NUTRIENT CONTENT AND YIELD IN THREE FLUSHES OF OYSTER MUSHROOMS (*Pleurotus sajor caju* and *Pleurotus Hk 35*)

BY

LINDIWE LUCIA MKHATSHWA

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DECLARATION

I Lindiwe Mkhatshwa declare that all the work presented in this dissertation is my work and has not been submitted for a degree at any University.

Signature..... L Mkhatshwa .....

Date ..... 30 - 10 - 2002 .....

APPROVAL

This dissertation of Lindiwe Mkhatshwa is approved as fulfilling part of the requirement for the award of the degree of Master of Science in Agronomy (Crop Science) by the University of Zambia.

Examiner's Name and Signature	Date
Irene Nawa	24/12/2002
	13.11.02
M. Phiri Doctor	24/12/2002

ABSTRACT

A study on oyster mushroom yield and chemical composition was carried out. The aim of this work was to provide more information on the yield and chemical composition of mushrooms of each flush of *Pleurotus sajor caju* and *P. Hk* 35 grown on water hyacinth, wheat and soybean straw. The data within each flush was subjected to the analysis of variance following the split plot design. Combined flush analysis was done to compare the yield and chemical composition of the three flushes and linear regression was used to compare the chemical composition of the substrate and mushroom.

The species and flush had a significant ($P \leq 0.05$) influence on the yield and chemical composition of the mushrooms. The type of substrate had an influence on the chemical composition of mushroom but not on the yield. Mushrooms grown on water hyacinth were high in protein (31.7%) and ash (10.4%) while those from soybean straw were high in carbohydrates (42.2%). Mushrooms grown on wheat straw were high in ash content (10.4%). Water hyacinth was the best straw since mushrooms produced were high in protein. It was also observed that the chemical composition of the mushrooms depends mainly on the substrates composition. *Pleurotus sajor caju* had higher ash content (10.29%) and *P. Hk 35* produced significantly higher yield (540g) than *P. sajor caju* (377g). Mushrooms from the three flushes differed in yield and chemical composition. Mushrooms from the first and second flush were the best since they had high yield and were high in carbohydrate, fiber and proteins while those from the third flush were high in fat and ash.

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1.0 INTRODUCTION

1.1 Background information

Mushrooms are fungi. They are often large, fleshy and lack chlorophyll. Most are saprophytes, others are mycorrhizas, but some are parasites. Edible mushrooms are a potential source of nutrients. They can convert nutritionally valueless substances into food. It is advantageous to grow mushrooms, because of the following reasons:

- They are a nutrient source
- Their production help create jobs
- They help improve family income
- They protect the natural mushroom flora
- They require less space
- Their production help control the burning of agricultural waste
- Their production help reduce air pollution
- Their production help keep fields, roadsides and forest margins clean
- Spent mushroom substrates improve the soil

Mushrooms were first cultivated in France in 1650, ever since that time mushroom production has been increasing worldwide at a very fast rate. From 1986 to 1991, oyster mushroom production increased from 169,000 to 917,000 tons (Royse, 1996). There has been an increase in production due to an increase in population and demand. Most people are attracted to mushrooms for their flavour, nutritive value and income generating potential (Kushalappa, 1998). In some parts of the world mushrooms do not get much attention because there are a number of naturally growing species that are edible but some may be poisonous and therefore people are afraid to eat them.

There are different types of mushrooms that are suitable for cultivation for example, *Auricularia species,* button mushroom (*Agaricus species*), *Ganodema species, Hericium erinacaeus,* the oak tree mushroom (*Lentinus species),* oyster mushroom (*Pleurotus species*), paddy straw mushroom (*Volvariella species)* and *Tremella fuciformis* (Atkins, 1972). The choice of species to grow depends on available waste materials, appropriate facilities, cost of necessary equipment, skill required to manage the life cycle of the fungus and the market for that species. Considering all the above requirements, *Pleurotus* and *Shiitake species* are the best choice for mushroom growers. Oyster mushrooms look like oysters (shell like appearance) and appear in different colours; they may be white, grey, brown, yellow or pink. The oyster mushroom can be grown in a wide range of temperatures (15°C to 30°C) therefore

it is suitable for areas with moderate and subtropical temperatures. It is possible to grow them throughout the year in controlled conditions.

Oyster mushrooms grow on wood in nature but can be cultivated on several substrates, which make them suitable even for small-scale producers. When using substrates, a variety of containers can be used e.g. baskets, bottles, jars and plastic bags. Preparation and pre–treatment of the substrate is necessary before packing in the containers. The spawn is distributed in the substrate and then packed in the containers. The containers are placed in a growing room where conditions are made to favor mycelium growth, and later production of the sporophores. Fruiting in mushrooms occur in flushes or breaks. With oyster mushrooms, once the first flush has been harvested, the next flush is expected to occur after 7-10days. Harvesting can continue as long as the substrate is still white. Mushrooms are highly perishable and are therefore used immediately or they can be conserved for future use.

1.2 Justification

Edible mushrooms are considered important for their flavor, nutritive value and income potential. If existing species of mushrooms are to be improved or new mushroom species are to be cultivated, the nutritional value and yield are important parameters to be considered. In a majority of nutrient studies on mushrooms general statements have been made that compare

mushrooms with other nutrient sources e.g. meat and vegetables. Some researchers say that the nutritional value of mushrooms lies between meat and vegetables. According to them mushrooms provide a rich addition to the diet in the form of proteins, carbohydrates, valuable salts and vitamins (Ju, 1994; Hussain, 2000). Others have even assigned values to the chemical composition of most mushrooms e.g. *Pleurotus florida* contain 58% carbohydrate, 11.5% fiber, 27% protein, 1.6% fat and 9.3% ash (Oei, 1991). The flush from which the nutrient composition was determined is not stated. Other researchers have mentioned that mushrooms can produce 3 to 4 flushes but the chemical composition of the mushroom and the yield from the first to the last flush is never stated. In any food source consumers are interested in good taste, texture, digestibility and the nutritive value whilst on the other hand producers are interested in high income (Chang and Hayes, 1978). It is important that the producer satisfies his own needs and also of the consumer. In doing so it is necessary to give more information on the nutritional value of the product.

A study was carried out to determine the nutrient content and yield of *Pleurotus sajor caju* and *Pleurotus Hk 35* in relation to the type of substrate, species and flush. Such information will help to enhance production and consumption of oyster mushroom.

The objectives of the study were:

1. To compare the yield and chemical composition of mushrooms grown on water hyacinth, soybean and wheat straw.

2. To determine the yield and chemical composition of *Pleurotus sajor caju* and *Pleurotus Hk 35*.
3. To compare the yield and chemical composition of the mushroom in the first three flushes.
4. To compare the chemical composition of the substrate and that of the mushroom.
5. To determine the most profitable straw for use in mushroom cultivation among the three types used.

2.0 LITERATURE REVIEW

2.1 Mushroom cultivation methods

The basic concept in mushroom cultivation is to start with a bit of mycelium and to expand that mycelial mass to the point that it has enough volume and stored up energy to support the final phase of the mushroom reproductive cycle, which is the formation of fruiting bodies or sporophores of mushrooms. To do this, either spores from a spore print or a fresh mushroom or a culture bought from a culture bank or other source is used. Growing out the spores is the sexual reproductive cycle and requires the combination of two genetically compatible spores to produce a new individual fungus. Reproduction from a culture or a fresh mushroom involves asexual reproduction. The original organism is cloned. According to Chadha (1992) the medium used for spawn production influences the mushroom yield. Gupta and Sharma (1992) observed that sorghum grain spawn is superior to straw spawn. Rathaiah and Shill (1998) compared the yield between parboiled paddy grain spawn and wheat grain spawn of oyster mushrooms. They found that the yield from parboiled paddy grain spawn was 759g/kg of straw and that of wheat grain spawn was 853g. Stamets and Chilton (1984) outlined the procedures for producing grain and sawdust spawn. They reported that the spores or a small piece of the mushroom or culture are placed on agar medium in petri dishes and the mycelium

is allowed to grow out. After the mycelium has colonized the petri dishes, usually after two weeks, it is transferred onto sterilized grain (rye, wheat, sorghum or millet). In 2 to 4 weeks it will then completely colonise the grain and this is called mother spawn.

The grain spawn can then be used to inoculate more grain for a larger quantity of grain spawn or can be used to make sawdust spawn or to inoculate the final substrate for mushroom production. Sawdust spawn is used to inoculate logs directly and to inoculate outdoor beds and is made by transferring grain spawn to previously sterilized hardwood sawdust. The mycelium will run through the sawdust in about 3 to 4 weeks and at this point it will be ready for use. The above procedures should be carried out in a sterile environment otherwise there will be a large percentage of contamination due to moulds and bacteria. The media used e.g. agar, grain, sawdust must be sterilized beforehand to give a competition free environment for the mycelium to grow.

The spawn produced is then used to inoculate the final substrate that will eventually produce mushrooms. The substrate can be logs, stumps, sawdust, wood chip mixtures, straw, cardboard and compost. The main thing is to introduce the fungus into a medium that is free of other fungi. If growing is done indoors on sawdust, wood chips or straw, the type of mushroom will determine the amount of processing of the substrate. *Shiitake, Maitake, Enoki* (*Flammulina velutipes*) are grown on sawdust and require sterilization of the substrate in an

autoclave because they do not compete well with moulds or bacteria. *Pleurotus species* and *Stropharia* are grown on a pasteurized substrate because they can consume the other organisms during their life cycle. Oyster mushrooms are versatile. They can grow on almost any substrate and can be cultivated in different methods. Substrates high in cellulose, hemicellulose and lignin can be used. They are amongst the easiest to grow (Volk, 1998). Cultivation methods that can be used in the growing of oyster mushrooms are cultivation on logs, use of pasteurized straw and sterilized sawdust in plastic bags.

(a) Cultivation on logs

According to Stamets (2000) almost any type of hardwood logs can be used for cultivating mushrooms e.g. oak logs. Species grown on logs include *Lentinus edodes* (shiitake), *Pleurotus ostreatus, Pleurotus pulmonarius*, *Ganoderma lucidum* and *Grifola frondosa.* Commercially the most common species grown on logs is shiitake (*Lentinus edodes*). This is due to the fact that log cultivation is usually less expensive than sawdust cultivation and shiitake gives a higher financial return than other types of mushrooms. For commercial cultivation oak is the preferred hardwood because it has a longer bark retention period than other species. The bark keeps the moisture in the log and keeps out competitor fungi. Once the bark has fallen off the log it becomes useless. Typically oak logs last an average of one year per inch of diameter. Winter cut logs are preferred due to the large quantity of sugars in

the wood. Stamets (2000) said that logs are inoculated by drilling a series of holes 3.8cm deep spaced about 12.7cm apart in a series of rows about 10cm apart. The holes are filled with sawdust spawn and sealed with molten wax. The wax prevents the spawn from drying out and dying. The logs are then stacked in the bush under some artificial shade until the mycelium takes over the whole log. Spawn run period varies from 6 to 8 months. After the spawn run, the logs are ready to produce fruiting bodies of shiitake, they are raised and leaned against a wire between two trees or posts; this is done so that the mushrooms do not come in contact with dirt. To induce the logs to produce, a sprinkler can be used to supply water or they can be soaked in water for 16 to 48 hours. The water is the stimulus needed by the mycelium to start the reproductive cycle. After fruiting, the logs are rested for a couple of months and then watered again to produce another crop. Oei (1991) reported that *Lentinus edodes* cultivated on wood logs produce higher quality mushrooms than when grown on sawdust.

(b) Cultivation on beds

Many species of mushrooms require stimuli, which are only available outdoors to produce fruiting bodies (Sarkarm *et al.*, 1995). It may be the bacteria in the soil, the pH of the soil, the varying temperatures, whatever it is they will not do as well indoors in a controlled environment. Sarkarm *et al.* (1995) described one outdoor growing technique that involves digging of a trench or making of a raised bed from soil, bamboo or wood in a suitable spot,

which may be under bushes, in the garden between the rows of vegetables or in a bush. They said that the trench is filled with substrate and then inoculated with grain spawn or sawdust spawn and finally covered with topsoil and watered. If kept moist, the mycelium will take over the substrate and a couple of months later will produce mushrooms. Suman and Sharma (1999) cultivated *Volvariella volvaceae* and *Pleurotus flabellatus* under natural conditions. They reported that 400-500g fruit bodies were harvested per flush in *P. flabellatus* at a temperature range of 20°C to 30°C and the biological efficiency (fresh weight of mushrooms compared to the air-dried weight of substrate) was found to be 92 to 95%. In *Volvariella volvaceae* an average yield of 750g to 1kg per flush per bed was obtained at a temperature range of 30°C to 42°C.

