownm, 1983).

Structure of cellulose

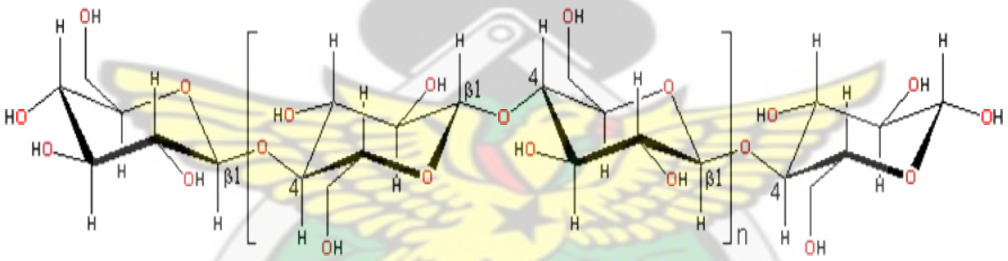


Fig 2.1 Structural units of cellulose (Brownm, 1983)

Cellulose (a polymeric β-glucoside) is the substance that makes up most of a plant's cell walls (Boxer, 1997). It is a polysaccharide consisting of tightly packed extended chains of D-glucopyranose molecules joined by β-1, 4 glycoside bonds and it is the most abundant of all polysaccharides (Schmid, 1996). Since it is made by all plants, it is also probably one of the most abundant organic compounds on Earth (Raven and Johnson, 1996). Since cellulose is the main building material out of which plants are made, and plants are the primary or first link in what is known as the food chain (which describes the feeding relationships of all living things), cellulose is a

very important substance (Raven and Johnson, 1996). In nature cellulose is usually associated with other polysaccharides such as xylan or lignin. Cellulose is a linear polysaccharide polymer with many glucose monosaccharide units (Raven and Johnson, 1996). The acetal linkage is beta which makes it different from starch. This peculiar difference in acetal linkages results in a major difference in digestibility in humans and other non ruminants (Boxer, 1997). Non-ruminants and humans are unable to digest cellulose because the appropriate enzymes to break down the beta acetal linkages are lacking (Boxer, 1997). Cellulose has been identified as the simplest of the polymers in the cellulosics, being composed of a continuous chain of D-glucose molecules linked in a ß1, 4 configuration (Brownm, 1983). These chains may contain more than 104 anhydroglucopyranose units giving a molecular mass of greater than 1.5Da (Brownm, 1983).

Cellulose micelles are bunched together to form thread-like microfibrils. The individual cellulose polymers are hydrogen bonded between the ring oxygen of glucose molecule and the hydroxyl groups at position 3 (Brownm, 1983).

The cellulosic fibrils are composed of highly ordered micelles possessing crystalline structure interspersed with disorderly areas of so-called amorphous cellulose (Brownm, 1983). Its density and complexity make it very resistant to hydrolysis without preliminary chemical or mechanical degradation or swelling (Thygesen *et al*, 2003). This is further supported by Puls and Schuseil (1993), who stated that cellulose cannot be enzymatically hydrolysed to glucose without a physical and chemical pre-treatment to break down the lignin and overcome the resistance of cellulose to hydrolytic cleavage due to its partly crystalline structure.

2.4.2 **Hemicellulose**

Hemicellulose is a particularly heterogeneous polymer in that it is composed variously of three hexoses, glucose, mannose and galactose and the two pentoses, xylose and arabinose together with their uronic acids (Brownm, 1983). Hemicellulose is more soluble than cellulose and is frequently branched with degree of polymerization (DP) of 100 to 200 (Kuhad *et al*., 1997; Leschine, 1995). Three well defined groups can be defined as xylans that have a basic backbone of poly ß 1, 4 xylan with additional side links to arabinose, glucuronic acid and galactans. The origin of lignocellulosic material defines the nature of the hemicelluloses (Brownm, 1983).

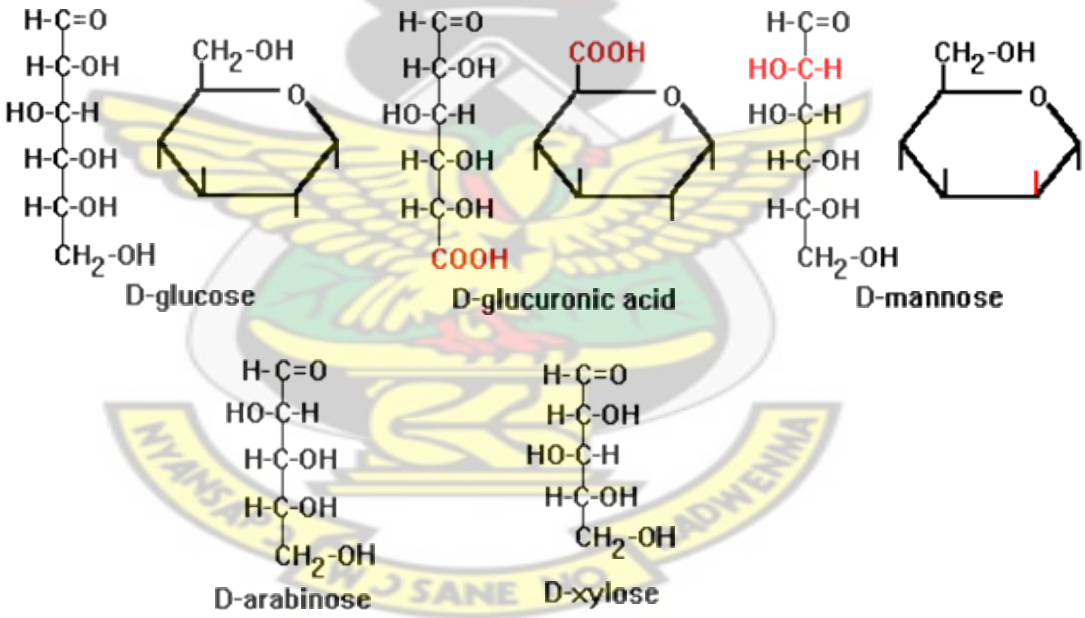


Fig 2.2 Some monomers of hemicellulose (Brownm, 1983).

Usually, all of the pentoses are present. There may even be small amounts of L-sugars. The hexoses as well as acids are formed by oxidation of sugars (Lebo *et al,* 2001). Mannose and mannuronic acid tend to be present, and there can be galactose

and galacturonic acid. The pentoses are also present in rings (not shown) that can be 5-membered or 6-membered. Xylose is always the sugar present in the largest amount (Raven and Johnson, 1996). In contrast to cellulose that is crystalline, strong and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. It is easily hydrolyzed by dilute acid or base (Lebo *et al,* 2001).

2.4.3 Lignin

Lignin is a complex chemical compound most commonly derived from wood and an integral part of the cell walls of plants (Lebo *et al,* 2001). It is a natural, complex, heterogeneous phenylpropanoid polymer comprising 25-30% of plant biomass (Arora *et al*.,2002) The term was introduced in 1819 by de Candolle and is derived from the Latin word "lignum", meaning wood (Boerjan *et al,* 2003).

It is one of the most abundant organic polymers on Earth, superseded only by cellulose, employing 30% of non-fossil organic carbon and constituting from a quarter to a third of the dry mass of wood (Boerjan *et al,* 2003). Lignin has several unusual properties as a biopolymer, not least being its heterogeneity in lacking a defined primary structure (Lebo *et al,* 2001). Lignin is three- dimensional polymer of phenylpropane unit (Lebo *et al,* 2001). It is a large, cross-linked, racemic macromolecule with molecular masses in excess of 10,000 atomic mass unit (Davin and Lewis, 2005). It is relatively hydrophobic and aromatic in nature (Hatakka, 2001). The degree of polymerisation in nature is difficult to measure, since it is fragmented during extraction and the molecule consists of various types of substructures which appear to repeat in a haphazard manner (Kawai and Ohashi 1993). Different types of lignin have been described depending on the means of isolation (Davin and Lewis, 2005). The true formulation of lignin in the natural state

can only be deduced from the theoretical reconstruction of the various degradation products (Brownm, 1983). There are three monmers that make up almost all lignin found in nature. They are biosynthesized in plants via the shikimic acid pathway and their structures and names are shown in fig 2.3.

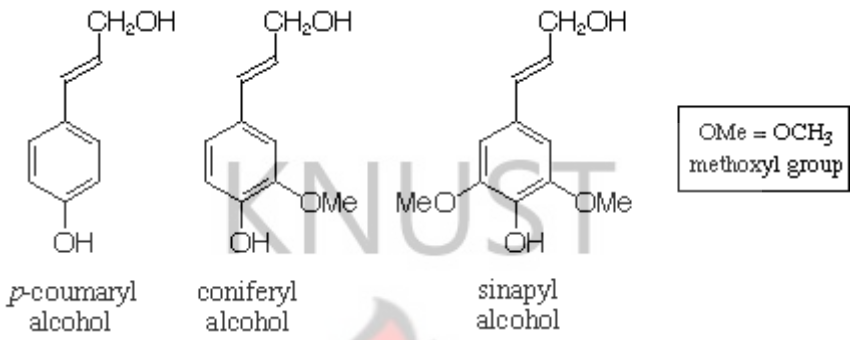


Fig 2.3 Structures and names of monomer units of lignin (Kawai and Ohashi 1993).

p-Coumaryl alcohol is a minor component of grass and forage type lignins (Davin and Lewis, 2005). Coniferyl alcohol is the predominant lignin monomer found in softwoods (Lebo *et al*., 2002). Both coniferyl and sinapyl alcohols are the building blocks of hardwood lignin (Davin and Lewis, 2005).

2.5. Lignocellulose biodegradation

Lignocellulose is a complex substrate and its biodegradation is not dependent on environmental conditions alone, but also the degradative capacity of the microbial population (Waldrop *et al*., 2000). The composition of the microbial community charged with lignocellulose biodegradation determines the rate and extent thereof (Tuor *et al*., 1995). The concerted actions of enzyme systems possessed by these microbial communities are required for complete hydrolysis and utilization of

lignocellulosic materials (Waldrop *et al*. 2000). Lignocellulose degradation is essentially a race between cellulose and lignin degradation (Reid 1989).

2.6. Lignocellulosic enzymes

2.6.1. Cellulases

Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze the cellulolysis (or hydrolysis) of cellulose.

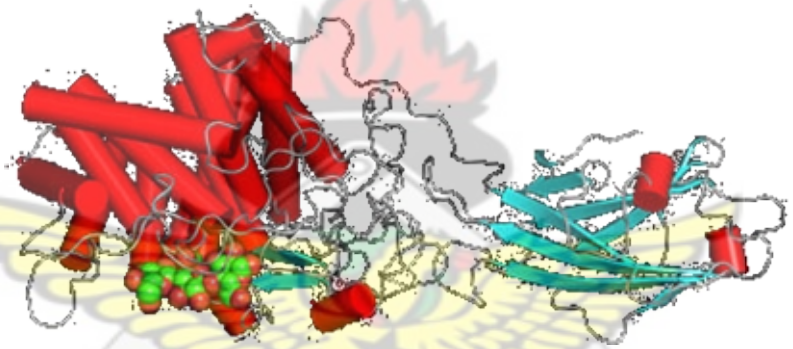


Figure 2.4 Model of cellulase enzyme (Chapin *et al*, 2002)

There are five general types of cellulases based on the type of reaction catalyzed namely: Endo-cellulase which breaks internal bonds to disrupt the crystalline structure of cellulose and expose individual cellulose polysaccharide chains, Exo-cellulase which cleaves 2-4 units from the ends of the exposed chains produced by endocellulase, resulting in disaccharides such as cellobiose (Deobald and Crawford, 1997). There are two main types of exo-cellulases (or cellobiohydrolases, abbreviated CBH) - one type working progressively from the reducing end, and one type working progressively from the non-reducing end of cellulose (Tomme, *et al*., 1995).

Cellobiase or beta-glucosidase, the third cellulase hydrolyses the exo-cellulase product into individual monosaccharides (Tomme, *et al*., 1995). Oxidative cellulases depolymerize cellulose by radical reactions, as for instance cellobiose dehydrogenase (acceptor). The last group of cellulase, cellulose phosphorylases work by depolymerizing cellulose using phosphates instead of water (Deobald and Crawford, 1997).

Mechanism of cellulolysis

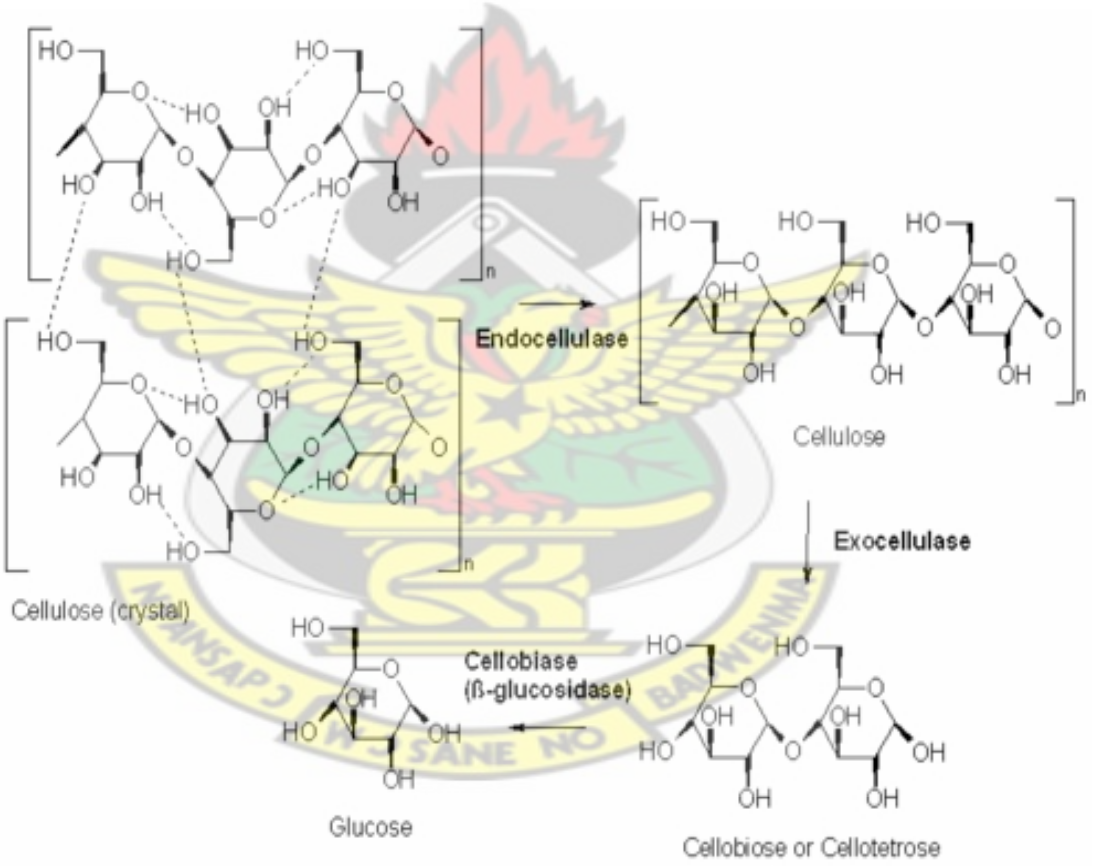


Figure 2.5: Mechanism of cellulolysis

Source: http://www.nationmaster.com/encyclopedia/hemicellulase

The three types of reaction catalyzed by cellulases: breakage of the non-covalent interactions present in the crystalline structure of cellulose (endo-cellulase),

hydrolysis of the individual cellulose fibers to break it into smaller sugars (exo-cellulase) hydrolysis of disaccharides and tetrasaccharides into glucose (beta-glucosidase) (Deobald and Crawford, 1997).

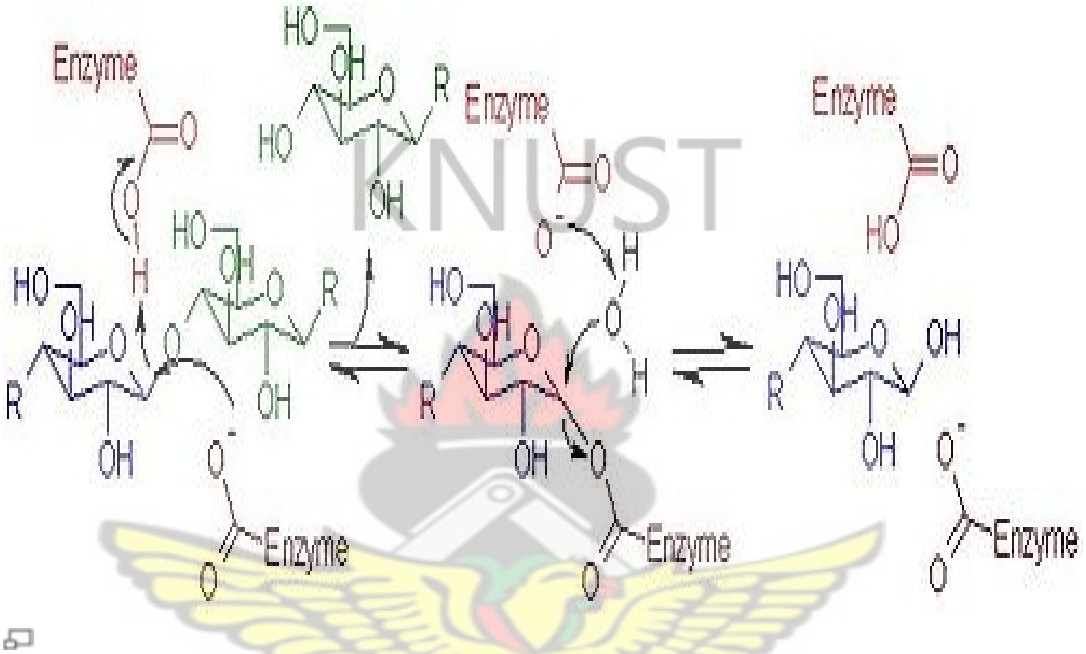


Figure 2.6: Mechanistic details of beta-glucosidase activity of cellulase

Source: http://www.nationmaster.com/encyclopedia/hemicellulase

Although similar types of enzymes are required for hemicellulose hydrolysis, more enzymes are required for its complete degradation because of its greater complexity compared to cellulose. Of these, xylanase is the best studied (Kuhad *et al*. 1997).

2.6.2. Laccase

Laccase is an enzyme which is able to degrade a lignin-cellulose complex (Kawai and Ohashi 1993). Laccase (EC 1.10.3.2, p-diphenol oxidase) is an extracellular blue oxidase capable of oxidizing phenols and aromatic amines by reducing molecular oxygen to water by a multicopper system (Thurston, 1994). Laccase occurs in certain plants and bacteria, but the enzyme is particulary abundant in

white-rot fungi and it is assumed to comprise a lignin biodegradable complex (Abdulkareem., *et al*, 2002). It degrades lignocellulosic materials by a simultaneous attack of lignin and cellulose / hemicellulose or selectively degrades lignin (Kuhad *et al*., 1997; Eriksson, *et al*, 1990). Laccase seems to be one of the most important enzymes in lignin degradation (Kawai and Ohashi 1993) since it can attack polymeric lignin, degrade the framework structure loosely, introduce additional hydrophilic groups, and produce water-soluble material (Iimura *et al*., 1991). In the presence of suitable redox mediators (e.g. 1-hydroxybenzotriazole), laccase is even able to oxidize recalcitrant non-phenolic lignin units (Li, *et al*., 1999). This capability has generally extended their use to a series of biotechnological applications, all of them related to the degradation of structurally diverse aromatic compounds (Kawai and Ohashi 1993).

