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Nutritional Composition of *Pleurotus sajor-caju* Grown on Water Hyacinth, Wheat Straw and Corncob Substrates

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Abstract: Substrate type is one of the critical factors that influence the composition of mushrooms such as the *Pleurotus sajor-caju.* This study evaluated the difference ($p < 0.05$) in nutritional composition of *P. sajor–caju* when grown on water hyacinth (*Eichornia crassipes*) and on two common substrates (wheat straw and corncob). The mushroom spawn was cultivated at random, with three treatments (water hyacinth, wheat straw and corn cob) and 15 replicates. Proximate analysis was done using the Association of official analytical chemists' methods. The protein content of the *P. sajor-caju*, (dry weight), varied from 16.1% (wheat straw), 13.4% (hyacinth) to 10.8% (corn cobs). The crude fat ranged from 3.70% (water hyacinth); 3.85% (corn cobs); 3.90% (wheat straw). The crude fiber was 15.8% (wheat straw); 16.1% (corn cobs) and 18.2% (water hyacinth). Of the nutritive elements analyzed, potassium was the most dominant with concentration as high as 11.5 mg/g from the water hyacinth substrate. The amino acids of protein identified on all three treatments using the circular paper chromatography were leucine and isoleucine; phenylalanine; alanine; glutamic acid and threonine; serine, glycine, and aspartic acid; arginine; lysine and histidine; cystine; Methionine and tryptophan were present in traces in the free amino acid fraction. It was concluded that use of water hyacinth as a substrate for growing *Pleurotus sajor-caju* edible mushrooms holds tremendous promise in complementing the protein and mineral supply deficits prevalent in low income countries.

Key words: *Pleurotus sajo-caju*, Water hyacinth, Nutritional composition

INTRODUCTION

Pleurotus sajor-caju can be obtained from the wild (as a forest product) in parts of Africa, which is highly appreciated for its meaty taste and biting texture[9]. However, with rapid urbanisation, these habitats with suitable agro climatic conditions are being destroyed alongside the germplasm of this fungus[15]. While it is recongnised that efforts towards their domestication are necessary, a major limitation to their domestication is the identification of suitable lignocellulose substrates for their propagation.

Water hyacinth (*Eichornia crassipes*), has been considered as a potential substrate for mushrooms as one way of reducing its presence in aquatic waters [18]. Water hyacinth is an aquatic macrophyte member of the pickerelweed family - Pontederiaceae[22]. It is listed as one of the most productive plants on earth and is considered the world's worst aquatic weed[16]. While the possibility of using water hyacinth has been demonstrated[18], the resulting nutritional composition is not well documented. Further there have been few such studies done within the East African region on possible use of lignocellulosic waste material (water hyacinth, wheat straw and corncob) substrates, found within the area.

Inadequate supply and high cost of animal protein necessitate the search for and cultivation of locally available and cheap protein sources. Since mushrooms are a potential cheap source of protein[10], their cultivation can enrich human diets and enhance incomes especially in low income countries where meat is rare and expensive[11]. The ability of fungus to degrade lignocelluloses wastes may provide a sustainable way of addressing protein deficiency via cultivation of mushrooms. Hyacinth like other wastes could thus be effectively exploited to yield useful food that can improve human nutrition[18]. The use of this

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lignocellulosic waste material and the consequential nutritional compositions is poorly documented. Such information is particularly lacking for studies in low income countries such as Kenya and more so for the edible mushroom *P. sajor – caju*. This study had the general objective of evaluating the nutritional composition of the *Pleurotus Sajor – Caju* when grown on water hyacinth and making a comparison when the mushroom is grown on two common substrates (wheat straw and corncob).

MATERIAL AND METHODS

This work was done in the department of Botany, department of food science, department of Animal science and department of Chemistry, Egerton University Njoro.

Wheat straw and corn cobs wastes were collected from plant breeding station Njoro area in Kenya. Hyacinth was harvested from Lake Victoria in Kisumu-Kenya. Mushroom seed (spawn) was imported from India and was available in Egerton University, Department of Biology. All reagents used were of analytical grade as required and were sourced from ET Monks - Kenya.

Experimental Design: Production on substrate - The experiment was laid out in a completely randomized design (CRD), with three treatments (control – wheat straw, hyacinth and corn cobs) and 15 replicates.

Establishing Spawn: *Pleuerotus sajor caju* spawn was made with mycelium from a stored culture. This was obtained from the department of botany, Egerton University from its collection of mushroom culture which is maintained on mycelial agar disks.