(c) Cultivation on sterilized sawdust in plastic bags

Most of the primary wood decomposers can be grown indoors on a block made up of sawdust, wood chips and bran. Davis and Aegerter (2001) said that *Lentinus edodes* and *Pleurotus species* could be grown on sawdust blocks. Sawdust in most countries has replaced the wood logs due to lack of suitable logs. Stamets (1993) described the cultivation of mushrooms on sawdust and reported that the ingredients are mixed together and placed in autoclavable polypropylene bags that have a breathable patch (a filter that allows the exchange of gases). The bags are then sterilized to kill all bacteria and competitor fungi. After sterilization, grain

spawn is mixed into the sawdust in a sterile environment and the bags are then sealed. The mycelium will run through this mixture in 3 to 4 weeks at 25°C. After full colonization, the blocks are moved to a growing room where the bags are removed. The growing room is kept at a constant temperature and high humidity thereby promoting the fruiting of the mushrooms. After the mushrooms are picked, the blocks are rested for two weeks and the cycle is begun again by sprinkling them with water.

(d) Cultivation on straw

Oyster mushrooms are very aggressive colonisers and can compete successfully with some bacteria. They grow very well on pasteurized straw of different kinds. Pasteurization only requires the substrate to be heated to 70°C for about an hour, which can be accomplished by simply boiling in water. According to Sohi (1989) grain spawn is mixed with the cooled down straw in a clean area and the mixture is bagged. The plastic bags are punched so that mushrooms can emerge through the openings. The mycelium will run through the straw in a couple of weeks and then the bags can be placed in a growing room and fruited. The fruiting period depends on the strain and substrate used.

2.2 Mushroom Growth

Mushroom life cycle is different from those of green plants. Since they do not have chlorophyll, they depend on living organisms or obtain their food by decomposing dead organic matter. Mushroom growth is divided into two stages, one vegetative and the other reproductive stage. The vegetative stage involves the growth of hyphae and mycelium whilst the reproductive stage involves the production of fruiting bodies (sporophores) and spores. The transition from the vegetative stage to the reproductive stage is affected by environmental factors e.g. light, carbon dioxide concentration, temperature and humidity. After spawning the substrate with the desired strain, the hyphae grow throughout the substrate and invade the entire substrate fully in about 15 days. The mycelium collects nutrients by breaking down the organic material. It excretes hydrolytic enzymes at the hyphal tip that hydrolyse the available nutrients of the substrate. The hydrolysed substances enter the mycelium across the cell membrane and stimulate growth and production (Beetz and Greer, 1999).

The mycelium extends into the substrate, increasing the accessibility of available nutrients. Such growth mechanism enables mushroom hyphae to penetrate solid substrates. After the substrate has been fully colonized by the mycelium, fruiting will occur if all the specific requirements have been met. The initial stage of fruiting is marked by the formation of

outgrowths that are the size of a pinhead and continue to expand and grow larger into mushrooms. These are the fruiting bodies that produce spores (Scott, 2001).

As the mushrooms grow, the chemical composition of the straw changes. During mycelial growth, amino acids, vitamins and biologically active substances accumulate in the straw. Changes in the chemical composition of wheat straw used for cultivating *Pleurotus ostreatus* was studied by Bilay and Bis'ko (1985). They observed that 15 days after inoculation, the rate of change of cellulose (16.4%) and lignin (15.9%) content in the straw was the same. The crude protein increased from 3.6% to 6.3%. Jadhav *et al.* (1998) also studied the biochemical changes in ten agro residues caused by growth of oyster mushrooms. They observed that the concentration of minerals such as nitrogen, phosphorus and calcium increased with a reduction in the carbon: nitrogen ratio. Their study showed that with mushroom growth there are several substances that accumulate in the straw and are taken up at different rates. Proteins and minerals kept accumulating as mycelium was growing. The decrease in C: N ratio showed that more carbon compounds were taken up and the increase in the minerals and protein content in the straw showed that more nutrients were available for mycelium uptake.

There are nutritional requirements that should be met in order for the mushrooms to grow. Carbon, nitrogen, lipids, minerals and vitamins are required for mushroom growth. According to Höfte (1995) carbon is for structural and energy requirement of the cell. Nitrogen is

essential for the synthesis of proteins, purines and pyrimidines. Vitamins and some minerals are constituents of membranes and function as coenzymes. Lipids are also essential in mushrooms since they serve as food reserves and as components of membranes. The substrate and mycelium serve as a source for the nutrients required by the mushrooms (Miles and Chang, 1997).

2.3 Substrates

The medium on which the mycelium grows is called the substrate. The properties of the substrate determine which species can be grown. Access to an economical substrate suitable to a species is also an important factor in deciding which species to grow (Volk, 1998).

Substrates high in cellulose and hemicelluloses can be successfully used. Substrates like banana leaves, citrus peels, coffee sawdust, corn (cobs and stalks), cotton straw, grasses, gum and pine sawdust and wood shavings, legume straw and pods, paper waste, water hyacinth straw, wheat straw and wood logs have been successfully used for cultivating oyster mushroom. Mushrooms are rich in enzymes such as cellulases, ligninases and proteases, which make it possible for mushrooms to biodegrade and assimilate a multitude of organic waste substrates (Poppe, 2000).

The internal condition of the substrate, determines if the mycelium can grow into the substrate. Solid substrates have a macromolecular structure (e.g. cellulose, starch, pectin, lignocelluloses and fibers). Each plant material contains variable proportions of chemical compounds, which are hydrolyzed by the enzymes. The chemical compounds vary in their linkages and also the easiness with which they can be broken down by enzymes secreted by the mycelium (Raimbault, 1998). Eswaran and Ramanujam (2000) studied the degradation rate of carbon sources in paddy straw and found that lignin and cellulose degrade at a faster rate than hemicelluloses. The maximum rate of degradation of lignin was 56.9%, cellulose 40.5% and hemicelluloses 17.5% (hot water pre–treatment).

Starchy substrates are more easily degraded compared to lignocelluloses. It is necessary to prepare and pre-treat the substrate in order to convert it to a form suitable for use. The pre-treatments include size reduction, sterilization, pasteurization, supplementation with nutrients and setting the moisture content and pH through a mineral solution. Such treatments pre-degrade macro molecular structures and make conditions favorable for mycelium growth and help eliminate contaminants (Raimbault, 1998). Several studies have been done on the use of nutrients by mushrooms. Höfte (1995), in an experiment on the depletion of nutrients from the substrate by *Coprinus cinereus,* concluded that this species depends on nutrients from both the mycelium and substrate. It was observed that the amount of mushrooms produced was proportional to the amount of nutrients made available. A decrease in dry weight of mycelium

was correlated with the formation of mushrooms. This study demonstrated that during fruiting, nutrients are depleted from the mycelium and these are replaced by nutrients from the substrates. Accordingly, it was concluded that with more easily accessible nutrients to the mycelium, the yield would be higher.

2.4 Yield

For successful mushroom production it is necessary to ensure that production is economical and efficient so that higher yields can be obtained. Yield depends on several factors such as the type of substrate, species, cultivation technique and environmental factors that enhance growth. The yield of *Pleurotus species* varies from 30% to 50% of the net weight of the substrate (Ju, 1994) and three to four flushes can be obtained at an interval of 7-10 days. However, the first two flushes give the maximum yield of about 80%. The interval between the flushes may be longer or shorter, depending on temperature, humidity, mushroom species and the stage of mushroom picking. Yield can be improved if the outside of the block is scraped off 1-2cm deep and sprayed with water (Pasamba, 1990) after each flush.

The environment that affect mushroom yield includes temperature, relative humidity, light and air. Thakur (2000) reported that the highest biological efficiency of 62% was obtained in *Pleurotus columbinus* at a temperature of 23.57°C and at minimum and average temperatures

of 13.93°C and 18.64°C respectively and at relative humidity of 63.33 to 84.34%. It was observed that correlation between yield and temperature was negative and positive between yield and relative humidity.

Substrates have different effects on the yield since their structures and chemical composition differ. Many workers have presented the benefits of different substrates on the cultivation of *Pleurotus species*. Pani and Mohanty (1998) emphasised on the usefulness of water hyacinth (*Eichhornia crassipes*) in the cultivation of *Pleurotus species*. They reported that *Pleurotus species* grow fast and give higher yield on water hyacinth. The biological efficiency of *Pleurotus sajor caju* has been reported to be 50% on water hyacinth. Quimio (1985) also examined the performance of oyster mushrooms on water hyacinth in combination with paddy straw and concluded that high yields can be obtained when the two straws are combined. The yield is much higher than when water hyacinth is used alone. The biological efficiency of paddy and water hyacinth has been reported to be as high as 78.3% to 80.0%.

Utility of agricultural wastes, namely paddy straw and sugarcane bagasse and coconut waste for culturing *Pleurotus citrinopileatus* was evaluated by Rajkumar and Dharmaraj (1999). They observed maximum growth rate and high yields of mushrooms on paddy straw and bagasse. Maximum protein and amino acid contents were recorded in mushrooms grown on paddy straw.

Yildiz (1999) showed the benefits of using peanut and sorghum straw in the cultivation of *Pleurotus florida*. *Pleurotus florida* cultivated on peanut straw took a short time to fruit and gave a high yield. The first, second and third flushes were obtained after 33.6, 47.2 and 63.8 days respectively. The yield from a 100g substrate for the first three flushes was 11.2, 7.7 and 4.8g respectively. A high percentage of the total yield came from the first flush (47%), 33% from the second flush and 20% from the third flush. When the same species was cultivated on sorghum straw, the yield was lower and harvesting was possible only after a long time. Harvesting was done after 63.5, 75.2 and 94 days for the first three flushes. The yield for the first three flushes in sorghum straw was 5.0, 3.3 and 3.1g respectively. The highest yield came from the first flush (44%) while 29% from the second flush and 27% from the third flush. The study showed that the use of different substrates resulted in differences in yield.

Singh (1998) cultivated *Pleurotus abalones, P.citrinopileatus*, *P. flabellatus, P. florida, P. ostreatus* and *P. sajor caju* on sugarcane residues (sugar cane green leaves and tops, trash, bagasse) and wheat straw and sawdust alone as well as in combination. He found that *P. florida* gave maximum yield on wheat straw (127.8g), bagasse (119g) and green leaves (37g) whereas *P. sajor caju* gave higher yield on trash (81.4g) and sawdust (38.2g). *Pleurotus florida* was found to be the best species for cultivation on mixture of sugar cane trash and wheat straw. Similarly sugarcane bagasse and wheat straw in 1:1 mixture produced significantly higher yield of sporophores as compared to straw and bagasse alone.

Thangamathu (1989) also recorded similar findings and reported that the biological efficiency of *Pleurotus species* on sugarcane bagasse was 100.2%, followed by paddy straw (80.4%) and wheat straw (79.8%).

The cultivation method is known to influence the production of sporophores (Smith, 1980 and Jandaik and Kapoor, 1974). Dubey (2000) reported that *Pleurotus species* can be successfully cultivated in polythene bags. He reported that polythene bags supported maximum yield and produced many more sporophores in *P. flabellatus*. The yield was 332.8g per bag and 130.2 sporophores per bag. The biological efficiency was 73.1% in polythene bags and 71.6% in nylon net bags. Maximum yield was obtained in polythene bags followed by nylon nets and the wooden mould. Dubey (2000) also determined the performance of *P. flabellatus* in straw treated in different ways and reported good performance of this species in steam-pasteurized straw. Steam pasteurization of the straw induced early pinning and the production of sporophores was very high and showed high biological efficiency.

Shukla and Biswas (2000) recommended the use of hot water and chemical treatment of straw for the cultivation of *Pleurotus species*. According to them, the yield was much higher. The biological efficiency in hot water treatment was found to be 107.75% and 73.5% in plain water. A much higher biological efficiency was obtained when the straw was sterilized in a

mixture of Bavistin and formaldehyde. The biological efficiency with this treatment was much higher and gave a value of 122.3%.

Se Jong (2002) assessed the effect of vinyl mulch on *Pleurotus ostreatus* and *P. sajor caju.* At spawning, the beds were covered with perforated transparent and black plastic sheets. This technique made picking quicker and also made more efficient use of labour since mushrooms occurred in bunches. The mean yield for *P. ostreatus* increased to 10.8kg/m^2 and was higher by 5.7%. The mean weight of a mushroom bunch from vinyl-mulched beds was 283g (33 fruit bodies) as compared to 117g (15 fruit bodies) obtained using the conventional growing methods. The total yield of mushrooms from both cultivation systems in *Pleurotus sajor caju* was 8kg /m^2. The mean weight of mushroom bunches from a mulched bed was 225g (79 fruit bodies) as compared to 197.5g (79 fruit bodies) obtained in the convectional method. Very few fruit bodies aborted after initiation with the mulch technique. In this study it was observed that mulching increased the number of fruiting bodies per bunch and also gave higher yield.

The yield can also be affected by the size of the substrate. Zhang (1998) studied the effect of straw size on mushroom yield. Rice straw and wheat straw was used for the cultivation of *Pleurotus sajor caju.* The study showed that the yield was high in *P. sajor caju* cultivated on

rice and wheat straw chopped up to 2.5cm, further size reduction lowered yield. Size reduction increases the accessibility of nutrients by mycelium.