2.6.3. Phenol oxidase

Lignin degradation by white-rot fungi is an oxidative process and phenol oxidases are the key enzymes (Kuhad *et al*., 1997; Leonowicz *et al*., 1999). Of these, lignin peroxidases (LiP) and manganese peroxidases (MnP) from especially white rot fungi (*P. chrysosporium, Pleurotus ostreatus* and *Trametes versicolor*) have been best studied (Borneman *et al*., 1990). LiP and MnP oxidize the substrate by two consecutive one-electron oxidation steps with intermediate cation radical formation (Borneman *et al*., 1990). Other enzymes that participate in the lignin degradation processes are H_2O_2-producing enzymes and oxido-reductases, which can be located either intra- or extracellularly (Kuhad *et al*., 1997). Bacterial and fungal feruloyl and p-coumaroyl esterases are relatively novel enzymes capable of releasing feruloyl and p-coumaroyl esters and play an important role in biodegradation of recalcitrant cell

walls in grasses (Kuhad *et al*., 1997). These enzymes act synergistically with xylanases to disrupt the hemicellulose-lignin association, without mineralization of the lignin per se (Borneman *et al*., 1990).

2.7 Feed preparation methods

2.7.1 Conventional animal feed production

Animals are fed on two main classes of food i.e. roughages and concentrates. The roughages consist of herbs and grasses whiles the concentrates are made up of the grains, cereals, legumes etc. During processing, these are taken through a host of procedures aimed at improving the nutritive quality, neutralizing toxic factors and improving the storage life. Some of the processes involved in storage life improvement include, hay making, silage and artificial drying. Examples of feed additives are vitamins, minerals, enzymes, hormones, probiotics and antibiotics. Antibiotics are the most commonly used feed additives in the poultry industry. Examples of antibiotics used as feed supplements in poultry, pigs and ruminants are chlorotetracycline, penicillin, erythromycin, neomycin and streptomycine (Prescott *et al*., 1999). The use and abuse of these antibiotics may lead to antibiotics resistance.

2.7.2 Chemical and Biological treatments of feed

According to Taniguchi *et al*, (2005), a number of different methods are available for the treatment of lignocellulosic materials. Chemical treatment of agricultural wastes is by far the most widely used when compared to physical and biological treatments with the aim of improving the nutritive value (Annan, 1998). Treatments

such as steam explosion, steam treatment with sulfuric acid or sulfur dioxide, alkali and biological treatment with white rot –fungi have been extensively investigated. Previously subcritical and supercritical water treatments have been of great interest because of their potential to induce direct conversion of biomass resources into simple and soluble sugar and gas without any additional conversion steps (Taniguchi *et al*., 2005). However, besides biological treatment, most treatment processes require expensive special instruments that have substantial energy requirements depending on the complexity of the process (Taniguchi *et al*., 2005). Chemical treatments have serious disadvantage in terms of the requirement for specialized corrosion-resistant equipment, extensive washing and proper disposal of chemical waste (Taniguchi *et al*., 2005). In developing country like Ghana, chemical treatment of crop residues cannot be afforded by farmers mainly because of cost and lack of skilled personnel to handle chemical and equipment (Annan, 1998). Biological treatment was previously reported as a safe and environmentally friendly method for lignin removal from lignocellulose (Taniguchi *et al.,* 2005). The most promising microorganisms for biological treatment are white-rot fungi that belong to the class Basidiomycota (Plat and Hadar, 1983).

2.7.3 Biodegradation

Biodegradation can be described as a process in which substrates are decomposed by known mono or mixed cultures of microorganisms under controlled environmental conditions with the aim of producing high quality products (Aderemi and Nworgu, 2007).

Enzymes from microorganisms, especially fungi have been indicated to be promising in degrading structural carbohydrates such as cellulose and hemicellulose

and in degrading or structurally modifying proteins and their nutritional properties (Rai *et al*., 1998). Biodegradation of lignocellulosic materials can be achieved through solid state fermentation with mushrooms.

With the advent of biotechnology, attempts have been made globally to make potential use of agro-industrial residues for added value by production of enzymes, organic acids, bioactive secondary metabolites and single cell protein through biodegradation (Pandey *et al*., 1999).

Solid state- fermentation (cultivation) is promising in this regard (Soccol and Krieger, 1998; Pandey *et al*., 2000). Lignocellulosic wastes can be degraded by mushroom-producing white-rot Basidiomycetes (Suki, 2000). White rot fungi are capable of selectively degrading lignin, thereby upgrading it (Hadar *et al*., 1992). These fungi gain nourishment from the cell wall structural polymers of lignocelluloses and produce edible mushrooms (Suki, 2000).

According to Smith (1993), mushroom cultivation represents the only current economically profitable biotechnology process for the biodegradation of plant residues (lignocelluloses) from forestry and agriculture. It is also the only microbial system that can bio-convert all of the major plant polymers; lignin, cellulose and hemicellulose (Wood and Smith, 1987).

Other microbial treatment systems, natural or man-made, utilizing lignocelluloses, such as waste digesters do not utilize lignin (Wood and Smith, 1987). Mushroom cultivation exploits the natural ability of fungi to bio-convert solid waste generated by industry and agriculture into food and other products (Chiu *et al*., 2000).

The use of fungi for the biodegradation of lignocellulose into food and feed rich in protein offers an alternative for developing unconventional source of proteins as food or feed (Vijay *et al*., 2007). Production of yeast and algal proteins require sophisticated techniques and heavy inputs whereas the beauty of mushroom 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 and animal feed (Vijay *et al*., 2007).

Pleurotus species are considered to be one of the most efficient producers of food protein (Ogundana and Okogbo, 1981). *Pleurotus* species grow fast; they degrade a variety of lignin as well as hemicellulose and cellulose in lignocellulosic wastes (Suki, 2000). By the activity of the enzyme systems in *Pleurotus ostreatus*, namely laccase, cellulase, celobiase, hemicellulase enzymes and others, the lignocellulose complex is decomposed into simpler organic compounds which the mushrooms use as nutritious substances (Adamovic *et al*., 2004).

Thus the chemical composition of substrata changes in comparison with the initial material and therefore the substrate is enriched with protein and the non- starchy polysaccharides are bio- converted into simple forms that can be utilized by poultry and other non- ruminants such as pigs. Through this biodegradation process, cheaper but quality and nutritious animal feed is made available whiles eliminating the problem of agricultural waste pollution.

2.8. Cassava in the Livestock Feed Industry

In Africa, most livestock animals are free range and under these conditions inadequate feeding is the main cause of poor productivity (Oke, 1992). Even for

ruminants that graze on herbage, concentrate feeds are required to optimize their productivity (Oke, 1992). In Ghana, livestock production lags behind crop production and this has lead to an inadequate supply of meat and livestock products for human consumption (Annan, 1998). In the animal feed market, cereals constitute between 40 to 55% of the bulk in standard feed rations and the availability of cereal grains has dominated progress in the livestock industry (Oke, 1992).The high demand for cereals by increasing human population and their use by millers for compounding livestock feed coupled with the need for livestock products have led to the use of unconventional feeds for animal production (Oke, 1992). These unconventional feed materials include sorghum spent grains and wheat offal (by products of sorghum and wheat malting respectively) as well as cassava peels (Oke, 1992).Cassava or tapioca meal has been extensively used as a replacement ingredient to corn (Damisa *et al*., 2007). It is widely recognized as a cheap source of food energy on the basis of some agronomic and economic advantages which the crop enjoys over grain crops (Adebowale, 1981). These include high dry matter and energy yields/hectare, low production cost and relatively low susceptibility to insect and pest attacks (Adebowale, 1981).

Given the current socioeconomic situation, prospects for improvements in the livestock productivity appear bleak due to the keen competition for cereals as human food, animal feed milling, and other industries such as brewing. Cassava is an ideal crop to provide bulk in animal feed, as its available production can easily be increased, and the whole plant has an attractive nutrients composition (Table 2. 5).

Table 2.5 Proximate compositions of cassava storage roots

Constituents	Peel	Storage roots
Dry Matter (%)	27.3-33.5 (29.6)	13.0-43.3(30.8)
Crude Protein (%)	2.8-6.5 (4.9)	1.5-3.5 (2.3)
Crude Fibre (%)	10.0-22.0 (16.6)	1.3-77.0 (3.4)
Ether Extract (%)	0.5-2.2 (1.3)	0.8-3.2 (1.4)
NFE (%)	62.5-72.9 (68.5)	88.0-94.1 (88.9)
Ash (%)	3.5-10.4 (5.9)	1.6-4.1 (2.5)

Source: Smith, 1992, NFE: nitrogen free extract, values in brackets are means.

Although the roots are deficient in protein, it has been demonstrated that for various livestock species including poultry, cattle, and pigs, cassava roots and peels can replace maize provided adequate supplementation of protein and micronutrients are ensured (Oke, 1992). About 3.5 million metric tones of cassava is produced annually in Ghana (FAO, 1990) and with an extraction rate of 20% (Kossila, 1984; Hahn and Chukwuma, 1986) about 665,400 metric tones of cassava peels are estimated to be produced annually in Ghana (Annan, 1998). These peels have virtually no other use apart from being fed to livestock (Annan, 1998).

2.9. The major limitation of cassava in livestock feeding

The main limitation of cassava in livestock feeding include, the cost of protein supplementation of cassava based rations, variation in quality of dried products in terms of contaminant, microbial proliferation, the dustiness of the dried cassava flour, which not only limits intake in stock, but also constitutes a problem in the milling industry and the cyanide content (Oke, 1992).

2.10. Cyanogenic glucosides in cassava

The ability to produce HCN (hydrogen cyanide) exists in more than 2,000 plant species grouped in 110 families, including ferns, gymnosperms and angiosperms and cassava is one of such plants. Free cyanide is usually not produced in the plant (Bokanga, 1993) but rather the plant accumulates cyanogenic glucosides (and cyanogenic lipids in some species). The cyanogenic glucosides of cassava, linamarin and lotaustralin, usually found in the ratio of 10 to 1, are synthesized from the amino acids valine and isoleucine, respectively (Bokanga, 1995).

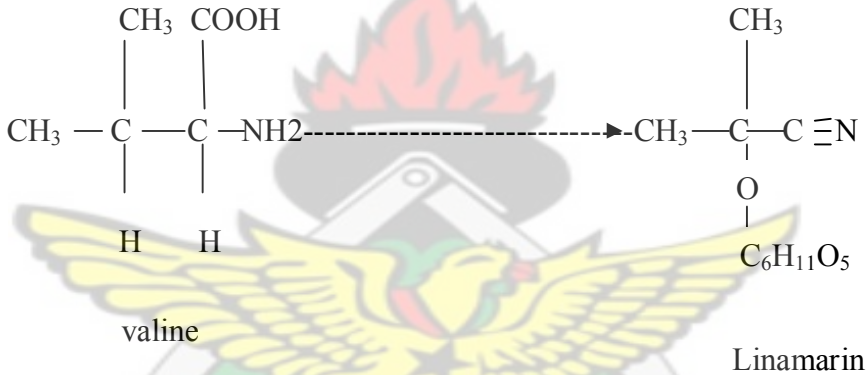
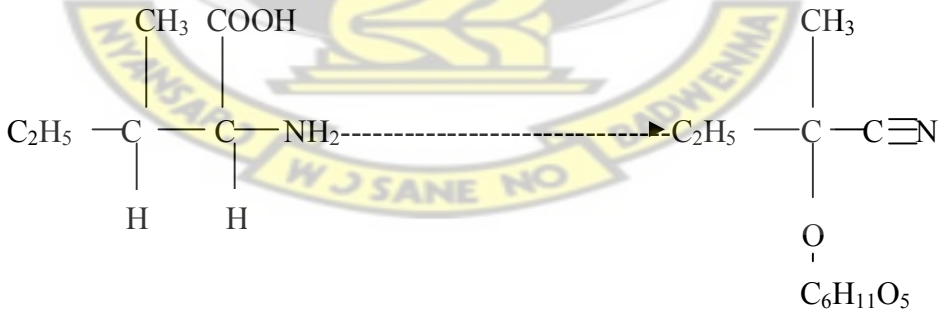


Fig 2.7: Linamarin and its biosynthetic precursor valine



Lotaustralin

Fig 2.8: Lotaustralin and its biosynthetic precursor isoleucine.

Linamarin and lotaustralin are stored inside vacuoles in the cytoplasm, while the enzyme linamarase, a β-glucosidase that can hydrolyze them, is located on the cell

wall. Generally in intact plant cells, the enzyme and the cyanogenic glucosides do not get into contact (Bokanga, 1995). When plant tissues are crushed (such as pounding the leaves or mashing the roots), the plant cell structure may be damaged to such extent that the enzyme comes into contact with the cyanogenic glucosides (Bokanga, 1993). The action of linamarase on linamarin and lotaustralin is the hydrolytic release of acetone cyanohydrins and 2-butanone cyanohydrin respectively (Bokanga, 1993). These products are relatively unstable at pH above 5 and therefore spontaneously decompose to their corresponding ketone and HCN. The two cyanogenic glycosides, linamarin and lotastraulin, are also know to be present in cassava peels with the former being present in much larger quantities, usually up to 90% for the total cyanogens (Aryee, 2002).

The high HCN content of peels of some varieties of cassava has made some researchers to suggest limits to which cassava peels can be included in diets of livestock (Annan, 1998). The release of HCN from the cyanogenic glycosides is known to trigger several toxic manifestations. Solomonson (1981) studied the inhibitory properties of cyanide on metabolism and concluded that cyanide can inhibit tissue respiration in animals. It is also known that, the lethal effects of cyanide result from impairment of the mitochondrial enzyme, cytochrome oxidase (Bell *et al*., 1976) and this inhibition may lead to a lot of health related problems (Jones, 1959).

2.11. Fermentation and detoxification of cyanide in cassava peels

Fermentation has been found to reduce the cyanide content drastically beyond toxic levels (Bokanga *et al*, 1993). Bokanga *et al*, 1990, found submerged fermentation to be the most efficient process for reducing the levels of cyanogens in cassava;

reduction rates of 95-100%. The removal of cyanogens from cassava during fermentation is the result of several factors.

The textural changes in the plant tissues that make it possible for vacuole-bound cyanogenic glucosides to diffuse and come into contact with the membrane-bound linamarase and for hydrolyzed and intact compounds to leach out accounts partly for the reduction in the cyanide. Also the increase in β-glucosidase activity in the cassava tissue during fermentation and the utilization of cyanogenic glucosides and their break down products by fermentation microorganisms contribute to the reduction of the cyanogens during fermentation (Bokanga, 1993; Balagopalan *et al*., 1988)

Other processes that are carried out before the fermentation process such as drying and pasteurization can also lead to a reduction of the cyanide content of cassava peels through volatilization since the boiling temperature of HCN (25.7^{o}C) is far below the pasteurization temperature of 100^{o}C.

2.12. Anti-nutritional factors in cassava peels

The presence of tannins, polyphenolic polymers that exert anti-nutritional effects (Kamalak *et al*., 2004; Seresinhe and Iben, 2003; Rittner and Reed, 1992) in by-products of tanniferous plants, is a major limitation to their use as feed ingredients. Several factors contribute to the anti-nutritional effects of condensed tannins. First, the binding of the polyphenolic compounds to cell wall polysaccharides reduces the digestibility of the latter in ruminants (Schofield *et al*., 2001; Kumar and D'Mello, 1995).

Furthermore, soluble tannins can 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; Quesada *et al*., 1995; Petersen and Hill, 1991). In addition, condensed tannins impart an astringent taste and depress feed intake and use by animals (Brooker *et al*., 1994), leading to growth depression. Tannin is known to be present in cassava and these tannins make it harder to digest proteins and to absorb iron and methionnine. Thus organisms eating large amounts of cassava may fail to absorb exactly the amino acids they need to protect themselves against its toxic effects. Therefore, 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, particularly cassava peels, with *Pleurotus ostreatus* fermentation is an effective means of reducing tannin content and upgrading polysaccharide digestibility.

2.13. The need for bioprocessing of cassava peels

Fermentation is one of the oldest applied biotechnologies, having been used in food processing and preservation as well as beverages production for over 6,000 years (Motarjemi, 2002). The fermentation process of staples serves as a means of providing a major source of nourishment for large rural populations and contributes significantly to food security by increasing the range of raw materials which can be used in the production of edible products (Adewusi *et al*., 1999).

Fermentation enhances the nutrient content of foods through the biosynthesis of vitamins, essential amino acids and proteins, by improving protein quality and fibre

digestibility. It also enhances micronutrient bioavailability and aids in degrading antinutritional factors (Achinewhu *et al*., 1998).

Two important biological wastes that may cause damage to the environment are generated during the traditional processing of cassava roots for gari production in Africa, namely, the cassava peels and the liquid squeezed out of the fermented parenchyma mash (Oboh, 2006).

Cassava peels derived from gari processing are normally discarded as wastes and allowed to rot in the open, thus resulting in health hazards. As a rough estimate, about 10 million tonnes of cassava are processed into gari annually in Nigeria alone (Ogundana and Okogbo, 1981). Since these peels could make up to 10% of the wet weight of the roots, they constitute an important potential resource for animal feeds if properly processed by a bioconversion-system (Antai and Mbongo, 1994).

Cassava peels is the skin of the peeled cassava and the peels and sieviate which are by- products of harvesting and processing constitute 25% of the whole plant (Aderemi and Nworgu, 2007). In harnessing these products as poultry feed ingredients, it has been discovered that they are high in fibre hence this limits their utilization (Aderemi and Nworgu, 2007). Cassava peels for instance contain high amount of non-starch polysaccharides mostly non- digestible carbohydrates such as cellulose and hemicellulose which have a high water holding capacity (Aderemi and Nworgu, 2007). This was observed to be poorly digested and bio- utilized by laying birds which resulted in depressed weight gain and reduced egg production (Aderemi *et al*., 2004). The digestibility of a feed for both ruminant and non-ruminant tends to decrease with crude fibre content (Aderemi and Nworgu, 2007). Typically, a 1%

increase in crude fibre content brings a 1% decrease in digestibility for ruminant and a 2% decrease for pigs (Aderolu *et al*., 2002).

Cassava peels are known to contain high levels of carbohydrate and very low levels of protein. In their use as animal feed they have contributed mainly to the supply of energy for their life processes, but this does not result in much physical growth because of the significant absence of proteins and high fibre content (Aderolu *et al*., 2002). One of the ways to improve its protein content is through the use of *Pleurotus ostreatus* fermentation. The increase in protein is as a result of the growth of the mycelia of the mushroom on the substrate, as the mushroom is known to be rich in protein (Taniguchi, 2005). *Pleurotus* spp. has the potential to convert cheap cellulosics into valuable protein at a low cost (Vijay *et al*., 2007).

Pleurotus ostreatus also degrades the hemicellulose, cellulose and the lignin compounds present in the peels (Zadrazil and Isikhuemhen, 1997). Once they have been degraded, they produce simple forms of the compounds and these can be easily ingested by the animal. Apart from the increased amino acid content, digestibility of the peels is also improved and the animals are able to digest the feed properly due to the bioprocess (Aderemi and Tewe, 2004). *Pleurotus ostreatus* is known to possess certain enzymes in the form of enzyme systems which are involved in the bioprocessing (Rai *et al*., 1998).