For mushroom seeding, sorghum grain was used as the base material as described by Oei,[20]. In brief, the grains were washed and dead and floating grains removed. The grains were then soaked overnight in water and then washed again. The grains were heated at 100°C with an equal amount of water (on weight basis) for twenty minutes after which they were spread on an inclined surface to drain off excess water. The grains were then mixed with calcium sulphate and calcium carbonate (2 % on weight basis). Five hundred (500) grams of the grains were then transferred into spawn glass bottles which were then plugged with cotton wool. The spawn bottles with contents were sterilized at 121^0C for 1 hour in an autoclave before use. Each spawn bottle was then inoculated with 2-4 mycelial agar disks of 5 to 6 mm in diameter cut from the edge of vigorously growing *P. sajor-caju*. After 21 days the spawn was ready for seeding into the experimental substrates.

Three different types of lignocellulose's wastes - corn, wheat and hyacinth were used. All fresh substrates, were screened and cleaned to remove extraneous substances, dried before any degradation had occurred. The lignocelluloses wastes were chopped into 3-5 cm long pieces. Each substrate then was soaked separately in water 24 h, for moisture absorption to achieve moisture content of about 60-65%. The substrates were placed on wire sieves to drain and left to stabilize for 2 days at 25°C.

Polypropylene bags were then filled with moist substrates whose, components and preparation were (1.0 kg wheat/hyacinth/corn cobs lignocelluloses wastes + 12.0 g $CaSO_4.2H_2O$+3.0 g $CaCO_3$+1.5 liter distilled water). The method of preparation involved: 1.0 kg of each respective lignocelluloses waste residues was boiled (100°C) in 1.5 liter of water for 15 min and left to cool for an additional 15 min. The water was poured off and 1000.0 g of the cooked waste was mixed with 12.0 g gypsum and 3.0 g $CaCO_3$. The calcium carbonate adjusts the Ph to 6.5-7.9 which is an optimal pH for Pleurotus [14]. The Polypropylene bags (30 by 45 cm in size, with holes of 1 cm diameter and 5 to 6 cm apart all along the sides) with contents were then sterilized for 20 min at 121°C and after cooling to 25ºC inoculated with the mushroom spawn. The bags were tied and placed in the mushroom growing room maintained at 23 to 28°C. The bags were watered regularly and incubated for 30 days and the emerging fruit bodies were harvested. Windows and the doorframe of the room were covered with wire gauze to bar insects and rodents; they were hung with black cotton curtains to create darkness. The room was kept humid by pouring 10 liters of water per day on the floor.

Substrates were subjected to fructification conditions when, the mycelium had sufficiently colonized them. Fructification conditions included opening the curtains to provide more light and ventilation). Under these conditions, they were given several days for pinhead formation. Conditions in the room were 23–28°C and relative humidity of 85–95%. Fruiting bodies were harvested when the caps reached 5 to 10 cm in diameter.

The substrates were discarded after the third flush. The fruiting bodies from different flushes (1-3) in the different experiments were collected and the pileus and stipe diameters as well as the stipe lengths measured.

Growth and Yield Parameters: Data was collected during the growth and fruiting phases of the

mushrooms. Mycelia growth for *P. sajor-caju* was measured for the days required against all treatments

Proximate Analyses: Proximate analyses of mushrooms were determined according to the Association of Official Analytical Chemists (1995) methods[2]. Nitrogen was determined by the Kjeldahl method. The TN (total nitrogen), NPN (non-protein nitrogen) contents were determined using a Kjeldahl block digester apparatus (Foss Electric, Denmark) and calculated as; TN = N x 4.38, NPN = N x 4.38, TP (total protein) = TN – NPN. Because of the significant content of non-protein nitrogen in mushrooms, the protein was determined by using the adjusted conversion factor 4.38 for mushroom protein[8]. Crude fat was determined by using the Soxhlet extraction method using petroleum ether as the solvent while ash content of 1 g powdered sample was determined as the residue of incineration at 500 C, until a clear ash was obtained[2]. The determination of the fiber content in the samples was done according to the enzymatic gravimetric method[2].

Minerals: The minerals calcium, Zinc, copper, Iron, phosphorous and magnesium were determined and using an atomic absorption spectrophotometer. Potassium and sodium were determined using flame emission spectrophotometry. All mineral assays were made after dry ashing the samples, as described by Perkin-Elmer,[21]. In brief, 1 g of each mushroom sample was digested using a mixture of 9 ml $HN0_3$ (65%) and 1 ml $HClO_3$ (70%). The mixture was left overnight in a closed beaker. Digestion was performed at 160-170 C for about 5 min on a hot plate until white fumes of $HClO_3$ were visible. Digestion procedure was performed in a fuming hood for safety. After cooling, each sample was filtered using a Whatman-40 filter paper and 10 ml 5 % Lanthanium solution added, then each sample was diluted with 5 % $HN0_3$ (V/V) to 50 mL in 50 mL volumetric flask prior to analysis. The silica was left to settle and the supernatant decanted.