2.5 Nutritional Attributes

The nutritional composition of mushrooms is affected by many factors. These include strain differences, composition of the substrate and the environmental factors. The genetic nature of a strain coupled with its metabolism, determine how it utilises nutrients from its substrate and what effect the substrate has on the composition of the mushroom (Chang and Hayes, 1978). Chang and Hayes (1978) also reported that the intrinsic compositional variability in the mushroom was a major factor contributing to the differences in the nutritional composition of strains. Chang and Quimio (1982) studied the nutrient composition of two *Agaricus bisporus* strains (*Hauser* and *Somycel 87*) and concluded that the chemical composition of the strains was different. The *Hauser* strain contained 21% crude protein whilst the *Somycel 87* contained 27 to 35%. Ell-kattan and Salama (1999) studied the nutrient composition of *P. ostreatus* and *P. florida* grown on rice straw and supplemented with legume waste. They observed that the dry matter and protein content of *P. ostreatus* and *P. florida* increased with a higher percentage of legume waste. *P. ostreatus* was more responsive to supplementation than *P. florida.* The highest beneficial effect on yield was achieved when rice straw was enriched with 50% legume waste. The biological efficiency was 121.3 and 116.2 for *P. ostreatus* and

P. florida respectively. This study showed that the species responded differently to supplementation, since their intrinsic make up was different.

Mushrooms are rich in carbohydrates and crude protein, moderate in fiber and ash and low in fat. The nutritional composition of *Pleurotus species* in a dried sample is 57.6 to 81.8% carbohydrates, 7.5 to 27.6% fiber, 10.5 to 30.4% crude protein, 1.6 to 2.2% fats and 6.1 to 9.8% ash (Oei, 1991). Mushrooms contain almost every nutrient present in their growth substrate. Patrabansh and Madan (1999) studied the mineral content of *P. sajor caju* grown on different substrates. The mineral content was found to differ with the substrate. There was an increase in mineral content when mushrooms were grown on substrates rich in mineral content. Among the eight minerals determined, the potassium content was the highest followed by phosphorus, magnesium and sodium. The mineral content of the substrates was determined before cultivation. The mineral contents per 100g substrate were Ca (347mg), P (151mg), K (1805mg), Na (127mg), Mg (227mg), Fe (53mg), Mn (10mg) and Zn (3.1mg). The mineral contents of the fruiting bodies per 100g ranged as follows; Ca (25.1mg to 35.3 mg), P (448mg to 602mg), K (2146mg to 2350mg), Na (139mg to 229mg), Mg (153mg to 224mg), Fe (9.74mg to 20.75mg), Mn (2.5mg to 4.0mg) and Zn (2.2mg to 3.1mg).

Mushrooms contain a higher proportion of proteins on a dry weight basis than any comparable fruit or vegetable (Oei, 1991). The protein content varies from 19 to 35 % on a dry weight

basis. Protein content is usually analysed by determining the nitrogen content of the mushroom. The total nitrogen depends on the kind and the concentration of the nitrogen source. An increase in the available nitrogen is accompanied by as much as three fold increase in nitrogen in the mushroom (Vetter and Rimoczi, 1993). Vetter and Rimoczi (1993) also reported that mushroom nitrogen content also varied with the developmental stage. They analysed the protein content in caps and stipes of *P. ostreatus* in four developmental phases. The examined phases were: A (cap diameter < 5 cm); B (diameter 5-8 cm); C (diameter 8-10 cm) and D (diameter > 10 cm). The study showed that *P. ostreatus* had relatively high crude protein content, the main part of which is digestible (average, 92%). During the four stages of fruit body ripening, stage B was the best, with the highest crude and digestible protein concentrations.

Mushrooms contain essential amino acids such as Leucine, Isoleucine, Valine, Tryptophan, Lysine, Threonine, Phenylalanine, Methionine and Histidine. They can thus supplement diets that lack protein (Jiskani, 2001). Mushrooms are called vegetable meat because their nutritive value equals that of meat and they are chewy like meat. On average, protein value of mushrooms is far higher than the protein content of most vegetables and fruits. A nutritional balance in a normal human being weighing 70kg can be maintained by eating 100 to 200g mushrooms (Jiskani, 2001).

Vitamins and minerals are compounds that the body needs in varying amounts for normal growth, development and functioning. Mushrooms are protective food and contain vitamins (Vitamin B complex, niacin, biotin and vitamin C). Oyster mushrooms are 3 to 6 times richer in vitamin B (Vitamin B_1 and B_2) than other natural mushrooms. Mushrooms also contain minerals like potassium, phosphorus, iron, calcium, sodium, magnesium, zinc, and copper (Kadylak, 1999). A study of the mineral element content in some edible mushrooms revealed that *Pleurotus species* were good sources of potassium, iron, aluminum, sodium, calcium and magnesium (Gupta, 1998). Silicon content was recorded highest in *P. pistilaris* since it grows in sandy soil. Gupta (1998) also found that *Volvariella volvacea* contained minimum iron whilst *Macrolepiota rachodes* contained high amounts. Sivaprakasam (1983) reported that phosphorus, sodium and potassium were predominant in sporophores of *P. sajor caju* while calcium and iron were present in relatively low quantities. The ash content in mushrooms varies with the substrate, species, environment and developmental stage. The environmental effects include the factors affecting the external concentration of an element. The type of nitrogen source and the concentration of nitrogen also influence the total ash content indirectly. Low nitrogen can restrict synthesis of nucleic acids and in that way affect the accumulation of phosphorus since it is a component of nucleic acids (Cochrane, 1958).

3.0 MATERIALS AND METHODS

3.1 Experimental procedures

Three different locally available substrates (water hyacinth, soybean straw and wheat straw) were used for the cultivation of *Pleurotus sajor caju* and *P. Hk 35*. *P. Hk 35* is a cross from *P. pulmonarius* and *P. cornucopiae* (Mpolomoka, 2000). The substrates used were those used by farmers countrywide and also water hyacinth. The water hyacinth was collected from the Kafue river, soybean straw from Lilayi farm and wheat straw from Mount Makulu Research Station. The mushroom species were selected from nine species that are used by farmers in Zambia. De Jonghe (2000) listed seven pure breeds and two hybrids used countrywide: *P. ostreatus, P. pulmonarius, P. cornucopiae, P. eryngii, P. citrinopileatus, P. florida, P. sajor caju, P. Hk 35* (hybrid) and *P. Hk 51* (hybrid). Two species were selected by stratified random sampling (species were divided into two groups: pure breeds and hybrids and one selected from each group).

The mycelium was cultured on potato agar and a sorghum grain–based spawn was prepared following the standard technique by Stamets and Chilton (1984). The substrates were chopped to 5cm pieces and pasteurized by hot water (70°C) treatment for 30 minutes. Ten kilograms of

cool substrate was mixed with 250g of spawn and filled into perforated plastic bags (40cm x 65cm). Six bags were filled with each type of substrate and half of the bags were inoculated with each species. The inoculated bags were incubated in a dark room at 10°C to 30°C. Relative humidity was maintained at 85% to 90%. After spawn run light was introduced to initiate fruiting by removing the grass covering the translucent roofing sheets.

3.2 Experimental Design

The split plot design was used for the experiment. There were three main plots and two sub plots. The substrates formed the main plots and the species formed the sub plot. The six treatments were replicated three times. Table 1 shows the layout of the experiment.

Table 1. Layout of experiment.

K, *Psc*	K, *PH*	S, *PH*	S, *Psc*	W, *Psc*	W, *PH*
S, *PH*	S, *Psc*	K, *Psc*	K, *PH*	S, *Psc*	S, *PH*
W, *Psc*	W, *PH*	W, *Psc*	W, *PH*	K, *Psc*	K, *PH*
Block 1		Block 2		Block 3	

Psc *Pleurotus sajor caju*

PH *Pleurotus Hk 35*

K Water hyacinth

S Soybean straw

W Wheat straw

Table 1 shows the layout of a 2x3 factorial experiment arranged in a Split Plot design, with three substrates as main plots (K, S, W) and two species as sub plots (*Psc, PH*) in three replications.

3.3 Data Collection

The nutrient content of the substrate and the mushroom was determined. Nutrients in the substrates were determined before cultivation of the mushrooms. Mushrooms were weighed and nutrients determined from each flush. Three flushes were collected. The nutrients determined were carbohydrates, fiber, proteins, fat and ash. The methods shown in Table 2 were used for determining nutrients:

Table 2. Methods used for nutrient analysis.

Nutrient	Analysis method used
Carbohydrates	AOAC official method 931.02 (1998) p22-23
Proteins	AOAC official method 988.05(1998) p12-17 (Kjeldahl Method)
Fats	AOAC official method 920.39(1998) p25-26 (Weibull Method)
Ash	AOAC official method 942.05(1998) p4
Fiber	AOAC official method 962.09 (1998) p26-27 (Weende Method)

3.4 Data Analysis

The data was subjected to two separate data analysis methods depending on the objective. Mean separation was done using Least Significant Difference (LSD). Significance of means was tested at $P \leq 0.05$. Genstat 5 Release 3.2 (General Statistic Program) was used for analyzing the data.

1. Comparison of the yield and chemical composition of mushrooms grown on water hyacinth, soybean straw and wheat straw.

Yield from the first flush, second flush and third flush was analysed.

The chemical composition of mushrooms in the first, second and third flush was determined.

The analysis of variance was computed for each flush following the split plot design.

2. Determination of the yield and chemical composition of *Pleurotus sajor caju* and *Pleurotus Hk 35*

The within flush analysis was done by computing the analysis of variance following the split plot design.

3. Comparison of the yield and chemical composition of the mushroom from the first three flushes

Computing the combined analysis of variance following the split split plot design did combined flush analysis.

4. Comparison of the chemical composition of the substrate and that of the mushroom.

Data was analysed using linear regression, to compare the nutrient status of the substrate and that of the mushroom. The coefficient of determination was determined. The t - test was used to test for significance of the regression co-efficient.

5. Determination of the most profitable straw for use in mushroom cultivation among the three types used.

The total costs and returns from each straw were calculated. The profit/loss was calculated by subtracting the total costs from the total returns.

4.0 RESULTS AND DISCUSSION

The water hyacinth inoculated with *Pleurotus sajor caju* and *Pleurotus Hk 35*, formed many more fruiting bodies, but of a smaller size. This substrate produced the first three flushes earlier than in wheat and soybean straw. The first, second and third flush occurred 39, 51 and 57 days after inoculation.

Soybean straw was colonised faster than the water hyacinth and wheat straw by *Pleurotus sajor caju* and *P.Hk 35*. Soybean bags were colonised earlier but the fruiting bodies appeared later than on water hyacinth. The first, second and third flush occurred after 43, 58 and 70 days respectively. Maggots attacked soybean bags and this encouraged the occurrence of ink caps (*Coprinus species*). Mushroom sporophores formed on soybean straw were relatively large and fleshy.

Wheat straw was the last to produce mushrooms, but the size of the fruiting bodies was large and only few fruiting bodies were formed. The first, second and third flush occurred after 45, 59 and 77 days respectively.

4.1 Comparison of the yield and chemical composition of mushrooms grown on water hyacinth, soybean straw and wheat straw

The results showed that the use of different substrates result in significant differences in the chemical composition of the mushrooms. The findings confirmed what Marimuthu *et al.* (1994) reported that mushrooms respond differently to different substrates. The yield values of mushrooms from the different substrates were different although they were not statistically different. Within the flush analysis showed significant differences in protein and fat content of mushrooms from the first flush produced on different substrates. The protein content of mushrooms grown on water hyacinth, soybean and wheat straw was 33.87%, 29.07% and 31.07% respectively. The protein content of mushrooms on water hyacinth was found to be higher. However, the protein content of mushrooms from wheat and soybean straw was not significantly different. The average fat content of mushrooms grown on water hyacinth was 1.28%, on soybean straw 0.67% and on wheat straw 1.20%. Mushrooms grown on water hyacinth and wheat straw had significantly higher fat content than those on soybean straw. The fat content of mushrooms from water hyacinth and wheat straw was not significantly different. Yield, carbohydrate, fiber and ash content were not significantly different in the first flush (Figure 1).

The second flush analysis also showed significant differences in carbohydrate, protein and ash content of mushrooms. The carbohydrate content of mushrooms grown on water hyacinth was 38.03%, on soybean straw 42.38% and on wheat straw 40.15%. Mushrooms grown on soybean straw had significantly higher carbohydrate content in comparison to those produced on water hyacinth. The carbohydrate content of mushrooms from soybean straw and wheat straw was not significantly different and the latter was not different from that of water hyacinth. Mushrooms grown on water hyacinth had significantly higher protein content of 31.38% than those from wheat straw. Mushrooms grown on soybean straw had 28.12% and on wheat straw 27.93% protein (Figure 2). The protein content of mushrooms from water hyacinth was not different from that of mushrooms grown on soybean straw and the latter was not different from those grown on wheat straw. The ash content of mushrooms grown on water hyacinth was 10.42%, soybean straw 8.62% and wheat straw 10.83%. The ash content was significantly higher in mushrooms grown on water hyacinth and wheat straw but not in those grown on soybean straw. The ash content of mushrooms grown on water hyacinth and wheat straw was not significantly different.