2.14. History of *Pleurotus species*

Pleurotus is a globally distributed wood inhabiting ligninolytic white-rot wood decay genus, belonging to the *Pleurotaceae*, within the *Agaricales* s.l (Suki, 2000). This classification is supported by the most current molecular systematic studies (Thorn *et al*., 2000). *Pleurotus* species are saprotrophic on hardwoods, particularly beechwood (Suki, 2000). These fungi are also commonly found in forests and woodland, where they grow on fallen branches, dead tree stumps, and on felled logs, forming basidioma that are fan-shaped, whitish, spore-bearing gills on the underside of the cap (Suki, 2000). Some of the mushrooms of the genus *Pleurotus* are; *P. ostreatus*, *P. florida*, *P. cornucopiae*., P *.djamor (P.salmoneostramineus*), *P. ostreatus var florida*, P *.sajor-caju*, *P. abalones*, *P. tuberregium*, *P. cystidiosus*, P *.eryngii* ,*P. ostreatus var colombinus* etc.

2.14.1. Cultivation of *Pleurotus ostreatus*

Pleurotus ostreatus which was initially cultivated by the Asians is now grown all over the world and is known to predominantly thrive in temperate countries such as Germany, U.S.A etc (Baldrian & Gabriel 2003). The oyster mushroom (*Pleurotus ostreatus*) appears to be the best candidates for production in the West African climate (Bailey *et al*., 2009). It is known to be one of the most cultivated mushrooms in Ghana (Achio, 2009). In Ghana, mushrooms grow widely and also intensively cultivated because they are a rich source of protein (Obubuafo, 2009).

P. ostreatus has been cultivated on dead tree logs to a certain extent and the attention has been shifted to the use of agricultural and industrial waste products

such as cereal straws, cotton waste, saw dust, food waste, corn cobs, rice straw, wood chips, paper, cocoa pod husk, cassava peels, coffee pulp, barley, wheat straw etc (Quimio, 1986). The oyster mushroom can actually grow on almost any substrate that was once alive (Bailey *et al*., 2009).

Due to several reasons, cultivation of *P. ostreatus* alongside other traditional mushrooms has been encouraged extensively in developing countries mainly because:

research into the medicinal value of mushrooms has shown that *P. ostreatus* and other types of mushrooms have antagonistic effect on tumour formation and also lowers blood cholesterol levels (Gunde and Cimerman, 1995).

Large scale cultivation of *P. ostreatus* in developing countries is very essential since meat protein is an expensive commodity. This is because cultivation does not require fertilizer, pesticides and sophisticated farming equipment (Oei, 1991). In developing countries like Ghana, where majority of the populace are farmers, tons of agricultural wastes are burnt, discarded and neglected. Due to this, the ability of *P. ostreatus* to convert these agricultural wastes into enriched animal feed is therefore very useful in the prevention of environmental pollution.

Over the years various publications have made the mushroom to be widely accepted and introduced into various countries in the world, especially in the tropics. In Ghana, mushroom is now being cultivated with *Pleurotus ostreatus* being one of the commonly grown species, (Sawyer, 1994). Factors that affect growth of *P. ostreatus* include temperature, light, climate, moisture and carbon dioxide (Oei, 1991).

Most of the 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 (Vijay *et al*., 2007).

Mushrooms get nutrition from cellulose, hemicellulose and lignin, which are abundantly available in cereal and other carbohydrates (Vijay *et al*., 2007).

Pleurotus species are fast colonizers that degrade a wide variety of lignin in different wood wastes (Suki, 2000). They produce extracellular enzymes (lignin peroxidases, manganese peroxidases, and laccase) that can modify and degrade lignin (Suki, 2000).

As a result of fungal ligninolytic action, the spent wood waste substrates can become a source of available hemicellulose and cellulose that can be used as carbohydrate sources for animal feed (Oei, 1996) or fertilizers (Youri, 2003). *Pleurotus ostreatus* is therefore known as a lignocellulosic organism (Zadrazil, 1978).

It also belongs to the group of white-rot fungi that, upon induction, produce lignin and cellulose degrading extra-cellular enzymes (Zandrazil *et al*., 1990). The fungus generates laccase and manganese peroxidase to degrade lignin. Degradation of cellulose is carried out by endo and exoglucosidases and xylanases which are produced and exported from *P. ostreatus* into the medium in significant quantities (Baldrian & Gabriel 2003, Baldrian, *et al*., 2005).

P. ostreatus is also known to be very rich in minerals, carbohydrates, crude fibres and B vitamins in varying concentrations (Gunde and Cimerman, 1995).

2.15. *In vitro* Digestibility studies

The evaluation of quality of feeds is important for the prediction of animal performance (Tatli and Cerci, 2006) and the productive potential of animals has increased spectacularly during the past decades, due to the genetic progress, which also triggered changes in the nutritional management (Cone *et al*., 1996). Thus there

is a general trend to increase the energy level of the ruminant diets in order to reach their productive potential (Trabalza *et al*., 1992). The energy value of feeds can be determined by enzymatic methods which do not require rumen fluid (Tatli and Cerci, 2006). *In vitro* digestibility studies employ enzymatic action on feed outside the body in a test tube to evaluate the utilization of the feed by livestock since *in vivo* measurements are expensive and accompanied by difficulties associated with the procedures (Tatli and Cerci, 2006). This is therefore done to ascertain the ease of digestion of feed by the enzyme system possessed by the organism outside its body as a means of comparism with the *in vivo* digestion. *In vitro* digestibility methods (Tilley and Terry, 1963; Brown *et al*., 2002) usually predict *in vivo* digestibility with a lower error than any chemical method.

Higher dietary energy levels are achieved by increasing the digestibility and the concentrate feeds to bulk forage ratio (Cone *et al*., 1996). Next to the level of ingestion, digestibility is a particularly important factor in determining the nutritive value of forages (Piva *et al*., 1995). Although proximate analysis is beneficial, studies on composition must be more extensive and must include quantitation of readily available nutrients such as sugars and starch (Chang *et al*., 1998). This is vital for the formulation of non-ruminant feedstuffs since non-ruminants do not harbour cellulolytic microorganisms and, therefore depend on non- structural carbohydrates as a major source of energy.

CHAPTER 3

3.1. MATERIALS AND METHODS

3.1.1. Experimental Design

The Randomized Complete Block Design (RCBD) was used to determine the effect of fermentation on both the proximate composition and the individual fibre components of the fermented cassava peels.

3.1.2 Materials

Sources of substrates: The cassava peels were obtained from some selected chop bars in Kumasi, Queens Hall canteen, Africa Hall canteen, both on KNUST campus and Twin City restaurant at Ayeduasi.

Drying equipment- Solar dryers

Location of solar dryers: Former Forestry Research Institute of Ghana (FORIG)

Pasteurization unit- A barrel with a stand, inner seater, middle-holed lid and re-enforcement hoop. Specifially constructed by workers of the Ghana Association of Garages, Suame Magazine, a suburb of Kumasi. Aluminium trays used for this project were obtained from Central market, Kumasi and jute sacks (108cm by 65cm) were obtained from the Biochemistry Department.

The mushroom spawn

The mushroom spawn was obtained from Rub Art Farms, a mushroom production farm at Kenyasi in Kumasi.

Plate: 3. 1 Mushroom spawn

Substrate pre-treatment

The cassava peels were washed and dried in a solar dryer for 4 weeks to a moisture content of about 2.84%. This was done to ensure that some of the indigenous microbes in the substrate are eliminated by the drying temperature. The dried cassava peels were broken up by pounding in a mortar to reduce the size to an average area of about 0.6cm^2 to allow best mycelia growth and proliferation.

Plate 3. 2 Drying of substrate in a solar dryer

Plate 3.3 Size reduction of substrate

3.1.3. Composting

During composting, the substrates were sprinkled with water until they attained 65-70% moisture content. At this water level, if a handful of the wetted substrate is taken and squeezed, no water droplets fall out as a stream. This principle was used to check to attain this moisture content in the substrate as this moisture content has been found to be suitable moisture content for the growth and proliferation of the mycelia of the oyster mushroom. The substrates were then heaped under plastic sheet to a height of 1.5m and covered with a plastic sheet to protect them from the direct effect of sun or rains. They were then left for four days to allow the internally generated heat to kill some of the indigenous microbes in the substrate. The outside of the heap was relatively cool and the substrates were turned every two days to ensure equal distribution of water and heat throughout. This also ensured uniform degradation and replenishing of oxygen. At the end of composting, the substrates

were then transferred into aluminium trays and covered tightly with aluminium foil.

3.1.4. Pasteurization

The aluminium trays were packed on a wooden rack seater placed at the bottom of metal drum steamer containing water to a height of 15cm such that the level of the water will be just below the bottom of the seater. The drum steamer was placed on fire. The time when vapour was found coming through a hole in the middle of the lid of the drum was noted and from this time the heating was allowed to go on for 10 hours. This was done in order to ensure maximum pasteurization time for almost all of the indigenous microorganisms in the substrate to be eliminated before inoculation with the spawn of the mushroom is carried out.

Plate 3. 4: Pasteurization of substrate

3.1.5. Inoculation

After cooling the pasteurized substrates, the spawn bottles were shaken to loosen the grains. The bottles were then cleaned from top to bottom using a piece of cotton soaked in alcohol and 60 grains of the spawn introduced onto the substrate in each tray. For each triplicate, one tray was not spawned and it served as a control experiment and also to indicate if the room still remained free from contamination otherwise the necessary correction was applied.

Plate 3.5 Inoculation room

Plate 3.6 Inoculation of pasteurized substrate

The following measures were taken in controlling contamination during inoculation.

Clean clothes were worn,

The trays were kept in the inoculation room and allowed to cool down without ventilation,

Inoculation was done on the next day to allow enough time for the pasteurized substrates to cool,

Spawning was done at night when the level of contamination in the air is usually low,

A flame was brought near the mouths of the spawn bottle and the trays to keep the environment more or less sterile.

3.1.6. Incubation

The spawn trays were kept in the incubation room and left undisturbed to allow the mycelia to grow through the substrate. Experiments were carried out in triplicate and fermentation was allowed to run up to 8 weeks since the optimum period for the

process was not yet known.

At the end of each week, the fermentation process was stopped by drying the substrates in the solar dryer for at least five days depending on the prevailing weather conditions. The dried substrates were then milled to uniform mixture and kept in the solar dryer for chemical analysis.

Plate 3.7 Incubation stage

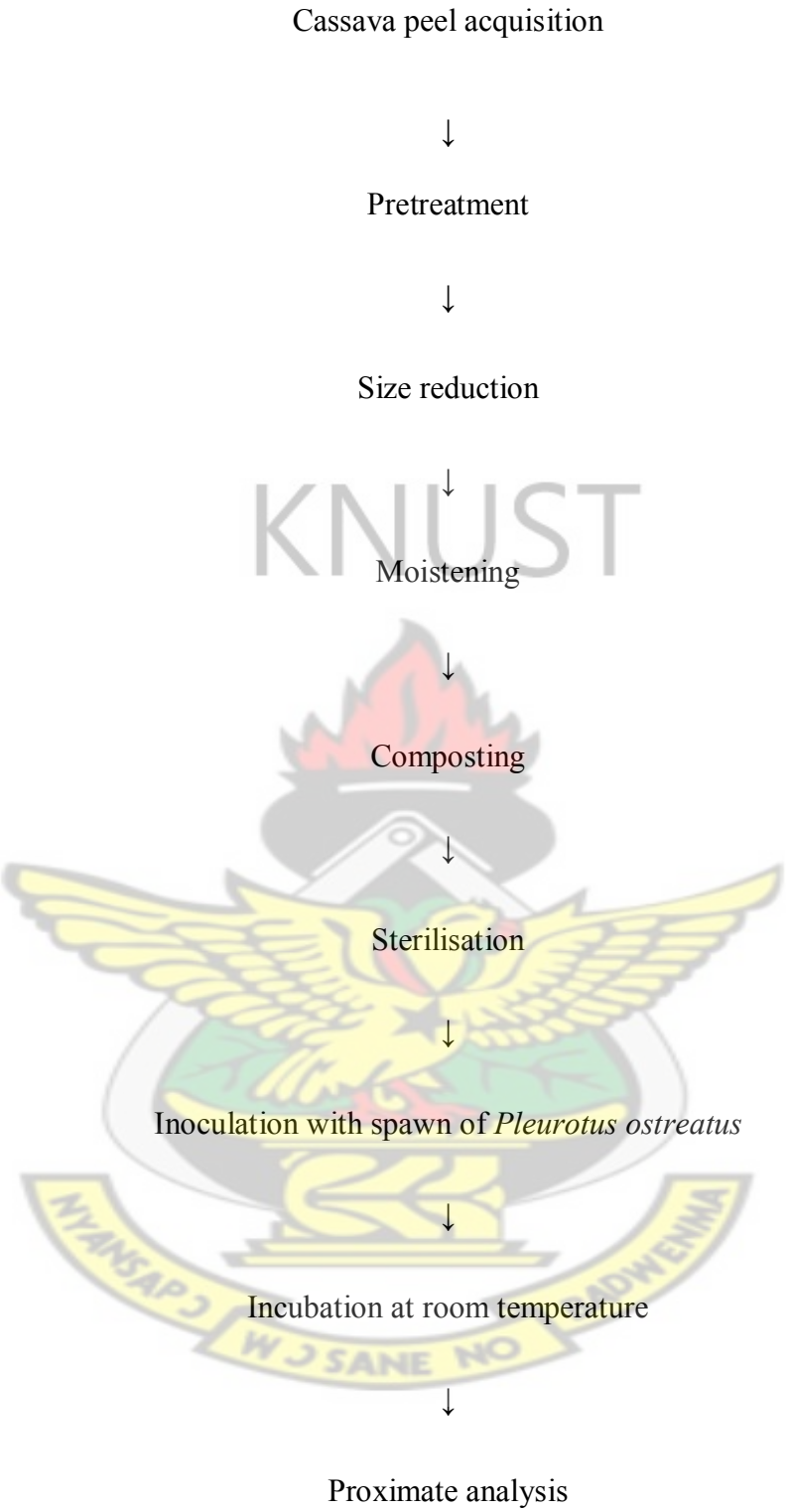


Fig 3.3.1 Flow diagram for the bioprocess

The chemical composition of the substrates determined by proximate analysis included moisture, fibre, ash and protein. The individual fibre components, namely, cellulose, hemicellulose and lignin contents were also determined.

3.2. Determination of Moisture

Two crucibles were each washed, dried and 2.0g of the raw sample, uninoculated and inoculated ones were transferred into a previously weighed dish. The dish was placed in an oven thermostatically controlled at 105ºC for 5 hours. The dish was removed and placed in the dessicator to cool and reweighed. The procedure was repeated until a constant weight was obtained. The moisture content was calculated by difference and expressed as a percentage of the initial weight of the sample.

3.3. Determination of Ash

Two (2.0) g of the raw and treated samples were transferred into a previously weighed and dried crucibles and placed in a furnace preheated at 600ºC for 2 hours. The crucibles were removed, cooled and reweighed. The masses of the crucibles and their contents were found by subtraction. The ash content was calculated by difference and expressed as a percentage of the initial weight of the sample.

3.4. Determination of mineral matter

After the ash has been weighed, it was transferred into a digestion tube, and 30 ml of Acqua-regia made up of 33.33% of HCl together with 66.66% of HNO3, was added to the ash and then digested inside a fume chamber until the solution reduced to about 10 ml. This was allowed to cool and transferred into a 50 ml volumetric flask and made to the mark with distilled water. The mineral contents were then measured

using the Atomic Absorption Spectrophotometer (AAS) (UNICAM 960 series), Belingham, England.

3.5. Determination of Protein

3.5.1. Digestion

Two (2.0) g of the raw, control and treated samples were put into a digestion flask and half of a Selenium-based catalyst tablet added. Concentrated sulphuric acid (25ml) was added and the flask thoroughly shaken to ensure that the sample was entirely wet. The flask was then placed on a digestion burner and heated slowly until bubbling ceased and the resulting solution was clear (normally after 24hours). The digested sample was transferred into a 100 ml volumetric flask and made up to the mark. The distillation apparatus was flushed out using distilled water boiled in a steam generator and the connections were arranged to circulate through the inner decomposition flask and out through the condenser for at least 10 minutes. Twenty five (25) ml of 2% boric acid was pipetted into a 250 ml conical flask and two drops of mixed indicator were added. The conical flask and its contents were placed beneath the condenser such that the tip of the condenser was immersed in the solution, 10 ml of the digested sample solution was also measured and poured into the funnel on the steam jacket and 20 ml of 40% NaOH added. The funnel's stopcock was then closed, as it was opened to allow for pouring of the digested sample and the NaOH into the funnel. Steam was forced through the decomposition flask in order to drive the liberated ammonia into the collection flask. This was done by shutting the stopcock on the steam trap outlet.

The boric acid changed to bluish green as soon as it came into contact with the ammonia and distillation was continued for another five minutes. The steam generator was taken off the burner and the condenser was then washed for thirty seconds using distilled water.

The distillate was titrated with 0.1N HCl solution. The acid was added until the solution became colourless and the appropriate titre values recorded.

3.6. Crude fibre determination

Crude fibre is normally determined on defatted samples or samples with negligible amounts of fat content. Since cassava is one with a negligible fat content, crude fibre determination was made without determination of the crude fat. Two (2.0) g of the sample was weighed into a 750 ml Erlenmeyer flask and 0.5g of asbestos was added and 200 ml of 1.25% sulphuric acid was added to the sample with the asbestos; the flask was set on a hot plate and then connected to a condenser. The contents boiled within a minute, continuing for thirty minutes. At the end of the thirty minutes, the flask was removed and the contents filtered through a linen cloth in a funnel. Boiling water was used to wash the sample during the filtration till the acid was totally removed. The distillate was discarded and the sample and asbestos on the linen cloth were put back into the Erlenmeyer flask using a spatula and 200 ml of 1.25% NaOH was added and the flask was connected to the condenser. Boiling was done for thirty minutes, after which the flask was disconnected and the contents filtered using the linen cloth. Boiling water was again used in washing the sample till the entire base in it had been completely removed. The residue was transferred into a porcelain crucible and placed in a furnace for thirty minutes. The sample was taken out after that period, cooled and weighed and the weight recorded (A.OA.C, 1994).

3.7. Fibre analysis

The concept behind the detergent fibre analysis is that plant cells can be divided into less digestible cell walls (contain hemicelluloses, cellulose and lignin) and mostly digestible cell contents (contain starch and sugars). Georing and Soest (1991) separated these two components successfully by use of two detergents: a neutral detergent (Na-lauryl sulfate, EDTA, pH=7) and an acid detergent (cetyl trimethyl ammonium bromide in 1N H_2S0_4). Hemicellulose, cellulose and lignin are indigestible in non-ruminants, while hemicellulose and cellulose are partially digestible in ruminants.

NDF = Hemicellulose + Cellulose + Lignin.

ADF = Cellulose + Lignin.

3.7.1. Neutral detergent fibre (NDF) determination

NDF solution: To 1 L of distilled water was added;

30 g of Sodium laurly sulfate (Sodium dodecyl sulphate)

18.61g of Disodium dihydrogen ethylene diamine tetraacetic dehydrate (EDTA-disodium salt)

6.81g of Sodium borate decahydrate (Borax)

4.56g Disodium hydrogen phosphate anhydrous (Na_2HP0_4)

10 ml of 2-ethoxy ethanol (ethyleneglycol monoethyl ether)

Amylase solution-Heat –stable α-amylase (Sigma N0. A3306-from the Dietary Fibre Kit)

Acetone

Refluxing apparatus

Whatman 541 filter paper

Aluminium pans

Sodium lauryl sulfate was dissolved in part of the distilled water and 2-ethoxyethanol was added. Sodium borate and disodium EDTA were put together in a large beaker, some of the distilled water added and heated until dissolved (i.e. complete solution obtained) and added to the solution containing Na-lauryl sulphate and 2-ethoxyethanol. Na_2HPO_4 was put into a separate beaker and distilled water was added, and heated until it dissolved. The two solutions were mixed and the remaining distilled water was added. The pH was maintained between 6.9 and 7.1 using NaOH or HCl (Georing and Van Soest 1991).