Appropriate stock standards for the minerals were made. The absorbances of the prepared standards were used to establish the calibration curves for the minerals. A corning® 410 flame photometer (Chem Tech Analytical®) was used. The µg/ml concentration of each sample was calculated from the calibration curve and concentrations of the minerals expressed as mg g-1.

Statistical Analyses: All samples were analyzed in triplicates and results were recorded as mean ± S.D. Means of readings from three replicates were determined and subjected to analysis of variance. Means then were separated using Duncan's Multiple Range Test with the aid of Minitab statistical package[19].

RESULTS AND DISCUSSION

Growth Characteristics: The mushrooms on all three substrates sprouted in clusters, and had the grayish-brown color that is characteristic for the species. The second flushes occurred 15 and 18 days after the first yield, while the third occurred between 21 and 25 days after the second flush. On average 10% of all plots stopped producing due to contamination of the substrate by competing microorganisms.

The spawn run on the hyacinth substrate could be observed after 12 days of incubation in the growing-room, with the formation of light pink halos around the spawn, indicating the beginning of degradation of the substrate by the fungus. The natural induction of primordia on the substrates occurred after 15 days (wheat straw), hyacinth (18 days) and 22 days (corn cobs) of incubation.

The first flush or harvest occurred after 20 days of incubation for wheat straw, 21 days for hyacinth and 26 days for corn cob (Table 1). These were averaged to the nearest day from the replicates.

From table 2, the mycelia growth was highest in the wheat substrate which also had the highest mycelia density. The hyacinth had good extension with moderate evidence of mycelia density.

Radial growth and mycelia density of *Pleurotus sajor-caju* when grown on various lignocellosic wastes was significantly ($p<0.05$) different indicating the substrate type has an influence on the radial growth and mycelial density.

Proximate Composition: The proximate compositions (g/100 g) (on dry weight basis) of the cultivated *P. sajor-caju* fruit bodies on the three substrates is shown in Table 3. Moisture content differed significantly ($p<0.05$) and the distribution ranged from 11.50±0.20% on the wheat straw to 12.40±0.01% from the hyacinth one and this difference was significant ($P<0.05$).

The mushroom which was grown on the wheat straw had a higher protein concentration (16.10±0.01%) than the hyacinth (13.40±1.20%) and the corn cob substrate (10.8±0.20%). This difference was significant ($p<0.05$) and suggests that cultivation on the wheat straw gives a better source of protein than the other two substrates used.

Table 1: Cultivation cycle (Average days from replicates) of *Pleurotus sajor caju* on the three lignocelluloses wastes.

Cultivation cycle	Wheat straw	Hyacinth	Corn cobs
Spawn running	11	12	14
Primordia/pinhead formation	4	6	8
To first fruit bodies	5	5	7
Total days to first flush	20	21	26
Second flush	15	18	18
Third flush	21	24	25
Total cycle for three flushes	56	63	69

Table 2: Radial growth and mycelia density of *Pleurotus sajor-caju when grown* on various lignocellosic wastes.

Treatments/ Lignocelluloses	Mycelia extension (mm)	Mycelia density
Wheat	78.81c± 0.2	+++
Hyacinth	67.6b± 1.0	0
Maize cobs	56.3a±0.1	0

+: Scanty mycelia growth.
++: Moderate mycelia growth.
+++: Very abundant mycelia growth.
Values followed by different letters are statistically significant different ($p < 0.05$) from each other.

Wheat straw and water hyacinth are considered to be model lignocelluloses material that contains highly crystalline cellulose and large amounts of arabinoxylan, the major hemicellulosic fraction[13]. For both substrates, enzymes from the mushrooms growing on them including cellulase and xylanase act on the hemicellulosic components converting them to a mixture of solubilized sugars, mainly oligomers. The sugars are then available as essential nutrients for the growth of the mushrooms. The fat concentration in the mushrooms grown on the hyacinth (3.70±0.03%) was lower than that in wheat straw (3.90±0.11%) and from the corn cob (3.85±1.00%) although this difference was not significant ($P>0.05$). The mushrooms grown on the water hyacinth contained a higher crude fiber content (18.2 ±0.20%) compared to the wheat straw (15.8 ±1.00%) and maize cobs (16.1 ±0.10%). This difference between the substrates was significant ($p<0.05$) and it may be due to the fact that the water hyacinth being a non cultivated substrate grows wild and the level of nutrients obtained could be their source of high fiber content.