The third flush analysis showed that mushrooms from the three substrates were different in respect to fiber and ash content. The fiber content of mushrooms grown on water hyacinth was 15.80%, on soybean was 17.82% and on wheat straw was 17.85%. Mushrooms grown on soybean and wheat straw had significantly high fiber content in comparison with those from

water hyacinth. The fiber content of mushrooms grown on soybean and wheat straw was not significantly different. The ash content of mushrooms grown on water hyacinth was 11.38%, soybean straw 9.42% and wheat straw 10.75% (Figure 3). Mushrooms grown on water hyacinth had significantly high ash content than those grown on soybean straw. There was no significant difference in ash content of mushrooms grown on water hyacinth and wheat straw.

The combined flush analysis showed that there was no significant difference in yield, fiber and fat content in mushrooms produced on the three substrates. However, significant differences were observed in carbohydrates, proteins and ash (Figure 4). Mushrooms grown on water hyacinth had an average carbohydrate content of 38.78% and those from soybean and wheat straw had 42.18% and 40.00% respectively. Mushrooms grown on soybean straw were significantly higher in carbohydrate content than mushrooms from water hyacinth. There was no significant difference in carbohydrate content in mushrooms grown on soybean and wheat straw. Mushrooms from wheat straw were also not different in carbohydrate content in comparison to those grown on water hyacinth. The protein content was significantly higher in mushrooms grown on water hyacinth and was 31.69%. Mushrooms grown on soybean straw and wheat straw had low protein content of 28.16% and 28.62% respectively. Mushrooms grown on soybean and wheat straw were not different in protein content. The ash content was also higher in mushrooms grown on water hyacinth and wheat straw and was 10.41% and 10.41% respectively. Mushrooms from soybean straw had low ash content of 8.60%.

Mushrooms grown on water hyacinth and wheat straw were not different in ash content. Water hyacinth was the best straw since mushrooms produced were significantly higher in protein and ash. Mushrooms grown on soybean and wheat straw were at par in carbohydrate and protein content except in ash content, which was higher in mushrooms from the latter substrate and that makes it to be the second best substrate among the three different substrates used in this study. ANOVA Tables are shown in the appendix.

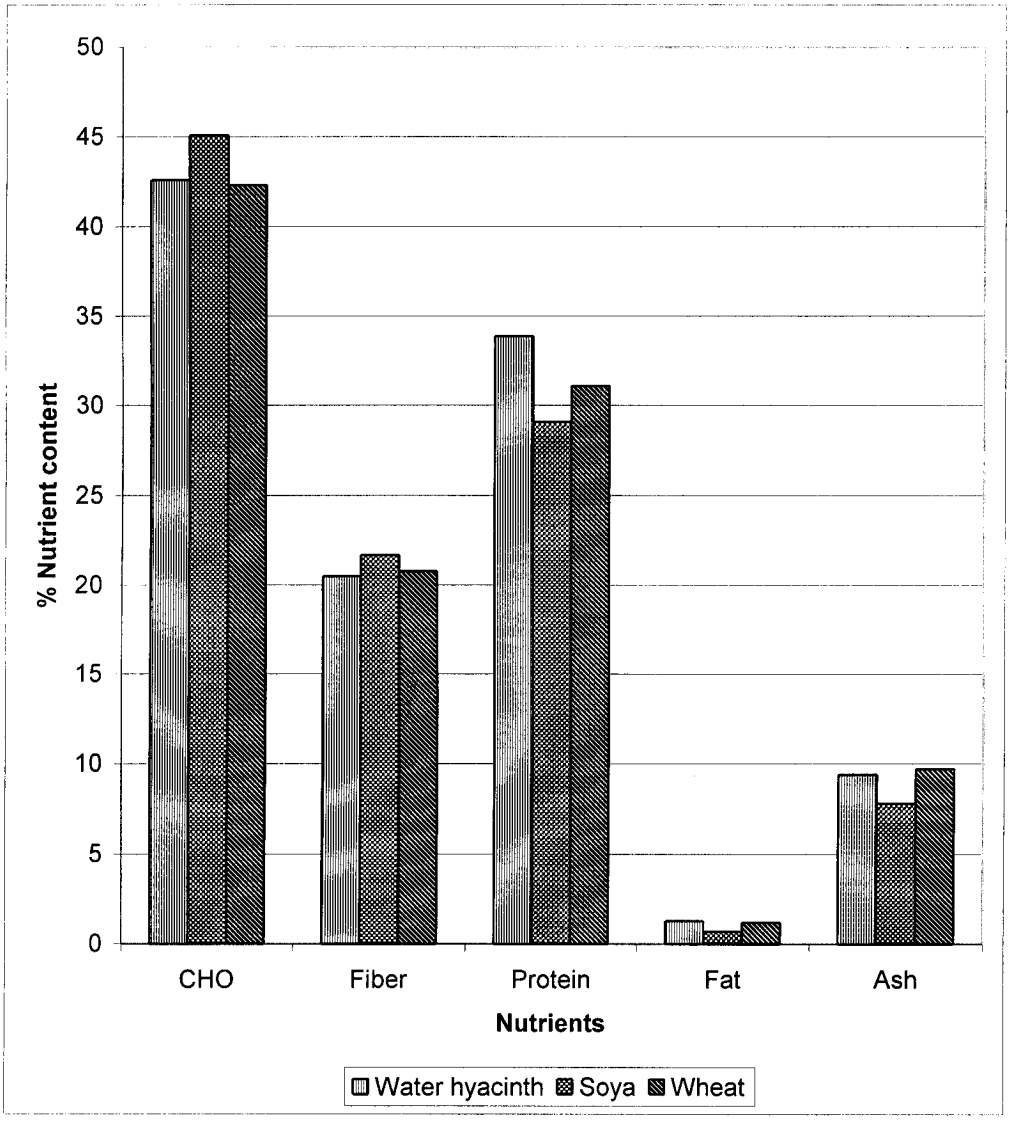

Figure 1. Chemical composition of the first flush mushrooms grown on water hyacinth, soybean and wheat straw.

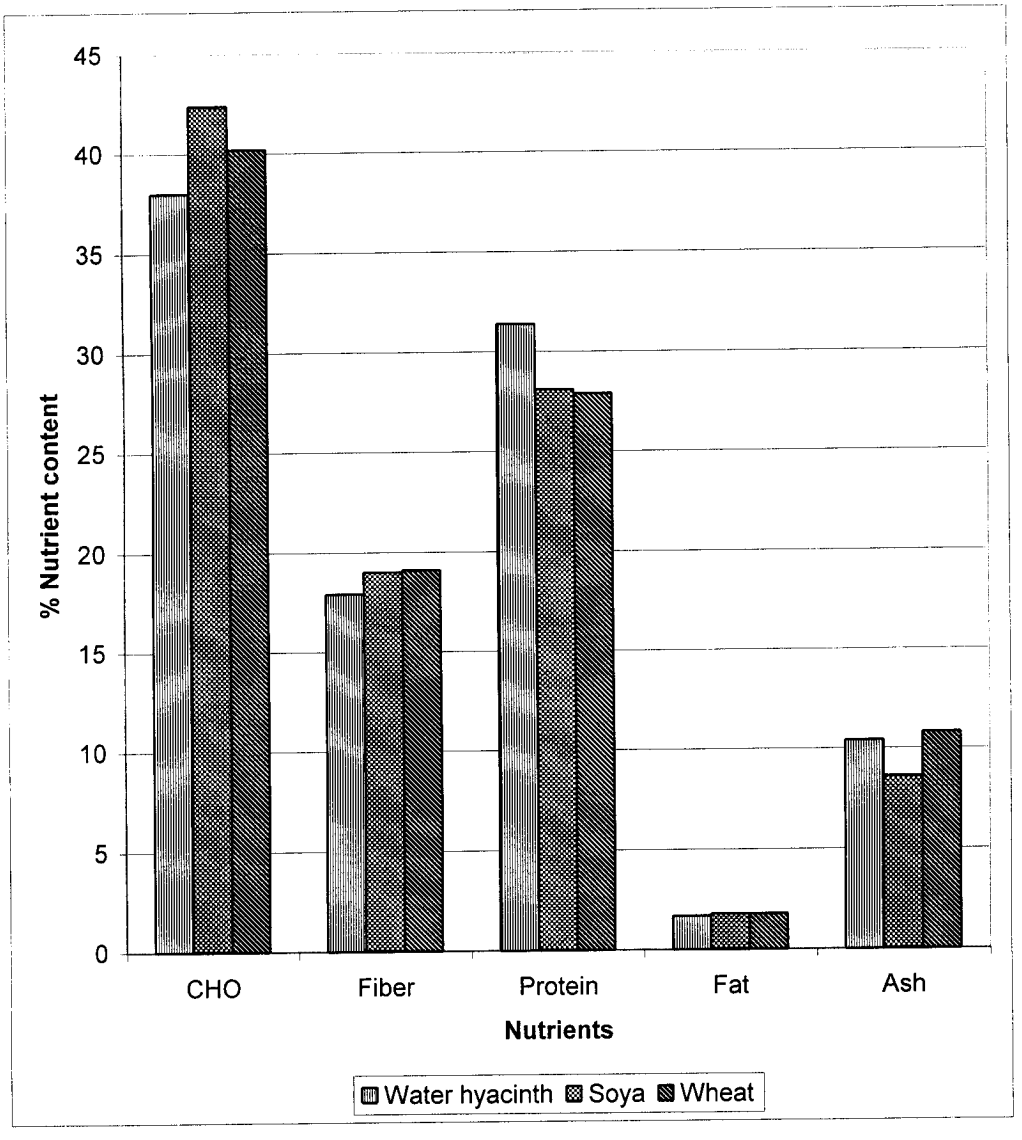

Figure 2. Chemical composition of the second flush mushrooms grown on water hyacinth, soybean and wheat straw.

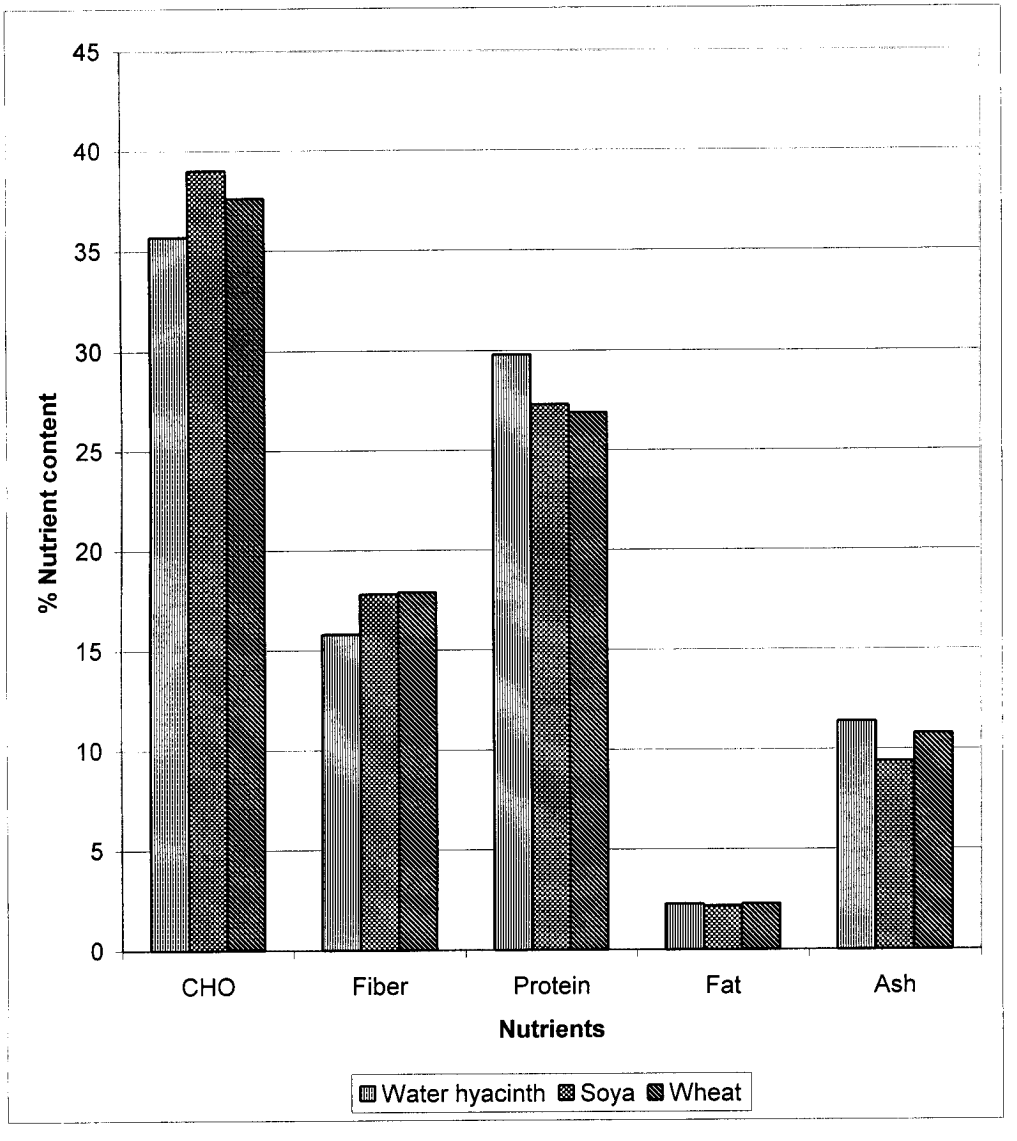

Figure 3. Chemical composition of the third flush mushrooms grown on water hyacinth, soybean and wheat straw.