Procedure

0ne (1.0) g of the sample was placed in 600 ml Berzelius beaker, 100ml of the neutral detergent fibre solution was added and heated to boiling (5 to 10 minutes). The heating was decreased as boiling begins and allow to boil for 60 minutes. After 60 minutes the content was filtered onto a pre-weighed Whatman 541 filter paper.

The content was then rinsed with hot water, filtered, and repeated twice. It was then washed twice with acetone, folded and placed in a preweighed aluminium pan. It was left to dry over night in 100 degrees Celsius oven after which it was cooled in a dissicator and weighed.

3.7.2. Reagent for the acid detergent fibre (ADF) determination

1. Acid Detergent Solution. 27.84 ml sulfuric acid (H_2SO_4) was added to a volumetric flask and volume brought to the mark with distilled water and 20 g of cetyl trimethyl ammonium bromide (CTAB) was then added to the solution.

2. Acetone

3.10.2 Procedure

0ne (1.0) g of air dried sample was transferred to Berzelius beaker. Hundred (100 ml) of acid detergent solution was then added and heated to boil (5 to 10 minutes) and then allowed to boil for exactly 60 minutes. After the 60 minutes, the content was washed with hot water 2 to 3 times. It was then washed thoroughly with acetone until no further colour was removed and filtered into a pre-weighed crucible. The sample was allowed to dry in 100 degrees Celsius oven, cooled in a dessicator, weighed and weight recorded.

3.7.3. Acid detergent lignin (ADL) determination

Reagent: 72% H_2SO_4 standardized to specific gravity of 1.634 at 20 degrees Celsius.

Procedure:

The ADF crucible was placed in a 50 ml beaker on a tray and the contents of the crucible covered with 72% H_2SO4. The content was stirred with a glass rod to a smooth paste in crucible, refilling hourly for 3 hours, each time stirring the contents of the crucible. After the 3 hours, the content of the crucible was filtered and washed with hot water until free of acid (minimum of five times), the rod was rinsed and removed. The crucible was dried in oven at 100 degrees Celsius for 24 hours, cooled in a dessicator, weighed and weight recorded. It was ashed in muffle furnance at 500 degrees Celsius for 4 hours, cooled in a dessicator, weighed and weight recorded.

3.8. Analysis of total phenols

Suitable aliquots of the sample (0.02. 0.05 and 0.1 ml) were taken into test tubes and made the volume made up to 0.5 ml with distilled water, after which 0.25 ml of

Folin- Ciocalteu reagent and 1.25 ml of sodium carbonate solution were added. The tubes were vortexed and absorbances recorded at 725nm after 40 minutes.

The amount of total phenols as tannic acid equivalent was determined from the calibration curve prepared (table 3.1) and expressed on dry matter basis (x%) (FAO/IAEA, 2000).

Table 3.1. Preparation of calibration curve

Tube	Tannic acid solution (0.1 mg/ml)	Distilled water (ml)	Folin reagent (ml)	Sodium carbonate solution (ml)	Absorbance at 725nm	Tannic acid (µg)
Blank	0.00	0.50	0.25	1.25	0.000	0
T1	0.02	0.48	0.25	1.25	0.112	2
T2	0.04	0.46	0.25	1.25	0.218	4
T3	0.06	0.44	0.25	1.25	0.327	6
T4	0.08	0.42	0.25	1.25	0.432	8
T5	0.10	0.40	0.25	1.25	0.538	10

3.9. Removal of tannin from the tannin-containing extracts (FAO/IAEA, 2000)

Hundred (100) mg of polyvinyl polypyrrolidone (PVPP) was weighed and put in to a test tube. One (1.0 ml) of distilled water was added to the sample. The tubes were

vortexed and kept at 4 degrees Celsius for 15 minutes. They were vortexed again and centrifuged (3000g for 10 minutes) and the supernatant collected. The non-tannin phenols content was determined on dry matter basis (y %). Percentage of tannins as tannic acid equivalent on dry matter basis= (x-y).

3.10. *In vitro* digestibility studies

Two (2.0) g of the sample was ground, filtered, washed with distilled water and oven-dried at 37 ^{0}C. All samples were then incubated in the presence of 1.3U.ml^{-1} α-amylase in 1M $NaHPO_4$ buffer, pH 6.7 at 37^{0}C for 4 hours. At the end of the incubation period, 1% (w/v) NaOH was added and the suspensions were filtered through Whatman No.1 filter paper. The amount of reducing sugars present in each filtrate was determined using a refractometer.

3.10.1. Measurement of sugar level

A refractometer (ABBE 60) was used to analyze the sugar level in degree brix. A drop of the sample was put on the glass surface of the refractometer and the sugar level subsequently determined.

3.11. Analysis of data

The results are presented as the mean standard values of three replicates each. A one-way analysis of variance (ANOVA) and the Least Significance Difference (LSD) were carried out. Significance was accepted at $P < 0.05$. All the data were also subjected to analysis of variance and significant mean differences were tested by the Duncan's New Multiple Range Test.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1.0 Proximate Composition

Analysis of proximate composition provides information on the basic chemical composition of foods or feeds. The components are moisture, ash, protein, crude fibre and carbohydrates. These components are fundamental to the assessment of the nutritive quality of the food or feed being analyzed.

4.1.1 Moisture

Moisture content of food or feed or processed product gives an indication of its shelf-life and nutritive values. Low moisture content is a requirement for a long shelf life. The moisture content for samples ranged from 2.84% for unfermented (raw peels) to a maximum of 5.70% by the 6th week of fermentation for the inoculated and then declined to a value of 4.16% by the 8th week. In contrast, the moisture content of the control (uninoculated) samples did not change from that of the raw sample over the entire 8 weeks of fermentation (Fig 4.1). The observed general increase in the moisture content of the inoculated peels from the 1st week to the 6th week is attributed to the mycelia growth. This is in agreement with Bano *et al* (1986) who stated that the mycelia of mushrooms contain appreciable amount of moisture. Sawyer (1994) also indicated that, water and carbohydrates constitute the main components of mushroom. The fruiting of the mushroom beyond the 6th week is the reason for the observed declined in moisture content of the inoculated peels as the mycelia are known to utilize both a lot of nutrients and moisture during the fruiting stage of growth (Rypacek, 1966).

Moisture is an important component in the feed and for that matter any food product. Very high levels (>12%) allow for microbial growth (mould, fungi etc),

thus low levels are favourable and give a relatively longer shelf life. There were significant differences ($P<0.05$) in the moisture content of the inoculated cassava peels. This could be attributed to the fermentation process. Meanwhile there was no significant difference in the moisture content of the control peels throughout the incubation period and this indicates that the observed significant difference observed in the inoculated peels over the fermentation period resulted from the growth of the mycelia of the oyster mushroom and not as results of differences in drying time or atmospheric conditions.

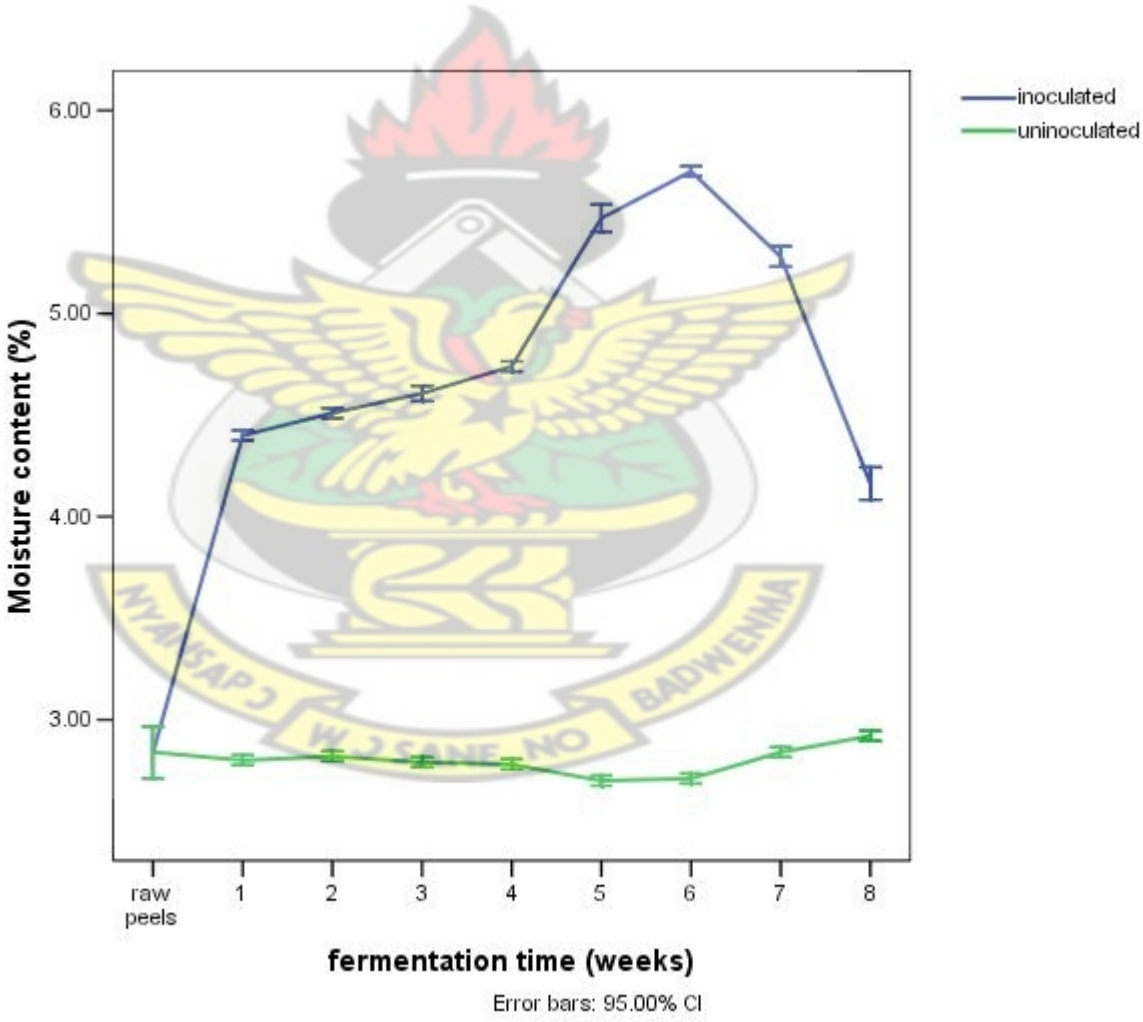


Fig. 4.1 Effect of fermentation time on moisture content of cassava peels

4.1.2 Ash

The ash content of the inoculated samples ranged from 4.16% to 7.99% whereas that of the raw sample had 3.49% (Figure 4.2). Significant differences ($P<0.05$) existed in the inoculated peels but there was no significant difference in the ash content of the control over the period of fermentation. The ash content of the raw cassava peels was within the literature value of 3.50-10.40% (Smith, 1993). The raw cassava peels had the lowest ash content of 3.49% with the eight week of inoculated samples having the highest ash content of 7.99% (Figure 4.2). There was a general increase in the ash content of the peels from the first week (3.90%) through to the 8th week (7.99%). The increase in the ash content could be attributed to the enrichment of minerals by the mycelia of the mushroom grown on the peel as Asmah (1999) also found the ash content of mushrooms grown on corncobs to be 8.42%, Aborbor (1998) reported 5.89% for *Pleurotus ostreatus* cultivated on cocoa pod husk and Crisan and Sands also observed 5.6% when cultivated on wheat straw. According to Sawyer (1994), mushrooms have richer supply of minerals than many meat products and double the amount in most vegetables. The fruit bodies of mushroom contain about 10% ash, on dry weight basis, which in turn represents the minerals (Bano *et al*., 1986).

Thus fermentation of the peels with the oyster mushroom impacts a positive effect on the mineral content of the peels as the control peels which were also fermented for eight weeks, but with no significant difference in the ash content. Thus the result of the control strongly supports the assertion that, observed increase in the ash content of the peels inoculated resulted from the growth of the oyster mushroom on the peels. The mineral elements perform critical functions in animals and therefore the treated peels used as feed will enhance and improve the development of animals

since inadequate amounts are associated with innumerable developmental and health-related problems (Church, 1976).

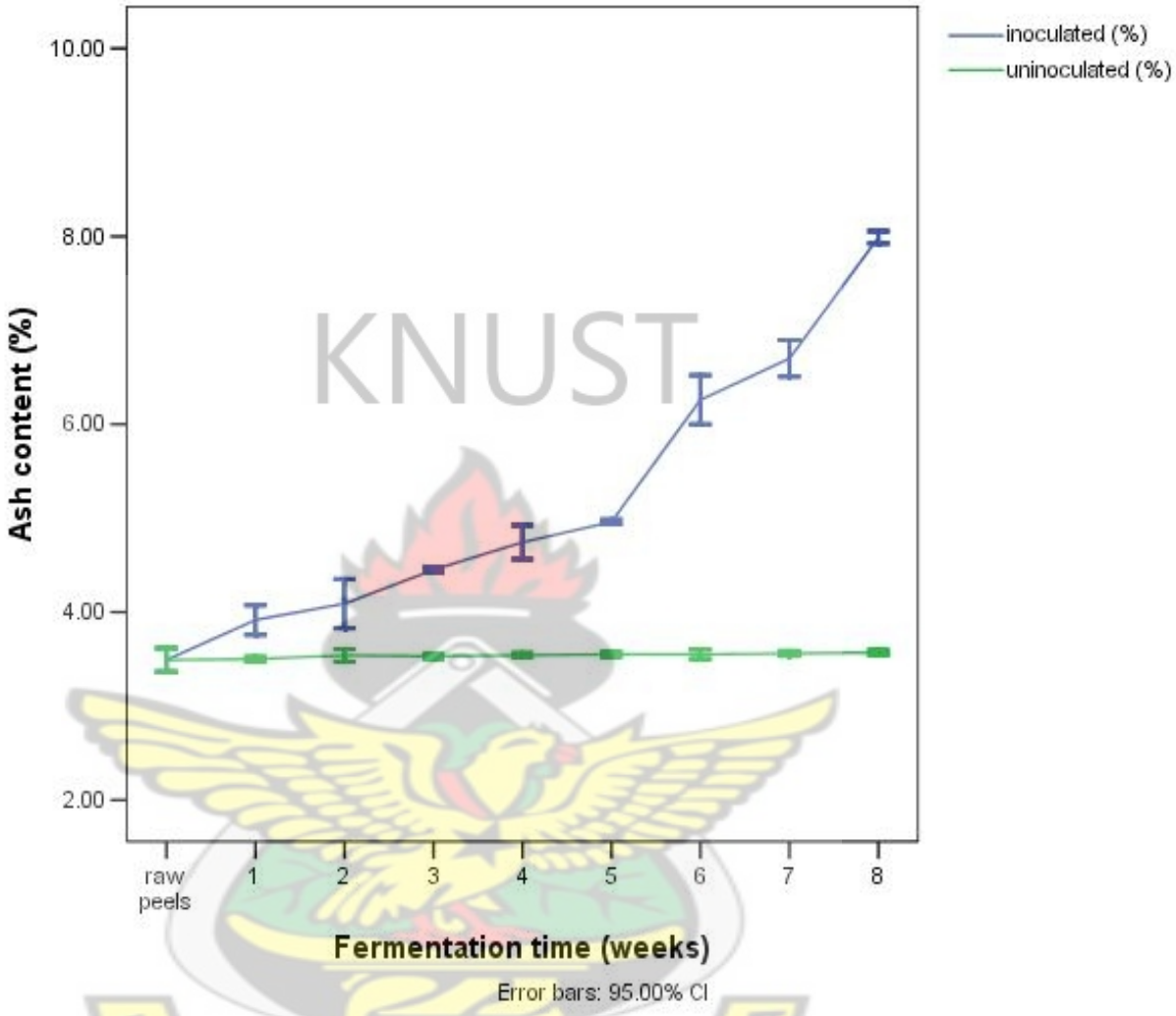


Fig 4.2 Effect of fermentation time on ash content of cassava peels

4.1.3 Protein

Analysis of the fermented cassava peels revealed that, there was an increase in the protein content of the peels fermented with *Pleurotus ostreatus* when compared to the raw cassava peels and the control (Fig4.3). This increase was highest in the peel fermented for six weeks (5.6%) and the first week of the fermentation registered the lowest crude protein level of 2.97% which was not significantly different from that

of the raw cassava peel sample of 2.18% (Fig 4.3). The crude protein content of the raw peels was found to be lower than the literature range of 2.8-6.5% (Smith, 1992) and this could be due to varietal effect of the cassava from which the peels were obtained.

The increase in the protein levels of the treated peels is in agreement with the report by Vijay *et al*., (2007) that, *Pleurotus ostreatus* has the potential to convert cheap lignocellulosic into valuable protein at low cost. Additionally, this result is supported by the report of Iyayi and Losel (2001) who reported the enrichment of cassava peels and pulp with protein when fermented with different fungi types. The protein content of the inoculated peels generally increased with fermentation time and peaked by the sixth week (5.6%) after which it declined to 4.28% by the eight week. The decrease in the protein content of the inoculated peels could be attributed to fruiting bodies formation by the mushroom. During fruiting stage of mushroom's life cycle, the mycelia utilize a lot of nutrients (protein) in forming the fruiting bodies and accounted for the observed decrease (Rypacek, 1966) after the 6^{th} week of fermentation.

There was a significant increase ($P < 0.05$) in the protein content of the cassava peels inoculated with the mushroom whereas no significant difference was found in the control peels throughout the fermentation period. The increase could be attributed to the possible secretion of some extracellular enzymes (proteins) such as, cellulase and also amylases (Oboh *et al*., 2002) into the cassava peels by the fermenting organisms in an attempt to make use of cellulose and starch respectively as sources of carbon (Raimbault, 1998).

Apart from this, the increase in the growth and proliferation of the fungi in the form of single cell proteins may also contribute to the apparent increase in the protein

content of the peels fermented with the mushroom (Antai and Mbongo, 1994; Oboh *al*. 2002). The present findings suggest that for the purposes of producing nutritive animal feed from cassava peels through *P. ostreatus*, six weeks of fermentation appears to be the optimal period since the decrease beyond the 6^{th} week is found to be statiscally significant. This result is further supported by Brimpong *et al* (2009), who also indicated that, the optimum period of fermentation with *P. ostreatus* on corn cobs for animal feed is after complete colonization of the corn cobs by mycelia. This observation is additionally backed by Alemawor *et al* (2009), who reported that, five weeks of *Pleurotus ostreatus* solid state- fermentation of Cocoa pod husk (CPH) amended with 0.075% (w/w) Mn^{2+} ion was found to be the optimum treatment to give the best results, a 30% (w/w) increase in protein.

In view of this significantly enhanced protein content in the *Pleurotus* fermented peels, the cassava peels so far regarded as having no economic value, could be integrated into animal nutrition provided it is acceptable and highly digestible in farm animals (Oboh, 2006).

Proteins occupy a unique position in metabolism. Not only may they serve as sources of energy for the organism, but they also constitute the most important raw materials out of which the complex structures of the body are built. While carbohydrates and fats are employed chiefly as fuels for the "protoplasmic fires," proteins, in a well balanced diet, serve mainly for purposes of synthesis. Like other biological macromolecules such as polysaccharides and nucleic acids, proteins are essential parts of organisms and participate in every process within cells (Schwarzer and Cole, 2005). Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism (Zagrovic *et al*., 2002). Proteins also have structural or

mechanical functions, such as actin and myosin in muscle and the proteins in the cytoskeleton, which form a system of scaffolding that maintains cell shape (Schwarzer and Cole, 2005). Other proteins are important in cell signaling, immune responses, cell adhesion, and the cell cycle (Zhang, 2008). Proteins are also necessary in animals' diets, since animals cannot synthesize all the amino acids they need and must obtain essential amino acids from feed (Zhang, 2008). Through the process of digestion, animals break down ingested protein into free amino acids that are then used in metabolism (Zagrovic *et al*., 2002). Thus the enrichment of the *Pleurotus* fermented peels with protein will enhance the general well being of the animals.