The findings suggest that using hyacinth substrates does not have a detrimental effect in terms of the proximate composition of the mushroom. Hyacinth substrate could offer viable means of cultivating mushrooms which could also offer one way of reducing their levels in the environment. The cultivation of edible mushroom using hyacinth lignocelluloses residues, which is also poses environmental problems, would be a value added process to convert the materials, which are otherwise considered to be wastes, into human foods.

Several agricultural residues have been used to produce the edible mushroom *Pleurotus sp*. Among the substrates used have included: rice hulls mixed with cotton residues, banana leaf, mixed with sugarcane bagasse or corn cobs and also cassava residues with sugarcane bagasse[3,6,7].

Mineral Composition of *Pleurotus sajor-caju* Fruit Bodies: Mineral composition (mg g-1) *of Pleurotus sajor-caju* fruitbodies on the cultivated substrtates is shown in Table 4. The results of mineral values of the mushrooms from all three substrates tested clearly indicate the potential for their use as sources of good quality food. The results of nutritionally valuable minerals show that the mushroom species is rich in potassium, calcium, magnesium and iron. The mineral levels, mainly potassium, phosphorous, sodium and iron in these mushrooms were higher than those reported for several cow pea varieties[1], but lower than those reported for fish, snails and broiler meat[1,5].

In this study, potassium was the most abundant nutrient followed by phosphorus and magnesium (Table 4). Fasidi and Kadiri[12] and Manzi *et al*.,[17] also reported that potassium was the most abundant mineral element in various species of edible mushrooms. Mushrooms are generally low in sodium concentration[4]. The low sodium and high potassium concentration is of significance as a Na/K ratio less than 0.6 suggests that the mushrooms will be suitable for diet formulation for hypertensives.

For the majority of the minerals the distribution was such that the mushroom grown on hyacinth had a higher concentration than that on the wheat and corncobs one (Table 4). Minerals in the diet are required for metabolic reactions, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance among others. From the study, it was observed that *Pleurotus sajor-caju* hold good promise in complementing the protein and mineral supply deficits prevalent in low income countries countries. Mushroom growing if successful can greatly improve the livelihood of the East African mushroom farmers. Availability of land is not a limiting factor since mushrooms require little space and can be grown under cover inside mushroom houses. Mushroom growing is also labor-intensive and therefore has employment potential. The technologies involved in mushroom growing are also simple and hence easily adaptable even by small-scale farmers.

Table 3: Mean ±SEM Proximate (g/100g) composition of *Pleurotus sajor-caju* fruit bodies from three substrates (water hyacinth, wheat straw and corn cob) on a dry weight basis (n=3)

Substrate	Moisture	Crude protein	Crude fiber	Fat	Ash
Water Hyacinth	12.40 ±0.20%	13.4±1.20%	18.2 ±0.20%	3.70 ±0.03%	10.95 ±0.10%
Wheat straw	11.50 ±0.20%	16.1±0.10%	15.8 ±1.00%	3.90 ±0.11%	10.50±0.20%
Corn cobs	11.76±0.10%	10.8±0.20%	16.1 ±0.10%	3.85 ±1.00%	11.40 ±2.10%
Significance	<0.001	0.004	0.009	ns	0.044

*Not significant (p>0.05)

Table 4: Mineral composition (mg g-1) of *Pleurotus sajor-caju* fruit bodies on the cultivated substrates*Analyzed on dry weight basis, (mean ±SD)

Mineral	Hyacinth	Wheat straw	Corn cobs	Significance
Ca	6.40±0.01	5.14±0.20	5.02±0.1	0
Fe	1.92±0.01	1.47±0.1	1.39±0.1	0
K	11.68±0.02	8.41±0.01	8.28±0.01	**
Na	2.93±0.01	1.63±0.03	1.51±0.10	**
Zn	0.30±0.00	0.34±0.02	0.31±0.10	ns
Cu	0.03±0.01	0.06±0.01	0.02±0.01	0
Mg	7.74±0.01	7.33±0.01	6.55±0.02	0
P	10.80±0.01	10.20±0.02	9.73±0.01	0

*and ** = significant at 5% and 1% levels, respectively; ns = not significant.

Conclusions: The nutritional composition of the mushroom *Pleurotus sajor-caju* grown on the hyacinth lignocelluloses compares favorably with that got when the mushroom is grown on common substrates. Cultivation of *Pleurotus sajor-caju* combined with waste utilization can be an economical and harmless method of waste disposal. It has nutritional benefits especially where nutritive foods are scarce and costly but lignocelluloses wastes are abundant. It can result in better nutrition for people in low income countries since mushrooms are a healthy food, poor in calories and fat but rich in vegetable proteins, vitamins and minerals.

Future research may entail the development of ready made or value added processed foods from mushrooms. The value added products will not only cater to the protein and micronutrient requirement but at the same time will enable the population to live a healthy life.

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