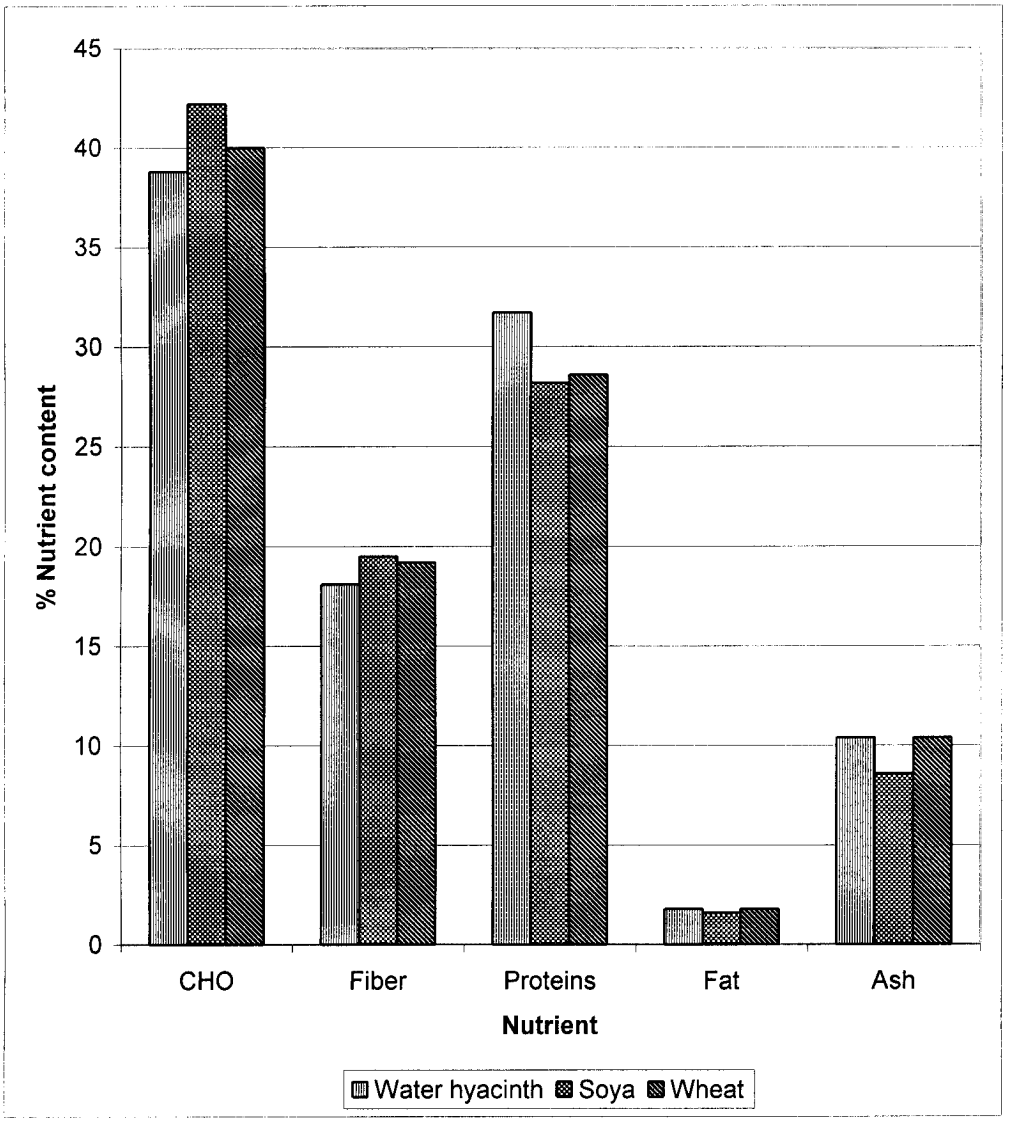

Figure 4. Chemical composition of mushrooms grown on water hyacinth, soybean and wheat straw.

4.2 Determination of the yield and chemical composition of *Pleurotus sajor caju* and *Pleurotus Hk 35*

Species differed in yield and chemical composition within a flush and when all flushes harvested were considered. Analysis of the first flush indicated that species differences cause differences in yield and ash content. *Pleurotus sajor caju* produced less than *P. Hk 35*. Mushroom yield was 514g for *P. sajor caju* whilst that of *P. Hk 35* was 827g. The two species also showed significant differences in their chemical composition. The ash content for *P. sajor caju* was 9.67% whilst that for *P. Hk 35* was 8.57%. *P. sajor caju* was high in ash content than *P. Hk 35* (Figure 5).

The second flush analysis also showed that species differences can cause significant differences in protein and ash content. *Pleurotus sajor caju* had significantly high protein and ash content than *P. Hk 35*. The protein content in *P. sajor caju* was 30.13% and 28.16% in *P. Hk 35*. The ash content in *P. sajor caju* was 10.42% whilst in *P. Hk 35* was 9.49%. All species varied significantly in their chemical composition as shown in Figure 6.

In the third flush differences in chemical composition occurred due to differences in species. *Pleurotus sajor caju* was found to be significantly high in protein whilst *P. Hk 35* was high in

fat. In *P. sajor caju* the protein content was 28.91% while in *P. Hk 35* was 27.08%. The fat content was 2.10% for *P. sajor caju* and 2.42% for *P. Hk 35* (Figure 7).

The combined flush analysis showed that species differences could cause significant differences in yield. *Pleurotus sajor caju* was the best nutritionally and *P. Hk 35* was the best in yield. *P. sajor caju* had a yield of 377g and *P. Hk 35* had significantly high yield of 540g. The species also varied significantly in their ash content. *P. sajor caju* had significantly high ash content of 10.29% and in *P. Hk 35* was 9.33% (Figure 8*)*. *P. Hk 35* was superior in mushroom yield and *P. sajor caju* was good nutritionally. Mushrooms from *P. sajor caju* were high in ash content, they were also high in protein content especially in the second and third flushes. The results were similar to those by Chang and Hayes (1978), who reported that the yield and chemical composition of mushrooms was affected by several factors, which included differences in species and substrates. Contrary to their findings differences in substrates in this study did not show statistical differences in yield.

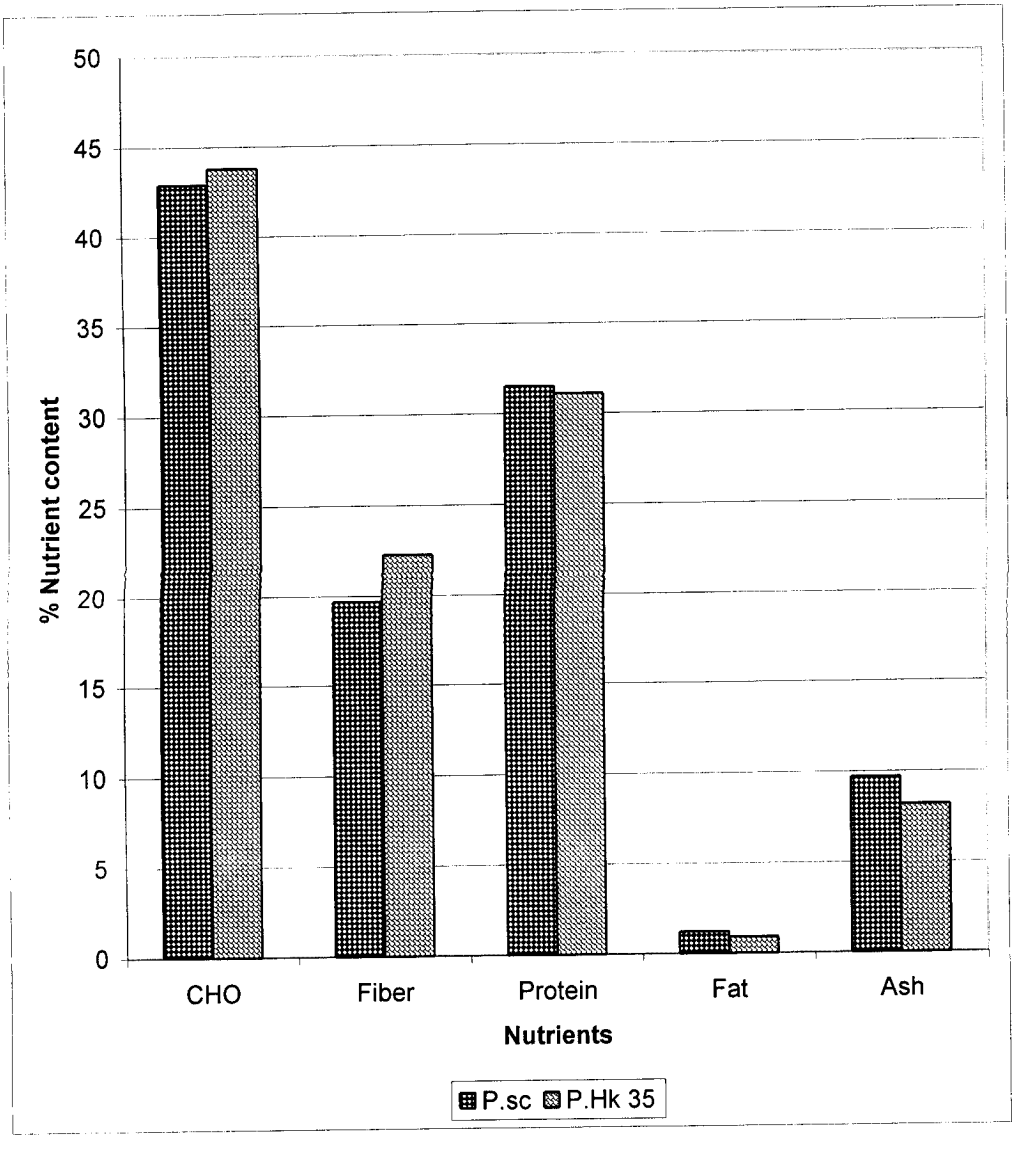

Figure 5. Comparison of nutrients in the sporophores of *Pleurotus sajor caju* (P.sc) and *Pleurotus Hk 35* (P.Hk 35) from the first flush.

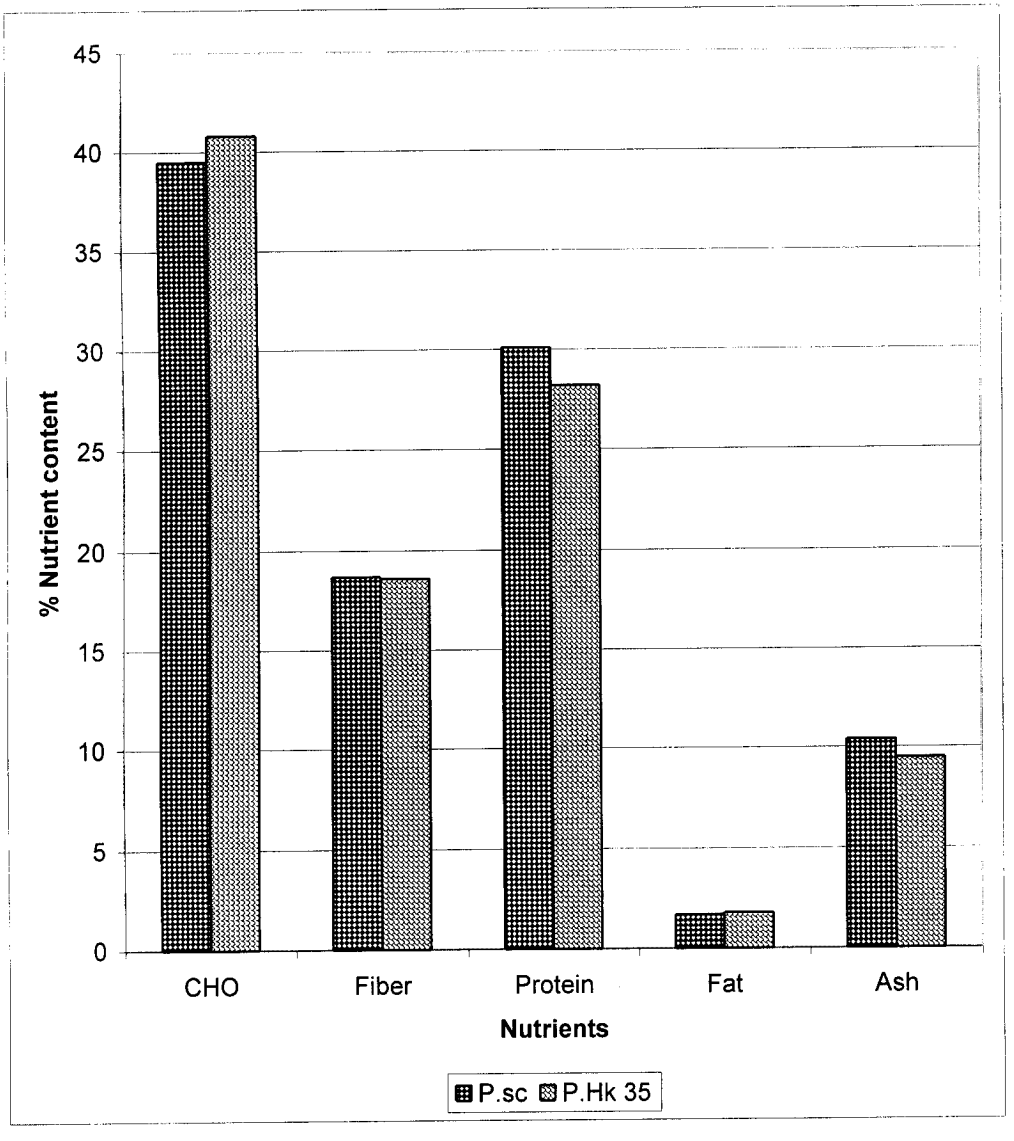

Figure 6. Comparison of nutrients in the sporophores of *Pleurotus sajor caju* (P.sc) and *Pleurotus Hk 35* (P.Hk 35) from the second flush.