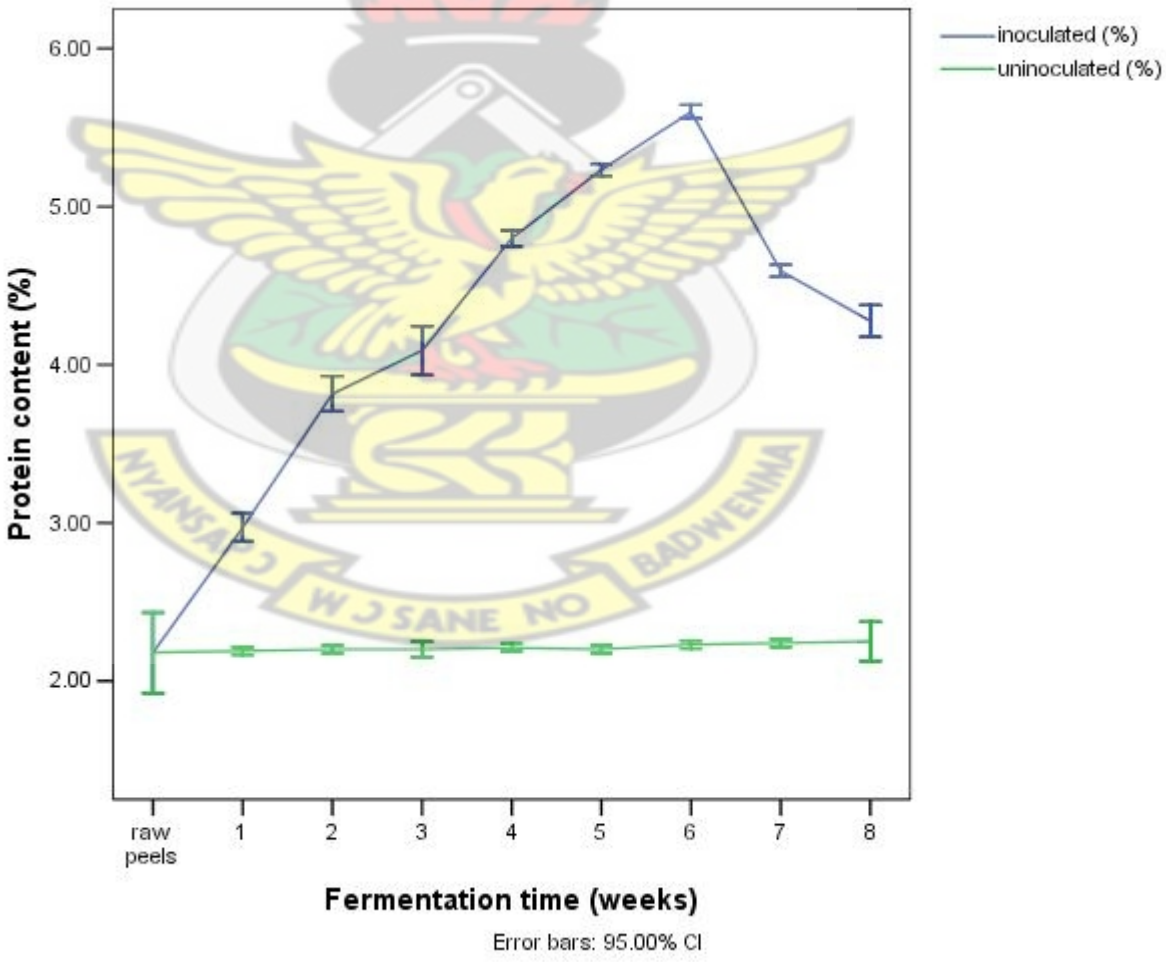


Fig 4.3 Effect of fermentation time on protein content of cassava peels

4.1.4 Carbohydrates

There was generally, an increase in the carbohydrate content of the *Pleurotus* fermented peels and the increase was statiscally different ($P < 0.05$) but there was no significant difference in the carbohydrates content of the control from that of the raw material throughout the fermentation period (Fig 4.4). The first week of fermentation had the lowest carbohydrate content with the eight week recording the highest value (73.27%) (Fig. 4.4). The initial decline in the carbohydrate content of the treated peels from day 0 to week 1 could be attributed to the ability of the fungi to hydrolyze starch into glucose and ultimately use the glucose as a source of carbon to synthesize fungal biomass rich in proteins (Oboh *et al*., 2002). These fungal proteins are then used to synthesize extracellular enzymes such as cellulases, hemicellulases, laccases etc (Raimbault, 1998) and these enzymes degraded the non-starch polysaccharides in the substrate to simple and soluble sugars (Iyayi, 2004). The increase in carbohydrate after the first week with fermentation time showed that more simple sugars were made available by the degradation of the fibre component of the substrates by the mushroom.

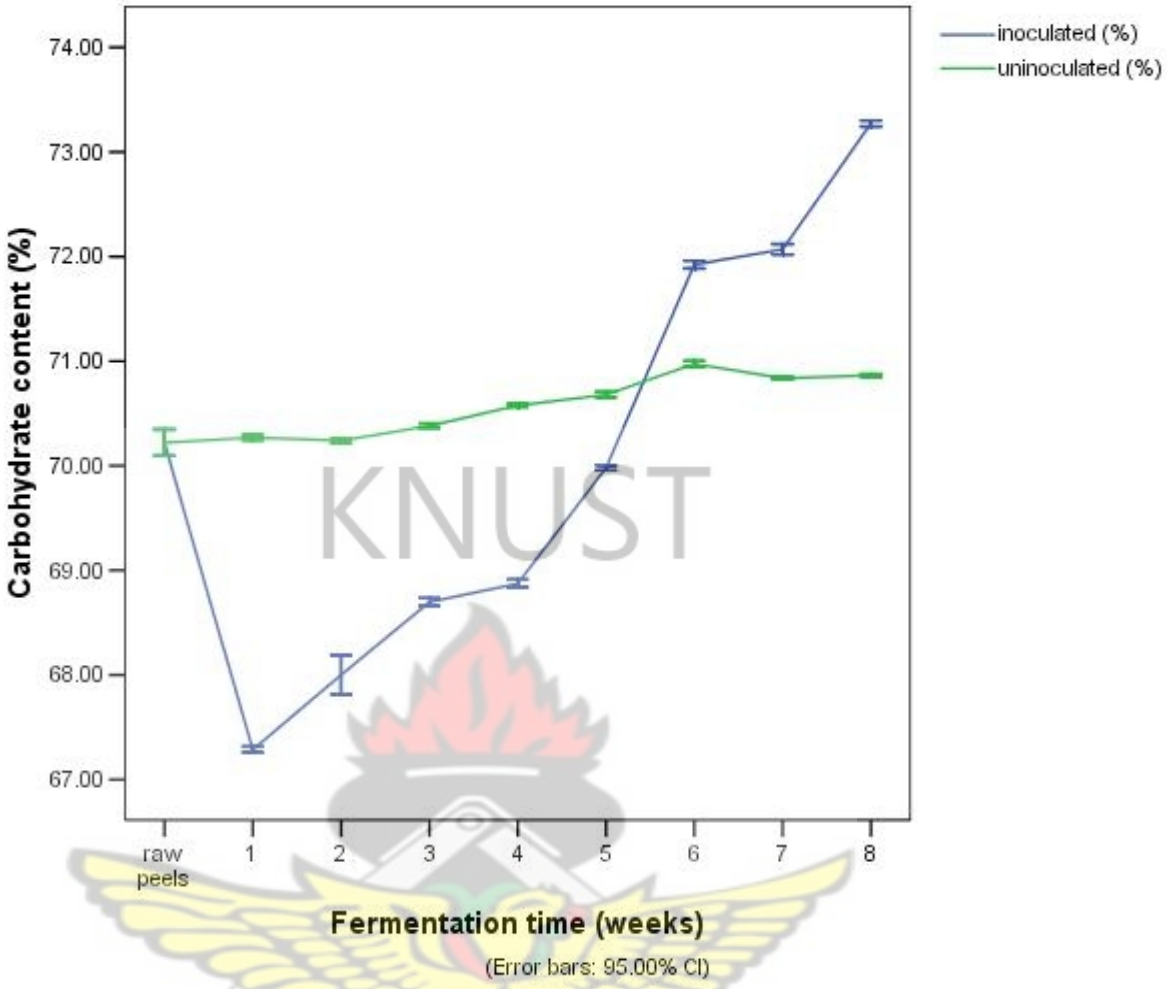


Fig 4.4: Total carbohydrates content of cassava peels fermented at different durations

4.1.5 Crude Fibre

The fibre content declined significantly ($P<0.05$) with fermentation (Fig 4.5) with values declining from 19.99 to 13.13 between the 1st and 8th weeks respectively for the inoculated samples. The decrease in fibre content resulted from the hydrolytic nature of the fungal enzyme systems used for the biodegradation as there was no significant difference in the fibre content of the control peels throughout the period of fermentation when compared with the raw material.

This observation is in agreement with that of Chesson (1993) who reviewed the early claim that disruption of cell walls and their degradation by fungi accounted for the decline in fibre content. He reported that, available cell wall carbohydrates not attacked by digestive enzymes are biodegraded by extracellular enzymes possessed by mushrooms with a corresponding decline in fibre.

He then stressed that, total breakdown requires the action not only of the enzyme responsible for the attack on the cell wall polysaccharide and glucan hydrolases but also of a second set of glucosidases able to reduce oligosaccharides to their monomeric units. Therefore during the biodegradation, the enzymes from the mushroom break down polysaccharides into less complex structures (Aderemi and Nworgu, 2007). It must be noted that the ease of degradation of any fibre component is a function of the enzyme composition of the mushroom and the physio-chemical properties of the substrates (Aderemi and Nworgu, 2007).

Further analysis of the fibre content of the treated peels also revealed that, there was no difference statistically between the fibre content beyond the 6th week. Based on this result, the optimum period for the greatest fibre degradation by the oyster mushroom is six weeks of fermentation since no significant decrease in fibre was observed beyound the 6th week.

Dietary fiber is the component in food not broken down by digestive enzymes and secretions of the gastrointestinal tract. This fiber includes hemicelluloses, pectins, gums, mucilages, cellulose, (all carbohydrates) and lignin, the only non-carbohydrate component of dietary fiber. Their degradation is beneficial since the cellulose, hemeicellulose and lignin contents of the cassava peels which hitherto

were not available to livestock because they lack the enzymes to act on them is converted into easily digestible components (Youri, 2003).

Thus fermentation of the peels with *P. ostreatus* improved tremendously the nutritional composition of the peels for use as livestock feed and also reduced the limitation of cassava peels in the livestock feed industry especially for non-ruminants who lack the complex stomach for the utilization of the high fibre content of cassava peels.

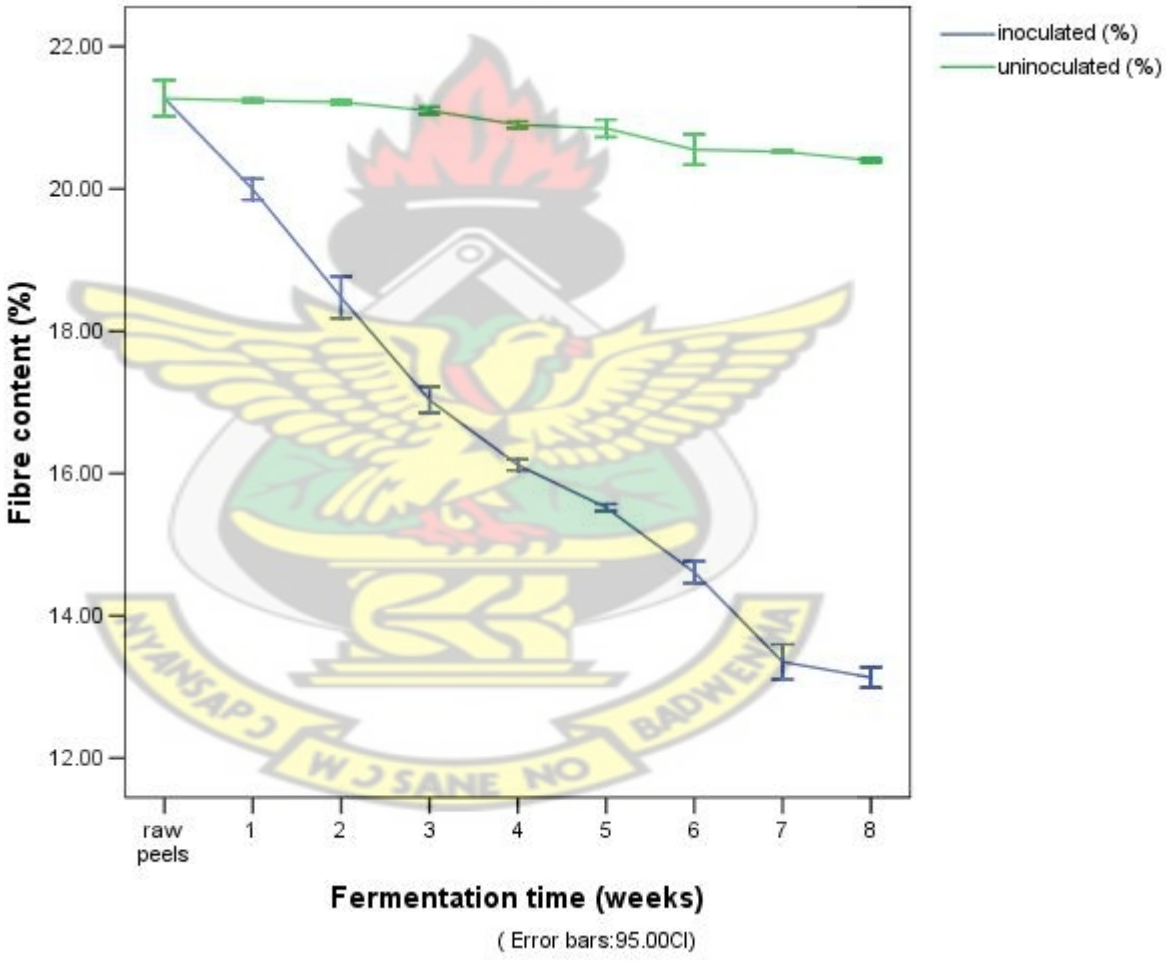


Fig 4.5: Effect of fermentation time on the fibre content of cassava peels

4.2. Lignocellulose degradation

The raw cassava peels was found to contain 22.51% hemicelluloses, 16.35% cellulose and 7.35% lignin. These values were slightly different from the work of Aderemi and Nworgu, (2007), who reported 21.65% hemicelluloses and 6.98 % lignin in the peels. This may be attributed to varietal differences. Degradation and utilization of cellulose, hemicellulose and lignin greatly affects the growth of *Pleurotus ostreatus* and the feed value of the spent substrate (Xiujin *et al*, 2001). Generally, these components are continuously degraded (Youri, 2003).

The appearance of the fungal mycelia on the substrate after the first week was an indication that the degradation has commenced (Aderemi and Nworgu, 2007). Neutral detergent fibre (Fig 4.6), acid detergent fibre (Fig 4.7) and acid detergent lignin contents (Fig 4.8) decreased with fermentation indicating the continuous breakdown activity of the fungi (Aderemi and Nworgu, 2007).

The higher the hydrolyzing or saccharifying ability of the microbes, the lower the neutral detergent fibre, acid detergent fibre and acid detergent lignin found in substrates (Aderemi and Nworgu, 2007). Neutral detergent fibre, acid detergent fibre and acid detergent lignin contents of the untreated peels were not significantly different when compared with the raw material over the fermentation period. From these results, the cellulolytic ability of the fungal enzyme was obvious and the biodegradation resulted in an improvement of the cassava peels, presumably making more reducing sugars available, since the biodegradation of the fibre components leads to an increase in the total soluble sugars (Aderemi and Nworgu, 2007).

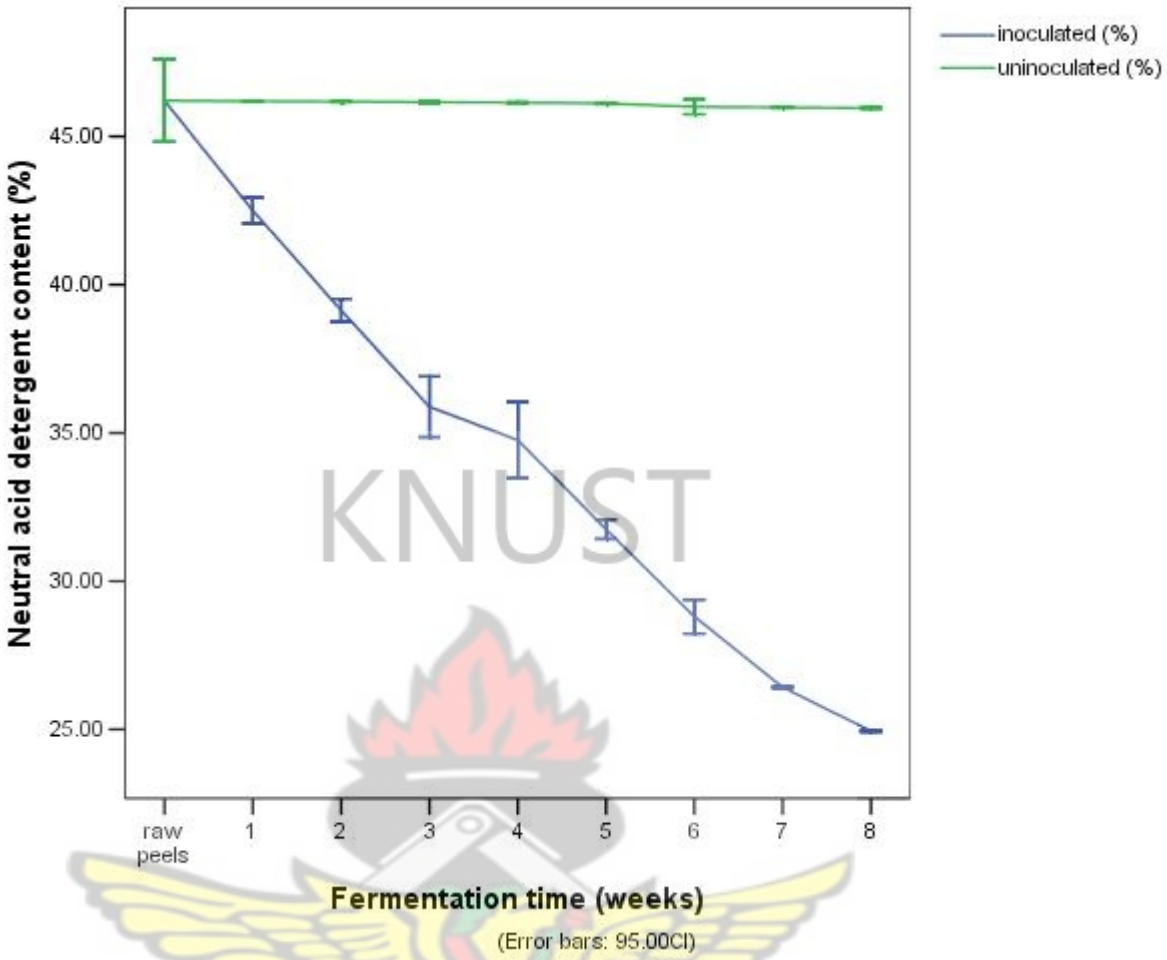


Fig 4.6: Effect of fermentation duration on the neutral detergent fibre content of cassava peels

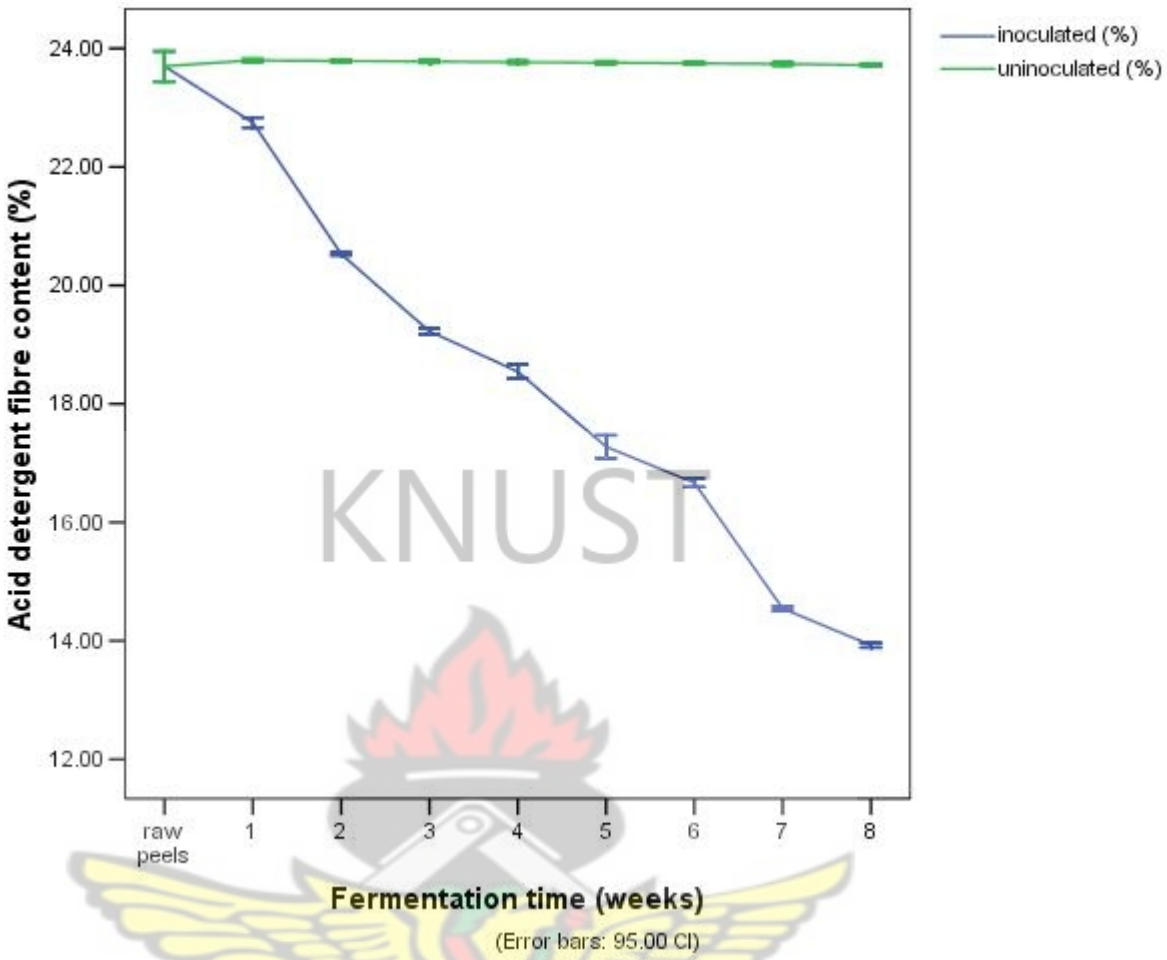


Fig 4.7: Effects of fermentation time on the acid detergent lignin content of cassava peels

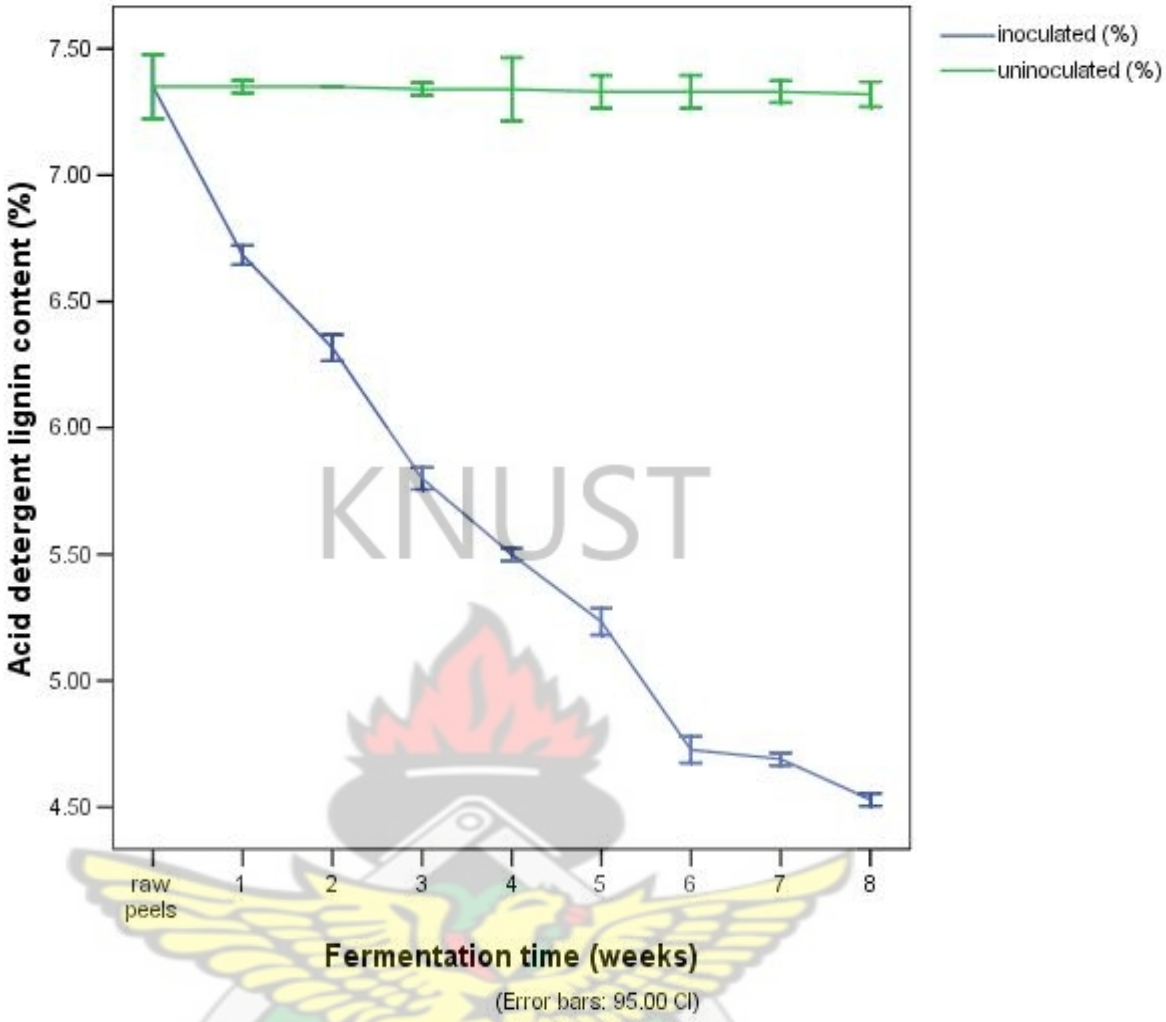


Fig 4.8: Effect of fermentation time on the acid detergent fibre content of fermented cassava peels.

4.2.1 Hemicellulose

The rate of degradation of hemicellulose was found to be the greatest among the lignocellulosic components of the substrate (Fig 4.9). The raw cassava peels and the control had hemicellulose content of 22.51% and 22.25% respectively. The decrease in the hemicellulose content of the inoculated peels was found to be significantly different from that of the control and the raw peels ($P<0.05$) but no significant difference was observed in the hemicellulose content of the control when compared with the raw peels during the fermentation period ($P<0.05$). It was also observed

that, there was no significant decrease in the hemicellulose content of the treated peels beyond the 6th week which indicates that, the best duration for hemicellulose degradation through *P.ostreatus* fermentation on cassava peels is six weeks after which no significant decrease was observed. Thus there was a 45.43% decrease in the hemicellulose content by the optimum fermentation period (6th week) when compared to the control. Among all the lignocellulosic materials, hemicellulose degradation was the highest during the fermentation with the oyster mushroom (Fig 4.9).

Xiujin *et al* (2001) observed a similar trend during the cultivation of *Pleurotus ostreatus* on cottonseed hull substrate. They concluded that this was probably due to the fact that hemicellulose is more easily degraded than cellulose and lignin and so more rapidly assimilated. *Pleurotus ostreatus* produces enzymes that will hydrolyze a variety of β-(1, 4) linked glucan substrates as well as various glycosides (Highley, 1976). This result is also strongly supported by Brimpong *et al* (2009) who also observed a 41% decrease in hemicellulose after complete colonization of corn cobs by the mycelia of the oyster mushroom with no further reduction thereafter. They also found hemicellulose degradation to be the highest among the lignocellulosic components of the corn cobs during the fermentation with the mushroom.

Thus the observed drastic reduction in the hemicellulose content in the peels treated with the mushroom will lead to an improvement in the digestibility of the peels if used as livestock feed since hemicellulose is one of the major components of fibre in the peels.

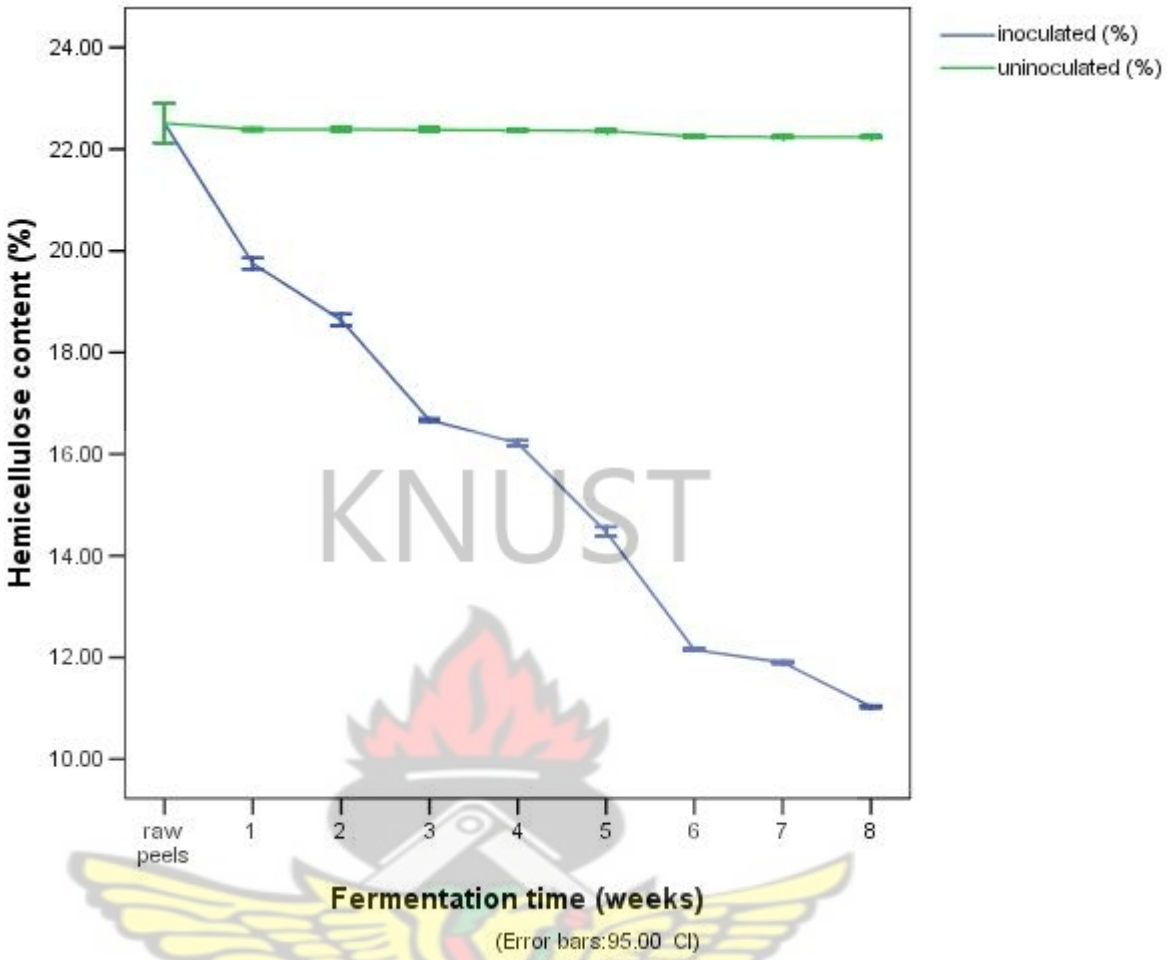


Fig 4.9: Effect of fermentation time on the hemicellulose content of cassava peels

4.2.2 Cellulose

The raw cassava peels recorded the highest amount of cellulose (16.35%) as shown in Fig 4.10. Cellulose was found to be the least degraded among the lignocellulosic components and statistical analysis of its degradation profile revealed no significant decrease between the 6th and the 8th weeks of fermentation with the mushroom though cellulose was generally degraded throughout the fermentation period. There was a 26.73% decrease in the cellulose content of the fermented peels at the end of the optimum fermentation period (6th week) when compared to the control. The

decrease in the cellulose content of the treated peels was found to be significantly different from the uninoculated and raw peels ($P<0.05$). This decrease is due to the effect of the enzymes produced by the mushroom as there was no significant difference in the cellulose content of the control peels over the period of fermentation when compared to the raw peels ($P<0.05$).

This result is similar to the work of Datta and Chakravarty, (2001) who stated that the ability to degrade cellulose has been attributed to the synergistic action of three types of hydrolases, collectively called cellulases.

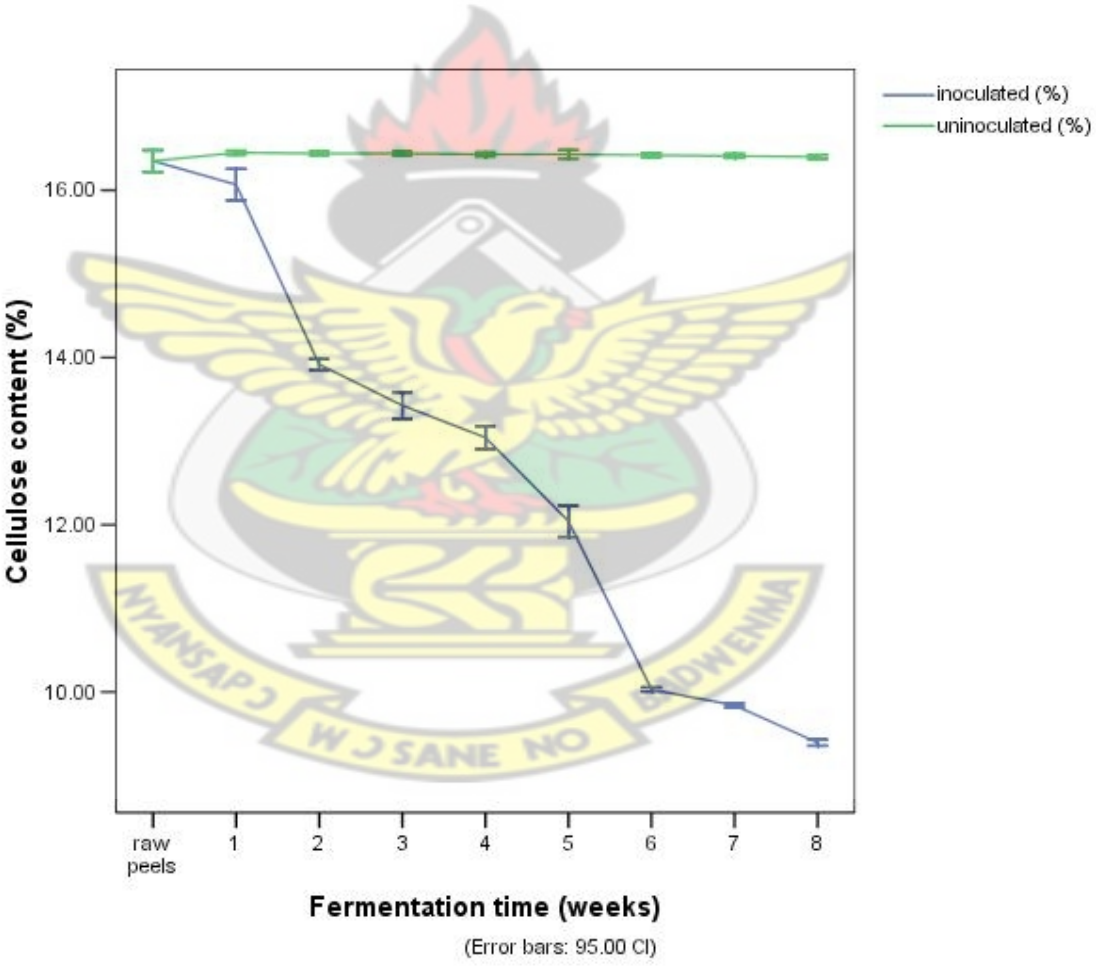


Fig 4.10 Effect of fermentation duration on the cellulose content of cassava peels

Fungi have the ability to produce a variety of enzymes (Iyayi, 2004). *P. ostreatus* has been reported to be a source of cellulases, hemicellulases and these enzymes help to degrade the non-starch polysaccharides in the substrate to soluble sugars (Iyayi, 2004). Thus with decrease in the amount of cellulose, a corresponding increase in soluble sugar content is normally observed (Iyayi, 2004). Therefore through the bioprocess, the high cellulose content of the peels which is poorly utilized by livestock is biodegraded, ensuring an adequate supply of digestible polysaccharides (Brimpong *et al*., 2009) and soluble sugars which can easily be utilized by livestock especially non-ruminants.

4.2.3 Lignin

The lignin content of the treated peels was found to decrease generally with fermentation time (Fig 4.11). Little degradation was observed after six weeks of fermentation and statistically no significant difference existed in the decrease of the lignin content of the treated peels beyond the 6th week. Thus lignin was found to be 35.78% degraded by the bioprocess by the optimum period of fermentation (6th week) (Fig 4.11). The decrease in lignin content of treated peels was found to be significant when compared to the control and raw material ($P<0.05$) but there was no significant difference in the lignin content of the control peels over the period of fermentation when compared to the control ($P<0.05$). According to the work of Brimpong *et al*., (2009), who investigated the action of oyster mushroom on corn cobs, lignin components of corn cobs declined by 42.3% after complete colonization of the corn cobs by the mycelia of the mushroom. They further stated that, there was no further reduction in lignin content of the corn cobs after complete colonization of the substrate with the mycelia of the oyster mushroom.

Lo *et al* (2001) explained that the lignin moiety of lignocelluloses can act as a barrier to cellulose and hemicelluloses degradation, and thereby restrict the availability of nutrients required for fungal growth. Therefore the degradation of the lignin serves to increase the availability of cellulose (Datta and Chakravarty, 2001). Degradation of lignin is accomplished by the production of extracellular enzymes that oxidize both the aromatic rings and the aliphatic side chains to produce low-molecular weight products that can be absorbed by the fungi (Youri *et al*, 2004).