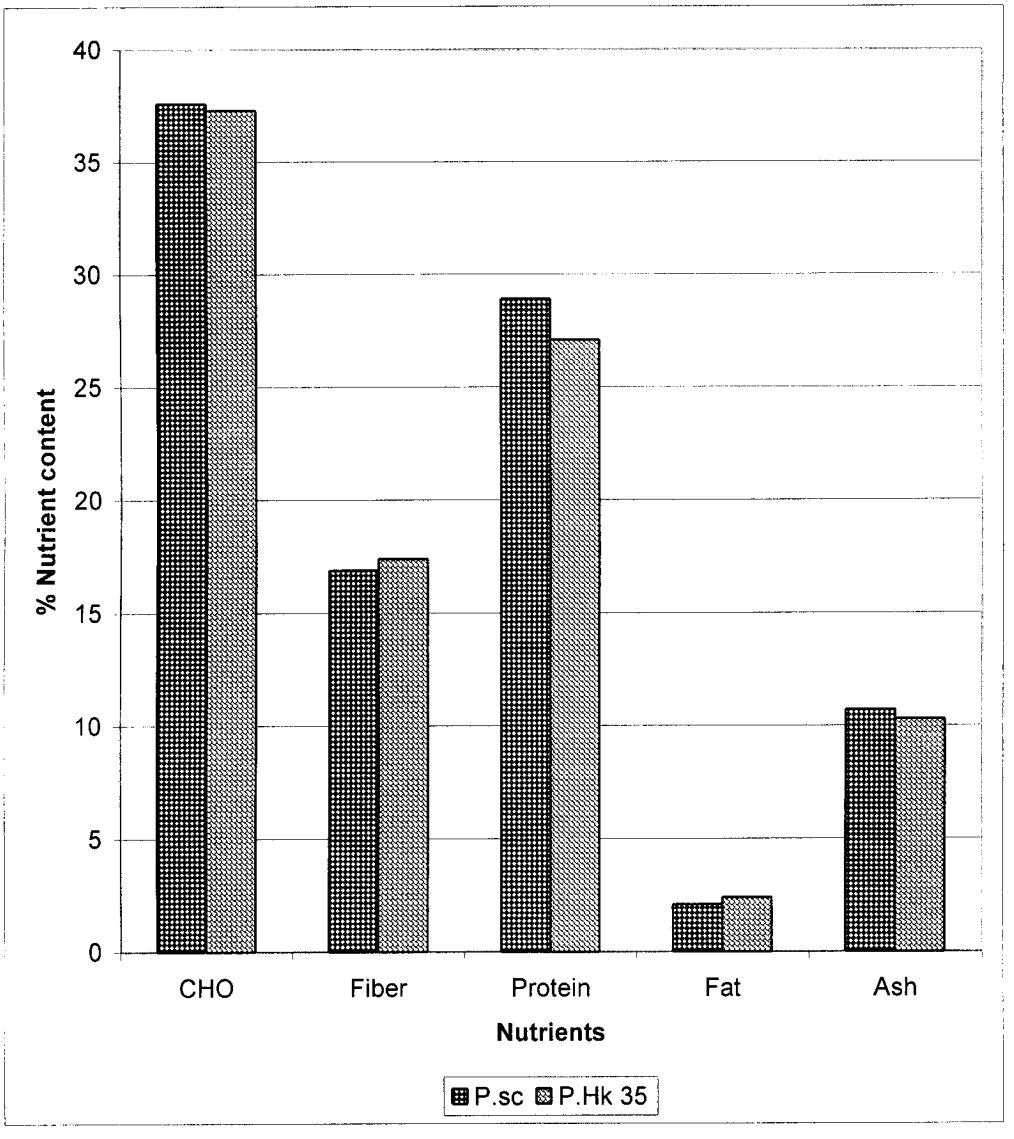

Figure 7. Comparison of nutrients in the sporophores of *Pleurotus sajor caju* (P.sc) and *Pleurotus Hk 35* (P.Hk 35) from the third flush.

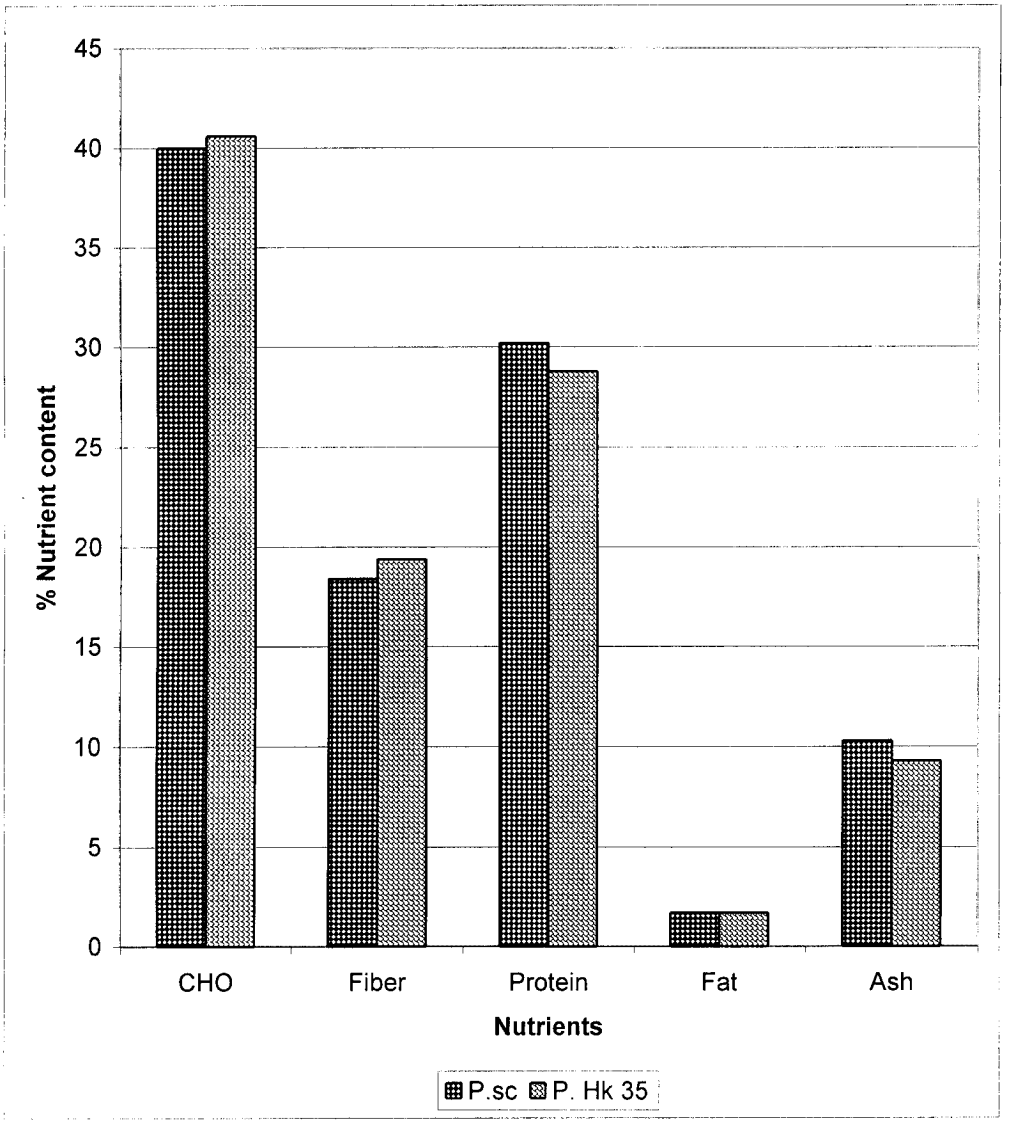

Figure 8. Effect of *Pleurotus sajor caju* (P.sc) and *Pleurotus Hk 35* (P.Hk 35) on the nutrient content in sporophores.

4.3 Comparison of the yield and chemical composition of mushrooms in the first three flushes.

When all three flushes were analysed, it was observed that there was a significant difference between flushes in respect to yield, carbohydrates, fiber, proteins, fat and ash content. The yield was highly different between flushes. The first and second flush gave the highest yield while the third flush gave the lowest. The mean yield was 671g (49% of the total yield) in the first flush, 436g (31%) in the second flush and 270g (20%) in the third flush (Figure 9). The first and second flush contributes 80% of the total yield (Pasamba, 1990). Yildiz (1999) in a study on mushroom yield reported that the highest yield comes from the first flush and the lowest from the third flush. The findings in this study were in agreement with his findings.

The carbohydrate content of mushrooms was significantly different between flushes. The carbohydrate content gradually declined in mushrooms in subsequent flushes. The mean carbohydrate content in the first flush was 43.33%, 40.19% second flush and 37.44% third flush. The first and second flush contained significantly higher carbohydrate content than the third flush (figure 10).

The fiber content was also significantly different between flushes. The first flush had a high fiber content of 20.98% but the second and third flushes contained significantly lower fiber

content in mushrooms and was 18.64% and 17.16% respectively. The fiber content of mushrooms from the second flush was higher than in third flush.

Mushrooms produced from the first and second flush had significantly higher protein content in comparison to the third flush. The protein content was 31.33% for the first flush, 29.14% second flush and 27.99% third flush. Protein content showed a decline from the first to the third flush (Figure 10).

The ash content in the mushrooms was observed to be highly significant between flushes. The first flush had a low ash content of 8.94% but the second and third flushes contained significantly higher ash content in mushrooms and was 9.96% and 10.52% respectively (Figure 10).

The fat content of fruiting bodies was significantly different between flushes. The fat content increased in fruiting bodies in subsequent flushes. The mean fat content in the first flush was 1.05%, 1.74% second flush and 2.26% third flush. The second and third flushes had significantly higher fat content than the first flush (Figure 10).

The results indicated that the first and second flushes were the best since they were high in yield and mushrooms produced were nutritionally valuable. Mushrooms from the first flush

were the best followed by the second flush. The mushrooms from the two flushes were higher in carbohydrates, fiber and proteins than the third flush mushrooms whilst the latter was higher in fat and ash content.