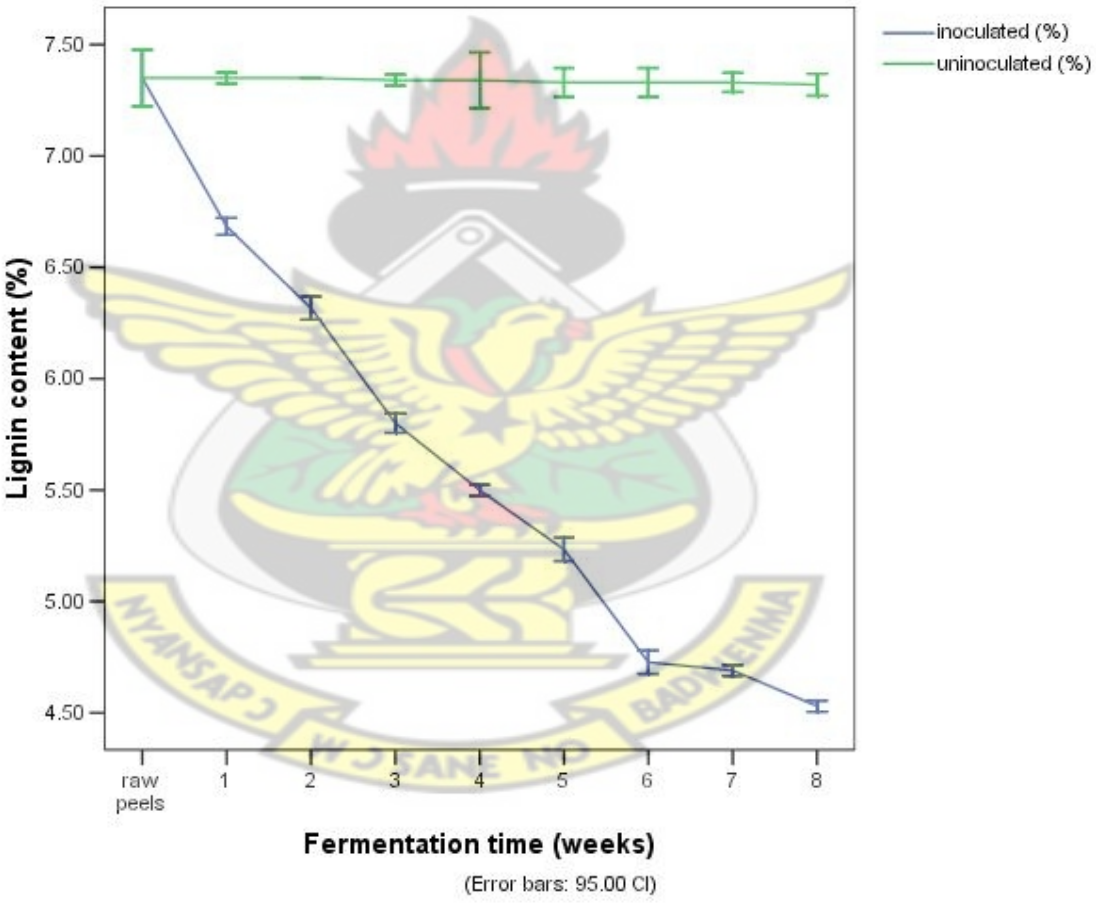


Fig 4.11 Lignin content of cassava peels fermented at different durations

Phenol oxidizing enzymes such as laccase, also are important in this respect and are produced in large quantity by white rot fungi (Lo *et al* 2001). Therefore the

observed lignin degradation obtained in the treated peels over the period of fermentation can be attributed to the secretion of these extracellular enzymes by the oyster mushroom with a corresponding decline in the lignin component of the peels. Since lignin is one of the major components of the high fibre content of cassava peels which limit the use of the peels in livestock feed industry, reductions in its content is of key importance to animal nutritionists and greatly enhance the feed value of the peels.

4.3. Changes in individual mineral contents following fermentation of cassava peels with *P. ostreatus*.

Since mineral elements perform critical functions in animals, the determination of mineral elements is an indispensable component of feed analysis. Three mineral elements namely calcium, potassium and phosphorous were analyzed after the fermentation of peels with the mushroom.

4.3.1 Calcium content

It was observed that there was an improvement in the calcium content of the peels fermented with the mushroom. The calcium content of the raw peels was found to be 1279.40 mg/kg whereas that of the control and inoculated peels recorded 1421.39 mg/kg and 3813.48 mg/kg respectively, an increase of 168.3% when compared to the 6^{th} week control (optimum period of fermentation) (Fig 4.12). This increase is attributed to the mycelia growth of the mushroom and subsequent enrichment of the peels with the mineral as the oyster mushroom is known to contain appreciable

amount of calcium (Sawyer, 1994). Oghenekaro *et al* (2009) also found mushrooms to be rich sources of minerals and vitamins.

Calcium, the most abundant mineral in the human body, has several important functions. More than 99% of total body calcium is stored in the bones and teeth where it functions to support their structure (Heaney *et al*., 2000). The remaining 1% is found throughout the body in blood, muscle, and the fluid between cells (Subar *et al*., 1998). Calcium is needed for muscle contraction, blood vessel contraction and expansion, the secretion of hormones and enzymes, and sending messages through the nervous system (Heaney *et al*., 1991). A constant level of calcium is maintained in body fluid and tissues so that these vital body processes function efficiently (Subar *et al*., 1998).

Calcium is also needed for normal functioning of cell membranes, for muscle contraction, and is also required for strengthening bones and teeth (Carruth and Skinner, 2001).

Thus the tremendous improvement in calcium content of the cassava peels through fermentation with the mushroom greatly enhanced the mineral content of the peels and the use of the treated peels as livestock feed will help maintain the general health of livestock and also promote their growth since calcium is also required for growth (Annan, 1998).

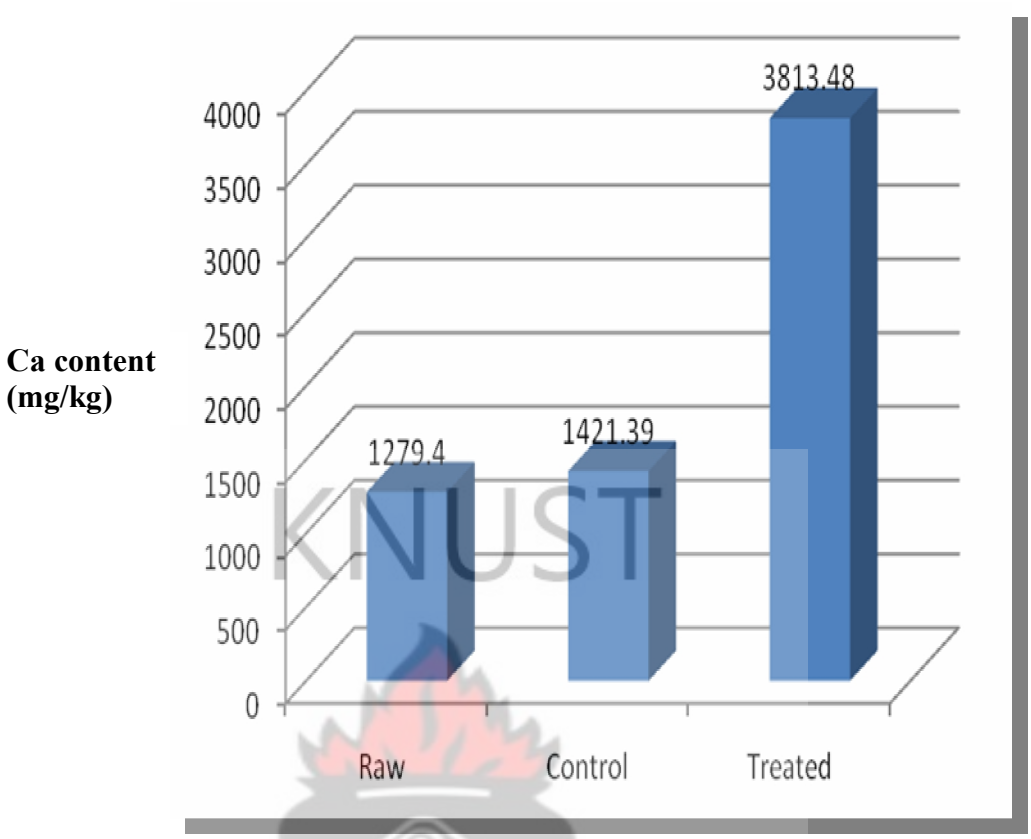


Fig 4.12: Changes in Ca content following fermentation of cassava peels with *Pleurotus ostreatus* after six weeks of fermentation.

4.3.2 Potassium content

The potassium content of the treated peels recorded more than two folds increase after six weeks of fermentation with the oyster mushroom when compared with the control. The treated, control and the raw peels had potassium contents of 1366.26 mg/kg, 413.28 mg/kg and 310.35 mg/kg respectively (Fig 4.13).

This increase is attributed to the mycelia growth of the mushroom and subsequent enrichment of the peels with the mineral as *Pleurotus ostreatus* is known to contain appreciable amount of the mineral element (Sawyer, 1994). The observed improvement in the potassium content of the treated peels supports the earlier work of Abbot *et al* (2009) who found mushrooms to be rich sources of proteins and

minerals such as potassium. Bano and Rajarathnam, (1982) also reported that, potassium, calcium and phosphorous are the main constituents of ash of *Pleurotus*.

Potassium is central to the maintenance of a potential difference across cell membranes and therefore plays an important role in the generation of nerve and muscle action potentials (Skelly, 2002). This mineral element is therefore essential for the proper functioning of the nervous system, especially nerve impulse transmission. Potassium is a primary constituent of primary cell metabolites such as nucleotide and co-enzymes such as flavin phosphate, pyridoxal phosphate and thiamine phosphate (Durand and Kawashima, 1980). It is essential for all energy transactions within animal cells, formation of adenosine di and tri phosphate (ADP and ATP) and guanine triphosphate (MacDonald *et al*., 1981). Potassium is also responsible for regulating DNA, protein and glycogen synthesis via its action as an enzyme cofactor (Skelly, 2002).

Potassium therefore plays an important role in the growth of animals and therefore the enrichment of substrate by this mineral element after the fermentation will help supplement the potassium nutritional requirement of livestock as reported by Campell and Roberts (1965).

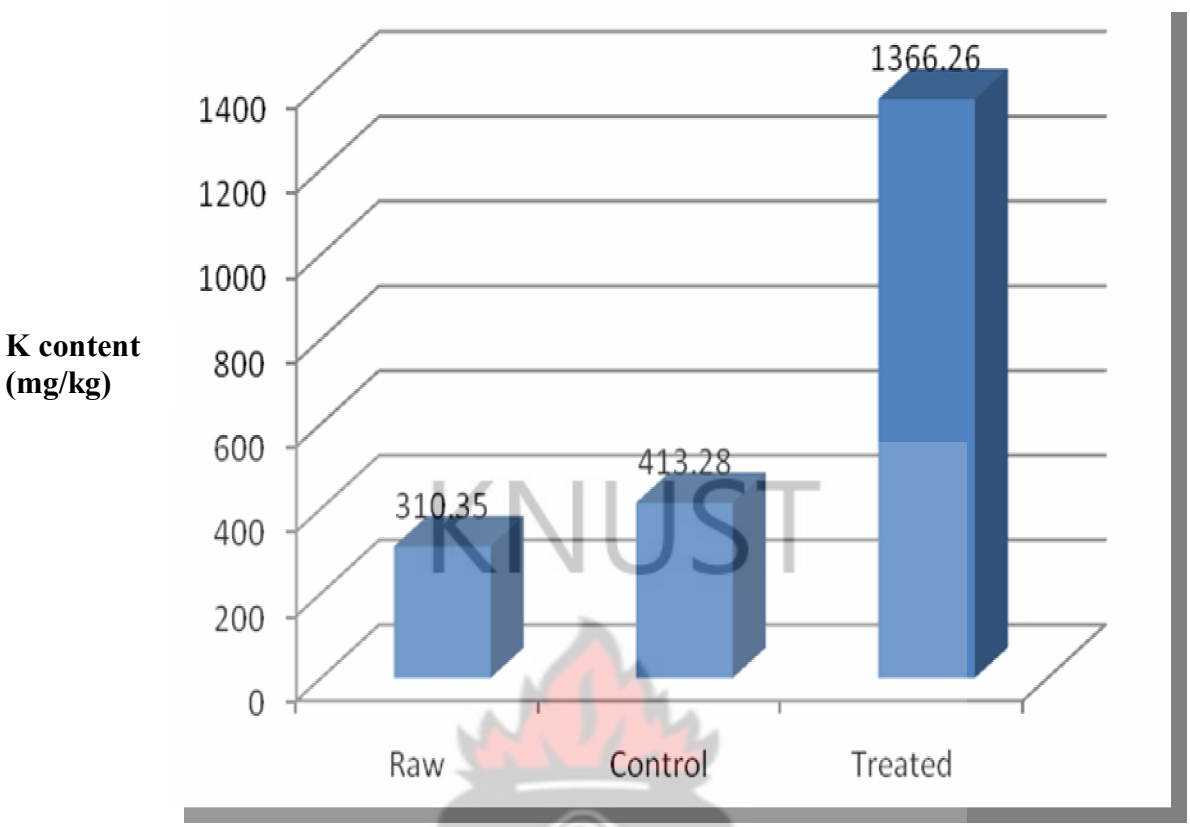


Fig 4.13: Changes in potassium content following fermentation of cassava peels with *Pleurotus ostreatus* after six weeks of fermentation.

4.3.3 Phosphorous content

Of the three mineral elements analyzed in the peels, phosphorous had the least improvement by the bioprocess, an increase of 46.3%. The respective values were 1197.21 mg/kg, 1244.47 mg/kg and 1821.12 mg/kg respectively for the raw peels, the control and the treated peels (Figure 4.14). This increase is attributed to the mycelia growth of the mushroom and subsequent enrichment of the peels with the mineral as *Pleurotus ostreatus* is also known to be rich in phosphorous and other elements needed by the body (Sawyer, 1994).

Phosphorus (P) is an essential nutrient for all animals. Deficiency of P is the most widespread of all the mineral deficiencies affecting livestock. In the animal body,

about 80 percent of P is found in the skeleton (Chase, 1999). Its major role is as a constituent of bones and teeth. The remainder is widely distributed throughout the body in combination with proteins and fats and as inorganic salts. Phosphorus constitutes about 22 percent of the mineral ash in an animal's body, a little less than one percent of total body weight (Chase, 1999).

It is essential in transfer and utilization of energy. Phosphorus is present in every living cell in the nucleic acid fraction. Calcium and P are closely associated with each other in animal metabolism. Adequate Ca and P nutrition depends on three factors: a sufficient supply of each nutrient, a suitable ratio between them, and the presence of vitamin D. These factors are interrelated. The desirable Ca:P ratio is often between 2:1 and 1:1. A liberal supply of Ca and P is essential for lactation (Skelly, 2002). Earliest symptoms of P deficiency are decreased appetite, lowered blood P, reduced rate of gain, and "pica", in which the animals have a craving for unusual foods such as wood or other materials. If severe deficiency occurs, there will be skeletal problems (Skelly, 2002).

Milk production decreases with P deficiency, and efficiency of feed utilization is depressed. Long-term P deficiency results in bone changes, lameness, and stiff joints (Chase, 1999). Thus the improvement of the P levels following the oyster mushroom fermentation of cassava peels will contribute positively to the general well being of livestock if used as feed.

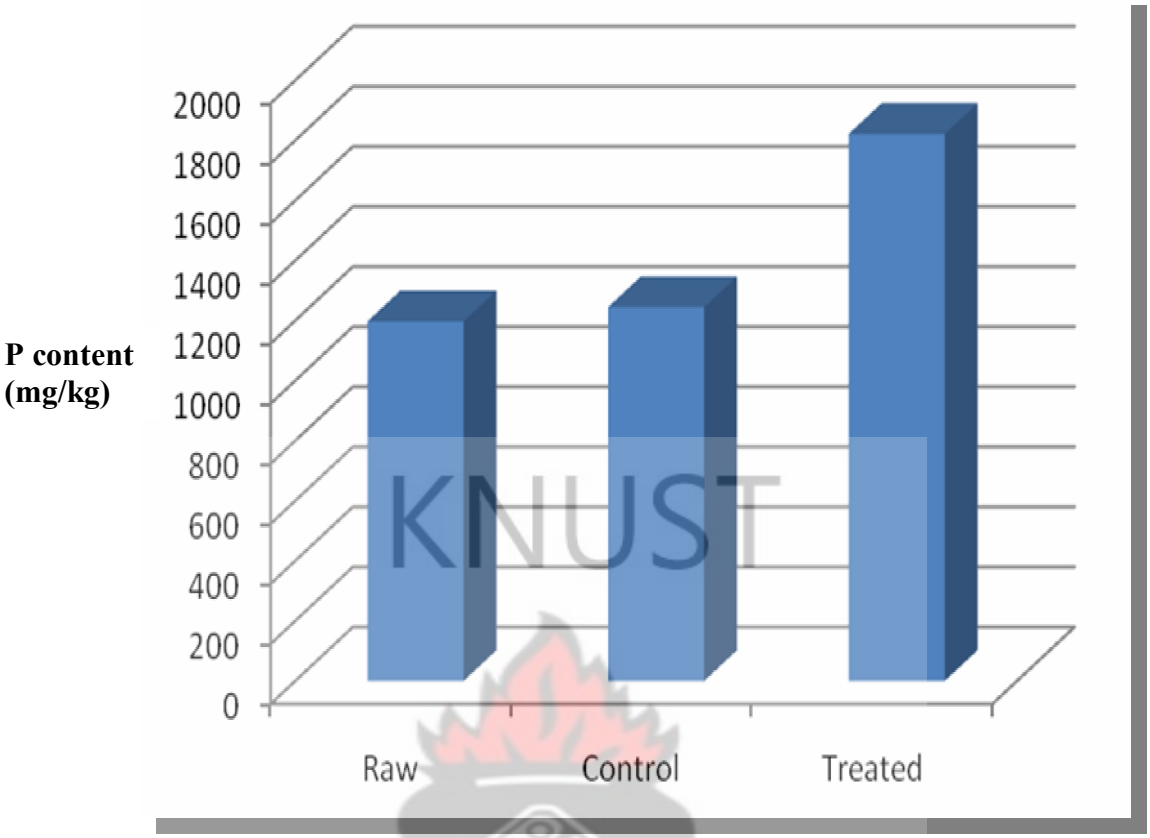


Fig 4.14: Changes in phosphorous content following fermentation of cassava peels with *Pleurotus ostreatus* after six weeks of fermentation.

4.4 Tannin

The levels of tannins in raw cassava peels, the control and treated peels were 0.94, 0.92 and 0.22 percent respectively (Fig 4.15). There was therefore a 75.5% reduction of the tannin levels in the *Pleurotus* fermented peels when compared with that of the control and this reduction is due to the action of the mushroom. The reduction is of key importance to the livestock feed industry and animal nutritionists because of the known anti-nutritional properties of tannins (Wheeler *et al.*, 1994). Waghorn & Jones (1989) reported that 0.17 percent tannin reduced soluble protein and ammonia in the rumen of cattle. Also, at levels greater than 1.5 per cent dry matter, tannin-containing diets fed to ducks caused endogenous protein wastage and inhibited gastrointestinal enzyme activity (Brooker *et al.*, 1994).

Furthermore, Wheeler, *et al* (1994) reported deleterious effects on organic matter digestibility caused by ingestion of 0.6 per cent tannin. Tannins tend to decrease metabolism, inhibit enzyme activity and also depress dry matter digestibility as stated by Hoover, (1986). Based on these observations, it would seem that the tannin levels of cassava peels are high, and are likely to cause adverse effects if the peels are used as animal feed without treatment. It is reasonable to expect that, this decrease in tannin content would be associated with a corresponding increase in the digestibility of the cassava peels as feed, as tannins inhibit the activities of digestive enzymes (Maitra and Ray, 2003).

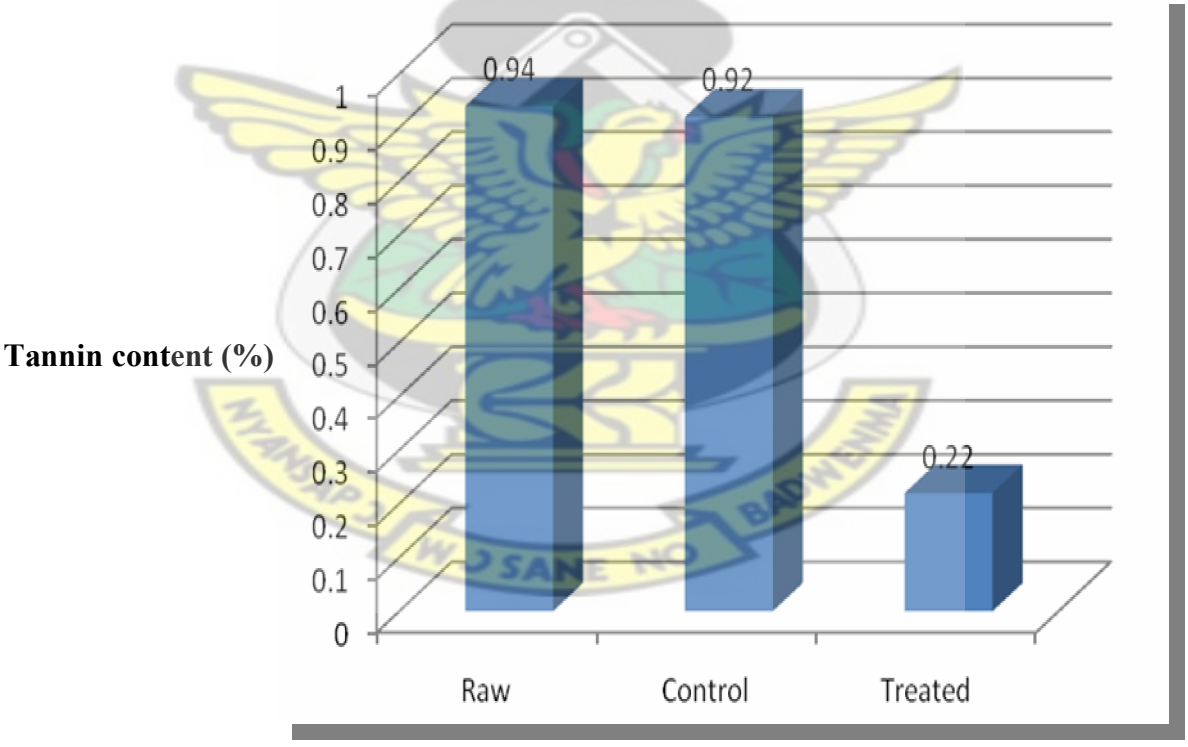


Fig 4.15: Changes in tannin content following fermentation of cassava peels with *Pleurotus ostreatus* for six weeks.

4.5. *In vitro* digestibility studies

The sugar content of the raw cassava peels, the control and the treated peels were found to be 0.5%, 0.7% and 5.5% respectively (Fig 4.16). The high level of sugar in the peels could be attributed to the biodegradation action of the enzyme system possessed by the mushroom. Thus through the enzyme systems in the oyster mushroom, the non digestible polysaccharides such as cellulose, hemicellulose and lignin were broken down to simple sugars which accounted for the observed high level of sugars in the mushroom fermented peels. The raw and the uninoculated peels recorded low levels of simple sugars (Fig 4.16). This observation confirms that, the high level of the sugars in the treated peels resulted from the secretion of extracellular enzymes by the oyster mushroom and subsequent break down of the non-digestible polysaccharides in to simple sugars. Simple sugars constitute an important source of readily available energy for non-ruminants and therefore the high level of simple sugars in the treated peels will readily supply energy to livestock if used as feed.

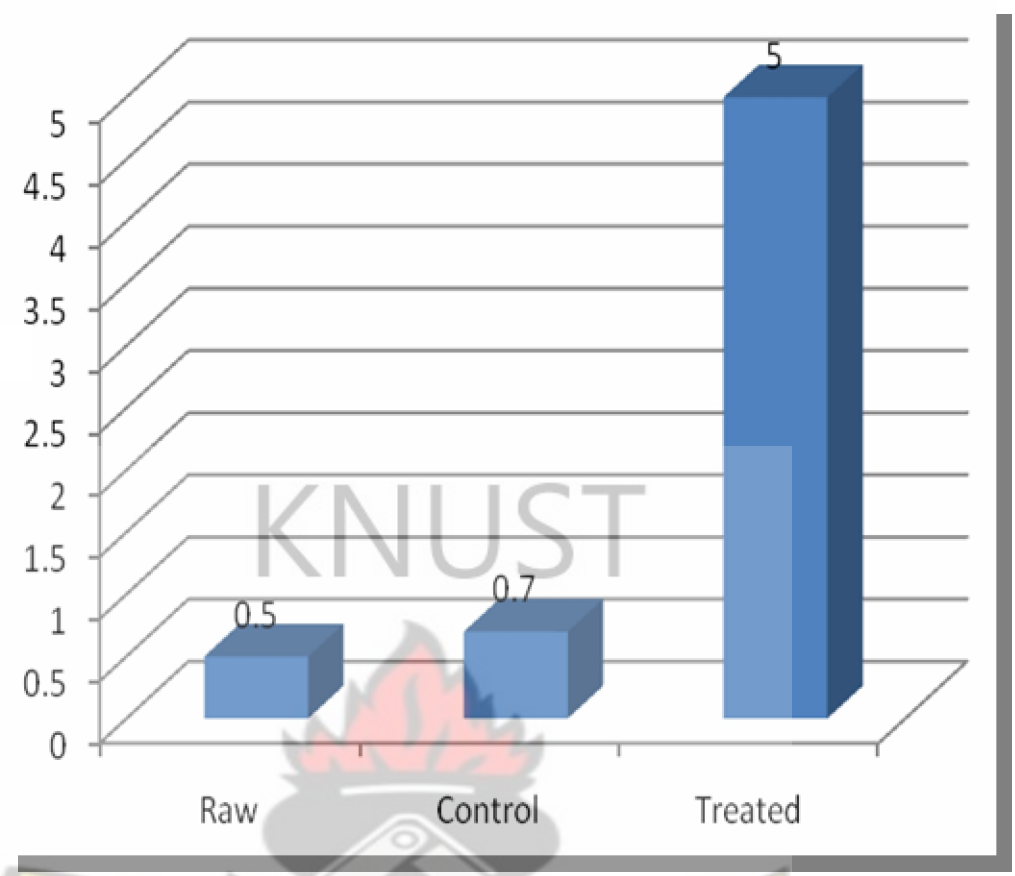


Fig 4.16: Changes in soluble sugar content following fermentation of cassava peels with *Pleurotus ostreatus*.

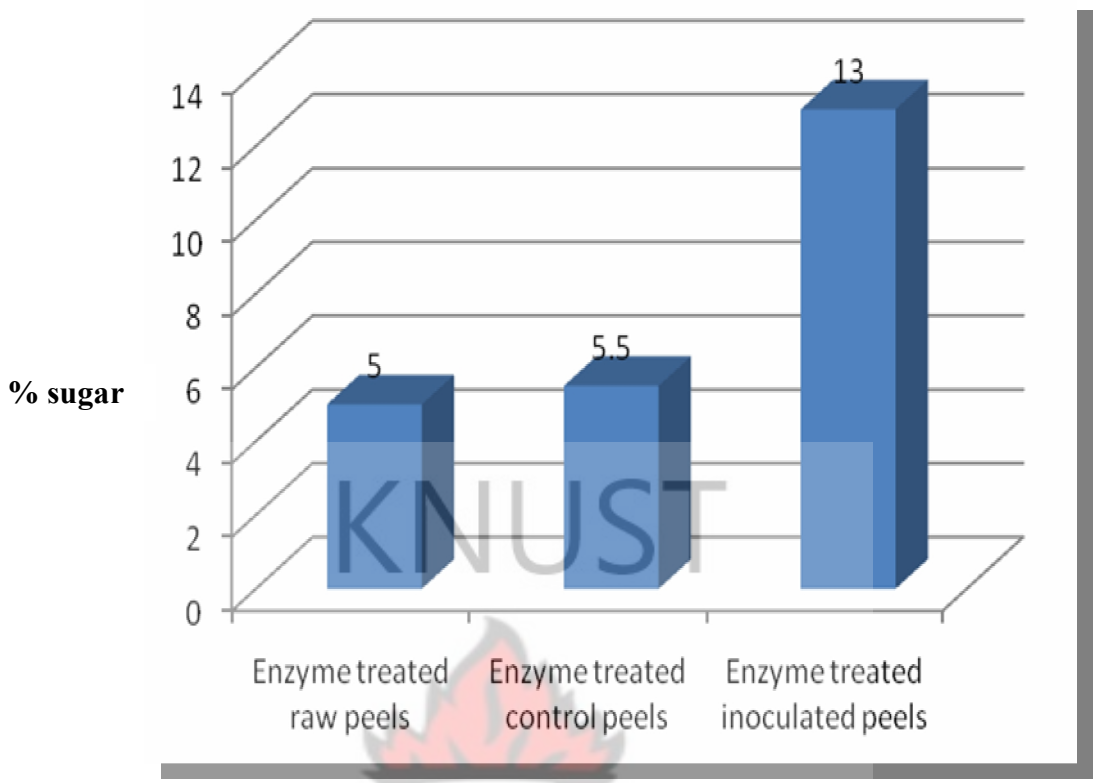


Fig 4.17: Changes in soluble sugar content of cassava peels after treatment with α –amylase.

The digestibility of animal feed is of utmost importance in determining the extent to which an animal would derive nutrients from it. Various approaches have been used in studying the digestibility of animal feed. In this study, the rate of starch digestion was chosen as an index of *in vitro* digestibility. Starch is hydrolyzed by α-amylase to yield disaccharides and oligosaccharides which act as reducing sugars. A comparison of the levels of reducing sugars obtained over a four hour period for the raw cassava peels, the control and peels fermented with oyster mushroom for six weeks revealed a 5.0%, 5.5% and 13.0% respectively (Fig 4.17). Thus the treated peels were about three times more digestible than the control and raw peels. The relatively low rate of digestion of the raw and uninoculated cassava peels is attributable to their high fibre content which was reduced in the mushroom fermented samples.

4.6. CONCLUSION AND RECOMMENDATIONS

4.6.1. Conclusion

Since the nutritional compositions of the peels were tremendously improved, it follows that other non conventional feed ingredients which are readily available can be improved upon using this method. Consequently, inclusion of such into livestock feeding will imply reduced cost of production.

The findings also indicated that fermentation of the cassava peels with *Pleurotus ostreatus* might be an effective means of detanninfying and improving its nutritional value for use as animal feed.

4.6.2. Recommendations

Based on the known high cyanide content of cassava peels, it is recommended that further studies be carried out to ascertain the effect of *Pleurotus ostreatus* fermentation on the cyanide content in cassava peels to verify if the fermentation process can reduce the cyanide content beyond toxic levels in livestock.

The use of the oyster mushroom was found to significantly increase the protein content of the peels statistically but it will be necessary to carry out further studies on the amino acid profile to find out the amino acids composition in the treated peels to ascertain if the enrichment of proteins by the mushroom will meet all the protein requirements of livestock.

Although drum pasteurization of substrates for 10 hours was effective, recent emerging technologies for sterilization of substrates like ash treatment method

should be studied further to ascertain its effectiveness and possible replacement due to the high fuel consumption and the laborious nature of the drum pasteurization method.

Finally it is strongly recommended that, livestock farmers must be educated on the importance of mushrooms as bio-processing agents of agricultural wastes into animal feed. This will help in the improvement of diet in animals nutritionally and also reduce the high cost of feed whiles preventing environmental pollution by agro-wastes.

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PHOTOGRAPH OF FERMENTED CASSAVA PEELS
APPENDIX 1

(A) FIRST WEEK

(B) SECOND WEEK

(C) THIRD WEEK

(D) FOURTH WEEK

(E) FIFTH WEEK

(F) SIXTH WEEK

(G)SEVENTH WEEK

(H) EIGHTH WEEK

APPENDIX 2A

FORMULAE USED IN THE ANALYSIS

1. % Moisture = $\frac{\text{loss in weight of sample} \times 100}{\text{Original weight of the sample}}$

2. % Ash = $\frac{\text{Weight of ash} \times 100}{\text{Weight of dried sample}}$

3. % Fibre = $\frac{\text{Weight of fibre obtained} \times 100}{\text{Dry weight of sample used}}$

4. % Nitrogen = $\frac{100 \times (VA - VB) \times NA \times 0.01401}{W \times 100}$

VA= Volume in ml of standard acid used in titration of the sample

VB= Volume in ml of standard acid used in titration of the blank

NA= Normalilty of the acid used

W= Weight of sample

% crude protein= % total nitrogen × 6.25

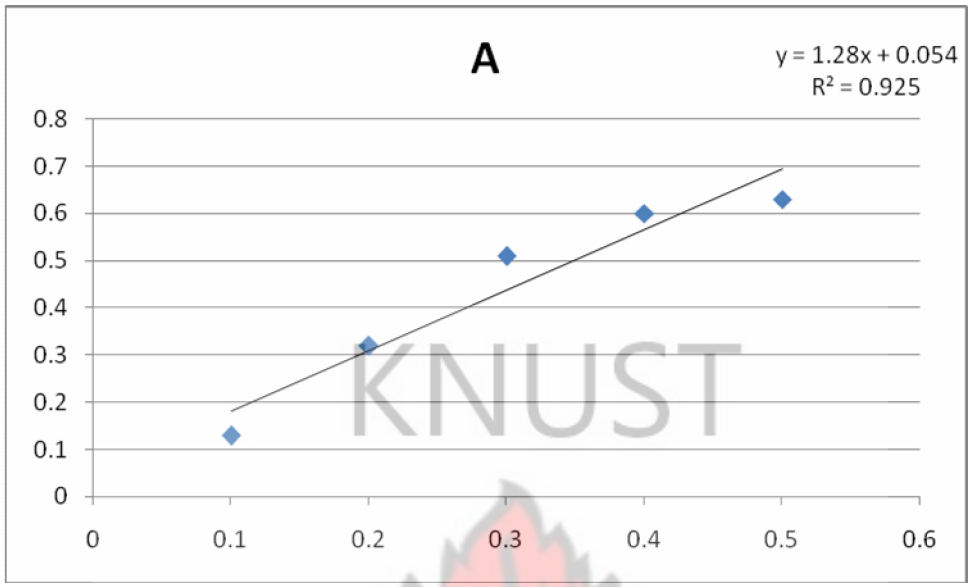
5. NDF = Hemicellulose + Cellulose +Lignin

6. NDF-ADF= Hemicellulose

7. ADF-ADL=Cellulose

8. ADL= Lignin

APPENDIX 2B: STANDARD TANNIC ACID CURVE FOR TANNIN DETERMINATION



APPENDICES 3 ANOVA TABLES FOR PROXIMATE COMPOSITES

APPENDIX 3A 1: MOISTURE ANOVA

Moisture (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.456	7	.922	2666.986	.000
Within Groups	.006	16	.000		
Total	6.462	23			

APPENDIX 3A 2: CONTROL-MOISTURE ANOVA

Control- moisture (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.104	7	.015	149.143	.315
Within Groups	.002	16	.000		
Total	.106	23			

APPENDIX 3B 1: ASH ANOVA

Ash (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	43.566	7	6.224	1484.787	.000
Within Groups	.067	16	.004		
Total	43.633	23			

APPENDIX 3B2: CONTROL-ASH ANOVA

Control-ash (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.015	8	.002	8.582	.110
Within Groups	.004	17	.000		
Total	.018	25			

APPENDIX 3C 1: PROTEIN ANOVA

Protein (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	23.757	8	2.970	2419.316	.000
Within Groups	.021	17	.001		
Total	23.778	25			

APPENDIX 3C 2: CONTROL-PROTEIN ANOVA

Control-protein(%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.012	8	.002	3.395	.163
Within Groups	.008	17	.000		
Total	.020	25			

APPENDIX 3D 1: FIBRE ANOVA

Fibre (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	249.721	8	31.215	6616.668	.000
Within Groups	.080	17	.005		
Total	249.801	25			

APPENDIX 3D2: CONTROL-FIBRE ANOVA

Control-fibre (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.260	7	.323	231.945	.144
Within Groups	.022	16	.001		
Total	2.282	23			

APPENDIX 3E1: TOTAL CARBOHYDRATE ANOVA

Carbohydrates (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	99.164	8	12.395	14499.255	.000
Within Groups	.015	17	.001		
Total	99.178	25			

APPENDIX 3E2: CONTROL-TOTAL CARBOHYDRATE ANOVA

Control-carbohydrates (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.676	7	.239	3023.609	.398
Within Groups	.001	16	.000		
Total	1.677	23			

APPENDICES 4 ANOVA TABLES FOR FIBRE ANALYSIS

APPENDIX 4 A1: NDF ANOVA

NDF (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1663.838	8	207.980	2916.086	.000
Within Groups	1.212	17	.071		
Total	1665.051	25			

APPENDIX 4 A2: CONTROL-NDF ANOVA

Control-NDF (%)

Control-NDF (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.190	7	.027	20.343	.607
Within Groups	.021	16	.001		
Total	.212	23			

APPENDIX 4 B1: ADL ANOVA

ADL (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25.131	8	3.141	1576.866	.000
Within Groups	.034	17	.002		
Total	25.165	25			

APPENDIX 4 B2: ADL CONTROL-ANOVA

Control-ADL (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.003	8	.000	.588	.775
Within Groups	.010	17	.001		
Total	.013	25			

APPENDIX 4 C1: ADF ANOVA

ADF (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	312.073	8	39.009	12028.202	.000
Within Groups	.055	17	.003		
Total	312.128	25			

APPENDIX 4 C2: CONTROL- ADF ANOVA

Control-ADF (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.022	8	.003	19.891	.413
Within Groups	.002	17	.000		
Total	.025	25			

APPENDIX 4 D1: Hemicellulose ANOVA

Hemicellulose (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	540.218	8	67.527	3140.514	.000
Within Groups	.366	17	.022		
Total	540.583	25			

APPENDIX 4 D2: Control-Hemicellulose ANOVA

Control-hemicellulose (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.166	8	.021	83.856	.912
Within Groups	.004	17	.000		
Total	.170	25			

APPENDIX 4 E1: Cellulose ANOVA

Cellulose (%)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	129.482	7	18.497	2836.658	.000
Within Groups	.104	16	.007		
Total	129.586	23			

APPENDIX 4 E2: Control-Cellulose ANOVA

Control-cellulose (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.017	8	.002	14.998	.664
Within Groups	.002	17	.000		
Total	.019	25			

APPENDIX 4 F1: Lignin ANOVA

Lignin values (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25.131	8	3.141	9768.880	.000
Within Groups	.005	17	.000		
Total	25.136	25			

APPENDIX 4 F2: Control-Lignin ANOVA

Control-lignin (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.003	8	.000	.588	.775
Within Groups	.010	17	.001		
Total	.013	25			