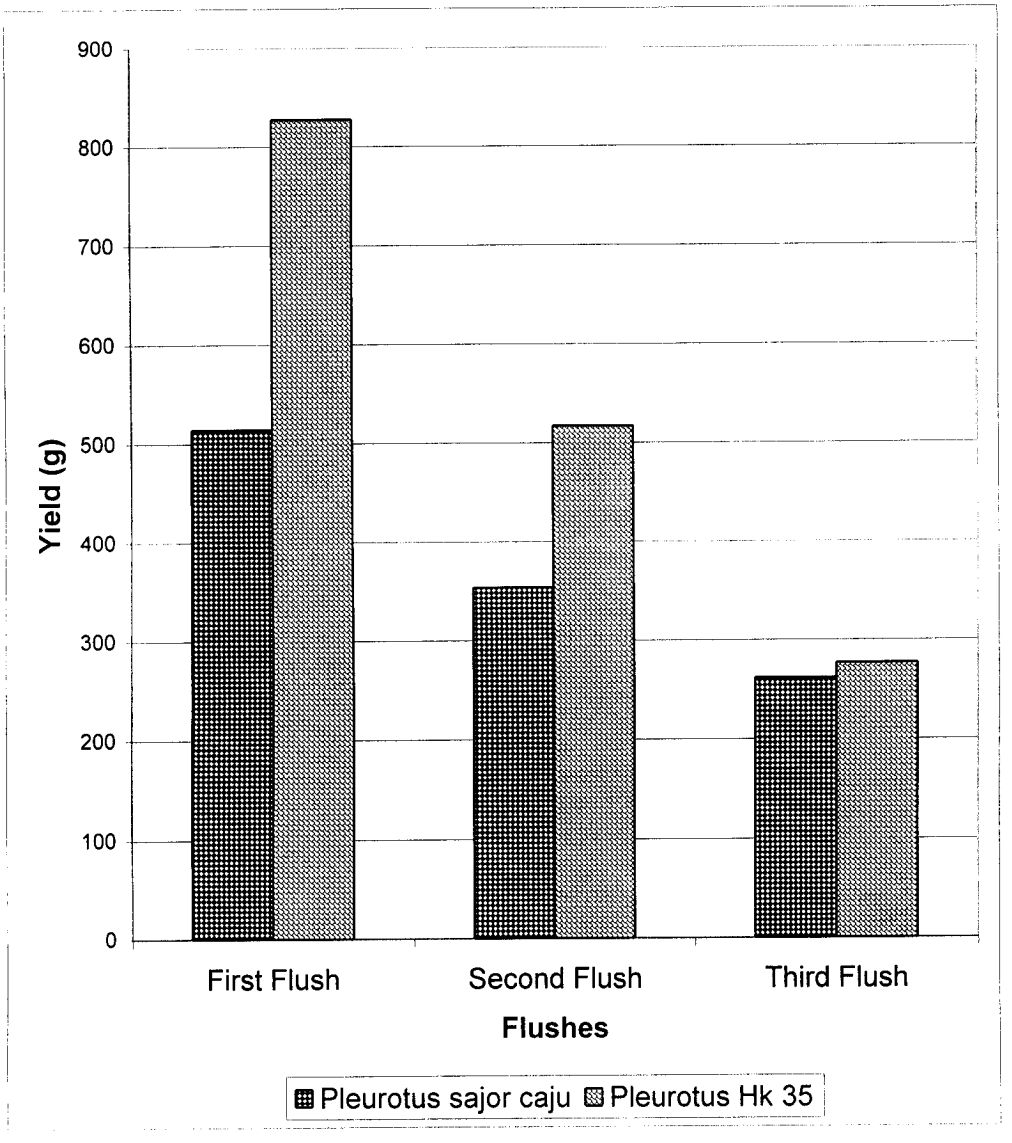

Figure 9. Yield in the first, second and third flush of *Pleurotus sajor caju* and *Pleurotus Hk 35.*

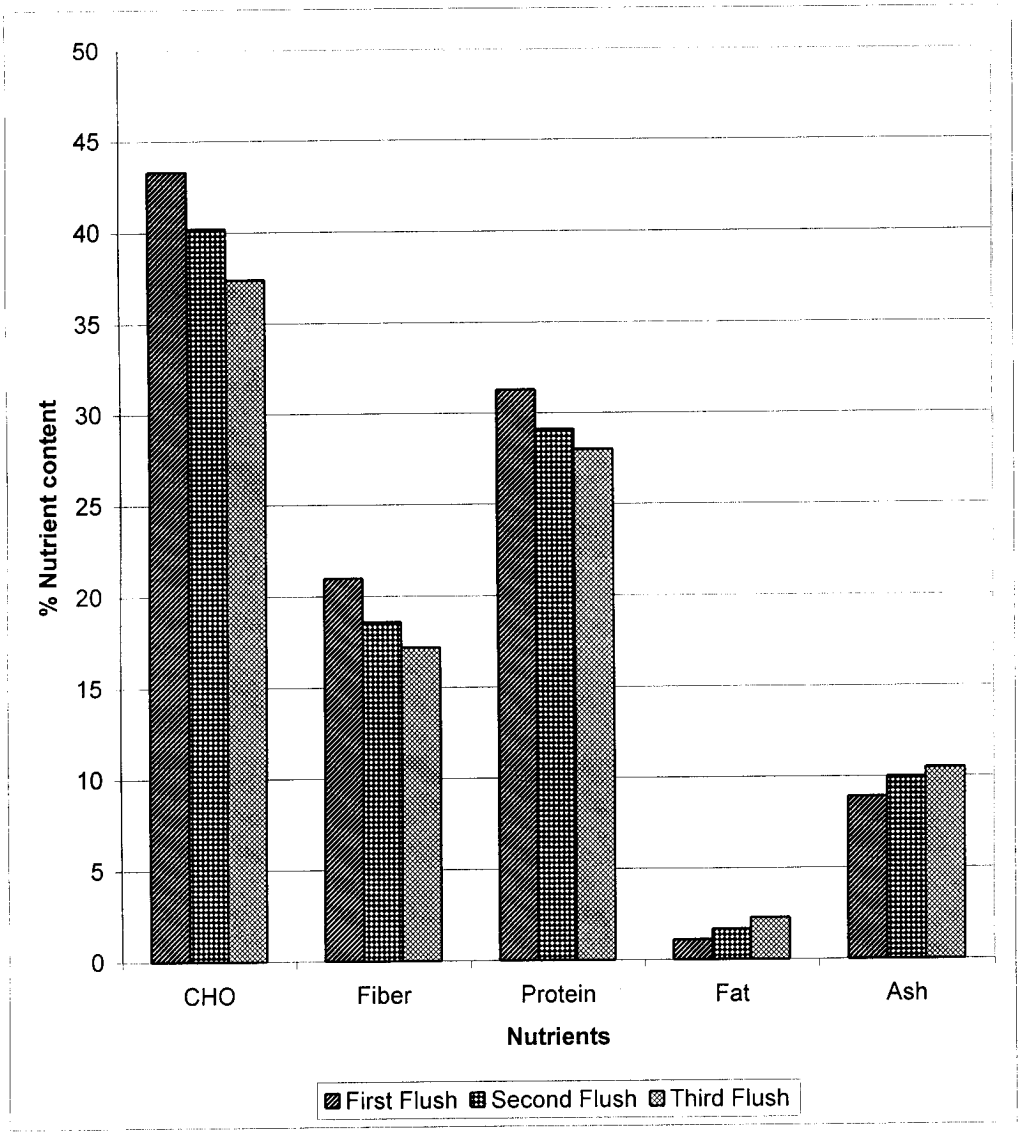

Figure 10. Comparison of nutrient profile in mushrooms from all three flushes.

The within flush analysis showed that there was an interaction between the substrate and species in the second flush. The interaction was found in the ash content analysis. There was no interaction between substrate and species in yield, carbohydrate, fiber, protein and fat content. The ash content of mushrooms grown on water hyacinth was 11.10% in *P. sajor caju* whilst in *P. Hk 35* it was 9.83%. Mushrooms grown on wheat straw gave an ash content of 10.43% in *P. sajor caju* and 11.23% in *P. Hk 35.* The ash content of *P. sajor caju* was 9.83% and that of *P. Hk 35* was 7.40% on soybean straw (Table 3). The analysis showed that all mushrooms from the different substrates were significantly high in ash content except *Pleurotus Hk 35* grown on soybean straw. The ash content in *P. sajor caju* and *P. Hk 35* grown on water hyacinth, soybean and wheat straw was not significantly different except in *P. Hk 35* grown on soybean straw.

The interaction between substrate and species also occurred in the third flush in the analysis of the ash content. The ash content in mushrooms grown on water hyacinth was 11.73% in *P.sajor caju* and 11.03% in *P. Hk 35.* The ash content in mushrooms grown on wheat straw was 10.00% in *P. sajor caju* and 11.50% in *P. Hk 35.* Use of water hyacinth and wheat straw resulted in significantly high ash content in *Pleurotus sajor caju* and *P. Hk 35. P. Hk 35* grown on soybean straw was low in ash content and the value was 8.37% than in other treatments (Table 3). The analysis also showed that the ash content in all mushrooms was not significantly different except in *P. Hk 35* grown on soybean straw.

The combined analysis exhibited that the substrate and species interacted in the ash content. In *Pleurotus sajor caju* the ash content of sporophores from water hyacinth was 11.03%, on wheat straw 10.10% and on soybean straw 9.73%. *P. Hk 35* grown on water hyacinth and wheat straw had significantly high ash content that is 9.78% and 10.72% respectively. Mushrooms from soybean straw contained 7.47% ash. Amongst the interactions between substrate and species, *P. Hk 35* grown on soybean straw contained low ash (Table 3). The combined analysis also showed that there was no significant difference in ash content in mushrooms except for *P. Hk 35* grown on soybean straw.

There was an interaction between the substrate, species and flush in the yield analysis. *Pleurotus sajor caju* produced a higher yield of 599g in the first flush when grown on soybean straw whilst *P.Hk 35* yield was significantly higher in all the straws used. The yield from soybean, water hyacinth and wheat straw was 931g, 662g and 888g respectively (Table 4). *P.Hk 35* also produced a higher yield of 772g in the second flush when grown on soybean straw. The yield for the two species grown on the three substrates was not significantly different in the third flush.

Table 3. Mushroom ash content (%) in three flushes of *Pleurotus sajor caju* and *Pleurotus Hk 35* cultivated on three substrates.

	First flush		Second flush		Third flush		Species/substrate	
Substrate/species	***P.sc***	***P.Hk35***	***P.sc***	***P.Hk35***	***P.sc***	***P.Hk35***	***P.sc***	***P.Hk35***
Water hyacinth	10.37a	8.47a	11.00a	9.83a	11.73a	11.03a	11.03a	9.78a
Soybean	8.90a	6.63a	9.83a	7.40b	10.47a	8.37b	9.73a	7.47b
Wheat	9.87a	9.43a	10.00a	11.23a	10.43a	11.50a	10.10a	10.72a
LSD	2.27		1.71		1.84		1.70	

Lsd significant at $P \leq 0.05$

P.sc - Pleurotus sajor caju

P.Hk35 - Pleurotus Hk 35

Means with the same alphabet are not significantly different.

Table 4. Mushroom yield in three flushes of *Pleurotus sajor caju* and *PleurotusHk35* cultivated on three substrates.

Substrate/flush	**First flush**		**Second flush**		**Third flush**	
	P. sc	***P.Hk35***	***P.sc***	***P.Hk35***	***P.sc***	***P.Hk35***
Water hyacinth	483	662*	346	425	217	227
Soybean	599*	931*	422	772*	294	324
Wheat	460	888*	293	354	276	279

* - significant at P≤ 0.05

P.sc - Pleurotus sajor caju

P.Hk35 - Pleurotus Hk 35

4.4 Comparison of the chemical composition of the substrate and that of the mushroom

A relationship was proved to be significant in carbohydrate content between the substrate and mushroom. The carbohydrate content of the water hyacinth, soybean and wheat straw was 36.35%, 39.78% and 37.72% respectively. The carbohydrate content of mushrooms grown on water hyacinth, soybean and wheat straw was 38.78%, 42.18% and 40.00% respectively. The percentage variance accounted for by the straw was 98.4%. This indicated that most of the carbohydrates in the mushroom came from the straw. The regression coefficient (0.95) was significant. This showed that a percent increase in carbohydrates in the substrate was associated with a 0.95% increase in carbohydrates in the mushroom.

The fiber content in the straw was also related to that found in the mushroom fruiting bodies. The fiber content of the water hyacinth was 31.30% and mushrooms 18.06%. In soybean straw it was 59.33% and mushrooms 19.50%. Wheat straw contained 35.25% fiber and mushrooms 19.22%. The percentage variance accounted for by the straw was 98.9%. The fiber content in the mushroom was contributed mainly by the substrate. The regression coefficient (0.18) was significant. Such a coefficient meant that a percent increase in fiber content in the substrate resulted in a 0.18% increase in fiber in the mushroom.

The protein content in the substrate was related to that in the mushroom fruiting bodies. The protein content of the water hyacinth, soybean and wheat straw was 8.54%, 4.95% and 5.69% respectively. The protein content of mushrooms grown on water hyacinth, soybean and wheat straw was 31.69%, 28.16% and 28.62% respectively. The percentage variance accounted for by the straw was 98.9%. This indicated that most of the protein in the mushroom came from the straw. The regression coefficient (1.0) was significant. This showed that a percent increase in protein in the substrate was associated with a 1.0% increase in protein in the mushroom.

The fat content in the substrate and mushrooms was also found related. The fat content of water hyacinth, soybean and wheat straw was 0.95%, 0.87% and 1.06% respectively. The fat content of mushrooms collected from water hyacinth, soybean and wheat straw was 1.75%, 1.56% and 1.75% respectively. The fat content of the mushroom was higher than in the substrate. The percentage variance accounted for by the substrate was 98.4%. A large portion of the fat content in the mushroom came from the substrate. The regression coefficient (1.04) was significant. The results show that a percent increase in fat content in the substrate resulted in 1.04% increase in fats in the mushroom. Table 5 shows the nutrient content in the straw and the mushroom.

A relationship was also proved to exist between the ash content in the substrate and that of the mushroom. The ash content of the water hyacinth was 14.4% and mushrooms 10.41%. In soybean straw was 4.21% and mushrooms 8.60%. Wheat straw had 14.80% ash whilst mushrooms produced had 10.41%. The ash content was observed to be lower in the mushroom than in the substrate except in soybean. A relationship was highly significant between the substrate and mushroom ash content and the variance occurring due to the substrate was 99.8%. The ash in the mushroom was mainly from the substrate. The regression coefficient (0.17) was significant. This indicates that a percent increase in ash content in the substrate results in a 0.17% increase ash content in the mushroom. Regression Analysis Summary is shown in Table 6 in appendix

The high coefficients of determination discussed prior showed that the chemical composition of mushroom depend mainly on the substrate. An increase in nutrients in the substrate resulted in an increase in nutrients in mushrooms. The percent increase in carbohydrates, proteins and fats in the mushrooms was almost similar to that occurring in substrates. Such observations are deduced from the regression coefficients 0.95, 1.0 and 1.04 for carbohydrates, proteins and fats respectively. A percent increase in fiber and ash content in the substrate resulted in a 0.18% and 0.17% increase of the nutrient in the mushroom. This indicated that a percent change in the nutrient in the substrate led to a very small change of the nutrient content in the mushroom.

Table 5. Chemical composition of the substrate and mushroom.

Nutrients	Water hyacinth		Soybean		Wheat	
	Straw	Mushroom	Straw	Mushroom	Straw	Mushroom
Carbohydrate (%)	36.35	38.78	39.78	42.18	37.72	40.00
Fiber (%)	31.30	18.06	59.33	19.50	35.25	19.22
Protein (%)	8.54	31.69	4.95	28.16	5.69	28.62
Fat (%)	0.95	1.75	0.87	1.56	1.06	1.75
Ash (%)	14.4	10.41	4.21	8.60	14.8	10.41

4.5 Determination of the most profitable straw for use in mushroom cultivation among the three types used

Soybean straw was the best straw in terms of yield. The total yield produced from six bags was 10.03kg from a 10kg bag of substrate. Soybean straw contributed 40.5% to the total yield. Use of soybean straw was more profitable than other straws, because US$11.44 was obtained from the mushrooms as profit (the price of 1kg mushrooms is US$2.38). Mushrooms from soybean straw contributed 56% of the gross profit. Wheat straw was the second best straw and gave a total yield of 7.65kg. Wheat straw contributed 31% of the total yield. US$5.79 was obtained from the mushrooms as profit, which was a 28% contribution to the gross profit. Water hyacinth straw contributed 28.5% to the total yield. The yield obtained from the latter straw was 7.07kg. The profit obtained from mushrooms grown on water hyacinth was US$3.22 and this was a 16% contribution to gross profit. Less profit was obtained from water hyacinth. The results on yield were in accordance with those reported by Thakur (2000). He found that soybean straw gave the highest yield followed by wheat straw when used for cultivation of *Pleurotus columbinus*. Paddy straw gave the lowest yield.

5.0 CONCLUSION

In the present study three factors substrates, species and flushes were undertaken for study of the yield and chemical composition of mushrooms. It was found that all factors studied had an effect on the chemical composition of mushrooms. The yield was affected by differences in species and flushes. Mushrooms grown on the three substrates differed nutritionally. Mushrooms grown on water hyacinth were high in protein and ash while those grown on soybean straw were high in carbohydrates. Mushrooms grown on wheat straw were high in ash content. The experiment showed that the water hyacinth straw was the best since mushrooms produced were high in protein and this indicated an increased need for the utilisation of the water hyacinth as a substrate.

The species used in the study differed in yield and chemical composition. *Pleurotus sajor caju* (pure breed) yielded lower than *P. Hk 35* (hybrid) irrespective of the substrate used. Use of *P. sajor caju* resulted in the production of mushrooms high in nutrients whilst *P. Hk 35* was high yielding. This showed that pure breeds and hybrids might be superior in different factors, which may require further improvement. Since the study revealed that the chemical composition of the substrate and that of mushrooms were related therefore there is a necessity of substrate improvement to enhance the productivity and nutritive value of the two species.

Out of the three flushes analysed the first flush mushrooms were the best since they were high in yield, carbohydrates, fiber and protein content in comparison with other flushes. The second flush mushrooms were the second best and the third flush mushrooms were the lowest in yield and the nutrients listed prior. The third flush mushrooms were higher in fat and ash content than mushrooms from other flushes. Such observations showed that mushroom producers should harvest the first, second and third flush in *Pleurotus sajor caju* and *Pleurotus Hk 35* because those are good nutritionally and in yield. Keeping the substrate further for more flushes may result in a loss and also in the consumption of mushrooms that are low in protein content.

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APPENDICES

able 6. Regression Analysis Summary.

	Carbohydrate	Fiber	Protein	Fat	Ash
)f Regr.	1	1	1	1	1
)f residual	1	1	1	1	1
)f Total	2	2	2	2	2
S.S Regr.	5.35168	14.32699	7.2335682	0.0198352	2.194409
S.S. Residual	0.02079	0.07968	0.0008318	0.0001648	0.001791
S.S. Total	5.37247	14.40667	7.2344000	0.0200000	2.196200
M.S. Regr.	5.35168	14.32699	7.2335682	0.0198352	2.194409
M.S.Residual	0.02079	0.07968	0.0008318	0.0001648	0.001791
M.S. Total	2.68623	7.20333	3.6172	0.100000	1.098100
F value	257.43*	179.82*	8696.42**	120.33*	1225.58**
R^2	98.4	98.9	98.9	98.4	99.8
Regr. Coeff.	0.95	0.18	1.0	1.04	0.17
T value	16.04*	13.41*	93.25**	10.97*	35.01**

* Significant at $P \leq 0.05$ ** Significant at $P \leq 0.01$

Table 7. First Flush ANOVA Table.

		YIELD			CHO			FIBER			PROTEINS			FATS			ASH		
Source	**df**	**ss**	**Ms**	**F value**	**ss**	**ms**	**F value**	**ss**	**ms**	**F value**	**ss**	**Ms**	**F value**	**ss**	**ms**	**F value**	**ss**	**ms**	**F value**
Reps	2	121519	60760	1.22	38.22	19.11	3.18	33.08	16.54	1.14	9.26	4.63	2.55	0.89	0.45	4.44	1.71	0.86	0.42
Substrate	2	140957	70478	1.41	29.43	14.71	2.45	4.86	2.43	0.17	69.76	34.88	19.20**	1.32	0.66	6.54*	12.65	6.32	3.09
Residual	4	199385	49846		24.07	6.02		58.07	14.52		7.27	1.82		0.40	0.10		8.18	2.05	
SPECIES	1	630550	630550	8.45	3.38	3.38	0.27	29.94	12.47	1.17	0.72	0.72	0.14	0.23	0.23	1.65	10.58	10.58	18.36**
Substrate x Species	2	144348	72174	0.97	47.90	23.95	1.93	24.94	12.47	1.17	12.52	6.26	1.19	0.42	0.21	1.48	2.82	1.41	2.45
Residual	6	447800	74633		74.44	12.41		63.96	10.66		31.63	5.27		0.85	0.14	3.46	0.58		
Total	17	1684558			217.44			214.57			131.16			4.11		39.40			

** - Significant at $P \leq 0.01$

* - Significant at $P \leq 0.05$

Table 8. Second Flush ANOVA Table.

		YIELD			CHO			FIBER			PROTEINS			FATS			ASH		
Source	**df**	**ss**	**Ms**	**F value**	**ss**	**ms**	**F value**	**ss**	**ms**	**F value**	**ss**	**ms**	**F value**	**ss**	**ms**	**F value**	**ss**	**ms**	**F value**
Reps	2	121519	60760	1.22	41.37	20.68	10.15	14.01	7.00	1.53	14.77	7.38	1.75	0.14	0.07	0.30	0.62	0.31	0.26
Substrate	2	140957	70478	1.41	56.78	28.39	13.94*	5.00	2.50	0.55	45.21	22.61	5.35*	0.06	0.03	0.12	16.65	8.33	7.04*
Residual	4	199385	49846		8.15	2.04		18.28	4.57		16.90	4.23		0.94	0.23		4.73	1.18	
SPECIES	1	630550	630550	8.45	7.74	7.74	1.18	0.01	0.01	0.00	17.60	17.60	5.02*	0.04	0.04	0.25	3.92	3.92	17.60**
Substrate x Species	2	144348	72174	0.97	15.74	7.87	1.20	13.93	6.97	1.77	2.55	1.28	0.36	0.86	0.43	3.08	7.96	3.98	17.87**
Residual	6	447800	74633		39.38	6.56		23.64	3.94		21.04	3.51		0.84	0.14		1.34	0.22	
Total	17	1684558			169.16			74.86			118.08			2.86			35.22		

** - Significant at $P \leq 0.01$

* - Significant at $P \leq 0.05$

Table 9. Third Flush ANOVA Table.

		YIELD			CHO			FIBER			PROTEINS			FATS			ASH		
Source	**df**	**ss**	**ms**	**F value**	**ss**	**ms**	**F value**	**ss**	**ms**	**F value**	**ss**	**ms**	**F value**	**ss**	**ms**	**F value**	**Ss**	**ms**	**F value**
Reps	2	121519	60760	1.22	54.27	27.14	2.78	1.94	0.97	1.94	3.63	1.82	0.36	0.03	0.02	0.07	0.26	0.13	0.11
Substrate	2	140957	70478	1.41	33.15	16.57	1.70	16.54	8.27	16.52*	30.45	15.22	3.03	0.01	0.01	0.03	12.09	6.05	5.05
Residual	4	199385	49846		39.09	9.77		2.00	0.50		20.10	5.02		0.84	0.21		4.79	1.20	
SPECIES	1	630550	630550	8.45	0.53	0.53	0.04	0.98	0.98	0.40	15.13	15.13	9.49*	0.47	0.47	10.01*	0.85	0.85	1.16
Substrate x Species	2	144348	72174	0.97	18.47	9.24	0.73	15.19	7.60	3.10	3.79	1.90	1.19	0.01	0.00	0.08	9.88	4.94	6.78*
Residual	6	447800	74633		76.21	12.70		14.69	2.45		9.56	1.59		0.28	0.05		4.37	0.73	
Total	17	1684558			221.72			51.34			82.65			1.64			32.25		

** - Significant at $P \leq 0.01$

* - Significant at $P \leq 0.05$

Table 10. Combined Flush ANOVA Table.

		Yield			Carbohydrate			Fiber			Protein			Fat			Ash		
Source	**df**	**Ss**	**ms**	**F value**	**Ss**	**ms**	**F value**	**ss**	**ms**	**F value**	**Ss**	**ms**	**F value**	**Ss**	**ms**	**F value**	**Ss**	**ms**	**F value**
Reps	2	204102	102051	1.20	121.37	60.68	8.25	38.86	19.43	1.43	20.25	10.13	1.08	0.18	0.09	0.18	1.39	0.70	0.20
Substrate	2	270943	135471	7.59	107.15	53.58	7.28*	20.97	10.49	0.77	132.37	66.19	7.08*	0.44	0.22	0.43	39.24	19.62	5.62*
Residual	4	341461	85365		29.42	7.36		54.27	13.57		37.41	9.35		2.06	0.52		13.96	3.49	
SPECIES	1	361916	361916	4.70*	5.04	5.04	0.21	13.40	13.40	0.99	26.60	26.60	3.58	0.05	0.05	0.29	12.62	12.62	23.61**
Substrate x Species	2	49079	24539	0.32	76.05	38.02	1.60	51.70	25.85	1.91	15.73	7.87	1.06	0.83	0.42	2.43	19.34	9.67	18.10**
Residual	6	461942	76990		142.84	23.81		81.37	13.56		44.59	7.43		1.03	0.17		3.21	0.53	
Flush	2	1216587	608293	24.27**	313.18	156.58	37**	134.03	67.02	29.16**	103.57	51.79	38.95**	13.23	6.62	82.24**	22.85	11.43	25.13**
Substrate x Flush	4	56984	14246	0.57	12.21	3.05	0.72	5.43	1.36	0.59	13.05	3.26	2.45*	0.94	0.24	2.93*	2.15	0.54	1.18
Species x Flush	2	321414	160707	6.41*	6.61	3.30	0.78	17.23	8.62	3.75*	6.85	3.42	2.58	0.69	0.34	4.26*	2.73	1.37	3.00*
Substrate x Species x Flush	4	254810	63702	2.54*	6.07	1.52	0.36	2.37	0.59	0.26	3.13	0.78	0.59	0.45	0.11	1.40	1.33	0.33	0.73
Residual	24	601552	25065		4.23			55.16	2.30		31.91	1.33		1.93	0.08		10.92	0.45	
Total	53	4140789			824.17			474.81			435.47			21.85			129.73		

** - Significant at $P \leq 0.01$ * - Significant at $P \leq 0.05$