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PROXIMATE COMPOSITION AND AMINO ACID PROFILE OF RICE HUSK BIODEGRADED WITH *PLEUROTUS OSTREATUS* FOR DIFFERENT PERIODS

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ABSTRACT

Native rice husk (NRH) was fermented with *Pleurotus ostreatus* for 7, 14 and 21 days to improve the nutritional values. The proximate composition and amino acid profiles were determined. The results showed that crude fibre (CF), nitrogen free extract (NFE), acid detergent fibre (ADF), and neutral detergent fibre (NDF) were significantly higher ($p<0.05$) in the NRH (with values 13.37%,42.93%, 17.45% and13.42%, respectively) while corresponding lowest values of 12.14%, 34.26%, 12.27% and 12.41% were obtained in the 14 days samples. Crude protein (CP) was significantly highest ($p<0.05$) in the 7 days fermentation (12.37%) but lowest in the 21 days (7.94%). The nitrogen free extract (NFE) values were similar and higher ($p<0.05$) in the NRH (control), 7 and 21 days fermentation that is 42.93%, 40.37% and 39.53%, respectively. The calculated metabolizable energy (ME) was similar and higher ($p<0.05$) for the fermented native rice husk at 7, 14 and 21 days that is 2289.19, 2308.77 and 2328.59 kcal/kg, respectively. Dry matter (DM), ash, calcium and phosphorous values were not influenced ($p<0.05$) by the fungi treatment of the NRH. Essential amino acids (EAA) values viz; lysine (4.94 g/100g), valine (7.34 g/100g), threonine (4.81 g/100g), iso-leucine (4.83 g/100g) and phenylalanine (5.59 g/100g) were higher ($p<0.05$) in the 14 days fermentation with *p. ostreatus* while others that are non-essential amino acids (NEAA) in this group include alanine (7.06 g/100g) and tyrosine (4.12 g/100g). Glycine (6.02 g/100g), serine (5.18 g/100g), proline (4.85 g/100g), leucine (7.44 g/100g), glutamate (14.54 g/100g) and arginine (8.51 g/100g) values were higher ($p<0.05$) in the 21 days while aspartate (8.70 g/100g), histidine (2.37 g/100g), methionine (2.69 g/100g), tyrosine (4.20 g/100g) and cystine (2.09 g/100g) were higher ($p<0.05$) in the 7 days treatment. All the amino acids with the exception of glycine, alanine and aspartate had lowest ($p<0.05$) values recorded in the native rice husk. The total amino acids (TAA), total essential amino acids (TEAA), total non-essential amino acids (TNEAA) were all significantly higher in the 7 days fermentation (95.43, 45.01 and 50.42, respectively) and the lowest values in the NRH. Percent EAA followed the same trend while %NEAA was highest in the NRH. Fermentation of native rice husk for 7 days improved its nutritive value and consequently increased its usage as a component of livestock feed.

Key words: Native Rice Husk**,** Fermentation, Proximate Composition, Amino Acids

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INTRODUCTION

Rice husk is a by-product of milling paddy rice, *Oryza sativa.* It consists of fibrous materials such as opaline and lignin, which makes up about 20 - 22% of the weight [1]. It is reported to contain 2.9-3.6% crude protein, 8-12% oil, 39-42% crude fibre, 15-22% ash and limited in usage as component of monogastric feed because of its high fibre content [2, 3].

The use of biotechnology to make farm wastes utilizable through degrading of their cell wall components has been extensively reported [3, 4, 5]. Some appreciable improvement in nutrient contents and amino acid profiles of some fibrous farm wastes namely, rice husk, corn cobs and cassava peels subjected to solid state fermentation (SSF) have been reported, which gave increment of about 20% crude protein, 25% metabolizable energy, 30% nitrogen free extract, with about 0.3% significant decrease in crude fibre content [4,5,6,7]. This is achieved through the use of microbes (fungi and bacteria) that can produce or biosynthesize enzymes capable of degrading the cross-linked bonds in ligno-cellulosic materials that are characteristic of fibre components of most agro wastes [8]. However, it has been established that fungi degraded lignin faster than bacteria and also enhanced the protein content of substrate through the addition of fungal protein [9]. *Pleurotus ostreatus* or oyster mushroom is a fungus which is capable of bio-conversion of varieties of lingo-cellulosic materials due to the secretion of extracellular enzymes [10, 11]. Available information on the nutritional changes when native rice husk (NRH) is fermented with fungus at different periods is limited. Hence, this study investigated the influence of solid state fermentation of native rice husk (NRH) at different periods using *Pleurotus ostreatu*s as the inoculums on the proximate composition and amino acid profiles.

MATERIALS AND METHODS

Procurement of the *Pleurotus ostreatu*s

Pure culture of *Pleurotus ostreatus* (oyster mushroom) was obtained from the Department of Applied Biological Science, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria. The fungus was maintained on Potato Dextrose Agar (PDA) slant, multiplied and stored in a refrigerator and later tissue cultured to obtain fungal mycelia in the microbiology laboratory of Ekiti State University, Ado-Ekiti, Nigeria [12].

Collection and processing of rice husk

Native rice husk (local variety of rice) was obtained from rice milling centre at Igbemo-Ekiti, Ekiti State, Nigeria. Ekiti State is located in the southwestern Nigeria. The native rice husk (NRH) was air dried to reduce the moisture content to about 12%. It was then packed in polythene bags and sterilized in the autoclave at 121ºC for 30minutes.

Inoculation and fermentation of rice husk

The autoclaved samples were cooled, moistened with distilled water at the rate of 300 ml per kg of NRH and inoculated with 5 plates of the cultured fungi (plate size 10cm diameter) [13]. Each inoculated sample was well mixed and then kept in a dark

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cupboard in the laboratory. The samples were subjected to fermentation for different periods: 7, 14 and 21 days after which the action of the fungus was stopped by oven drying at 80ºC for 24 hours.

Chemical Analyses

Proximate composition and amino acid profile determination

Samples of NRH fermented with *Pleurotus ostreatus* for the different periods were air dried, divided into triplicate and their proximate analysis determined to obtain values for dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), nitrogen free extract (NFE) and ash as described by AOAC [14]. Neutral detergent fibre (NDF) and acid detergent fiber (ADF) were obtained as described by Van Soest *et al*. [15]. The samples were mixed in a laboratory blender, hydrolyzed at 150^{o}C for about 90 minutes and the solution used for the determination of amino acids by the modified Waters 'Pictotag'system described by Bidlingmeyer *et al*. [16]. The metabolizable energy of the samples was calculated using prediction equation as reported [17].

M.E. = 35 x % CP + 81.8 x % EE + 35.5 x % NFE

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) at 5% probability level and means separated by Duncan Multiple Range Test using the minitab computer package software [18].

RESULTS

Proximate composition

The proximate composition of NRH fermented for different days is presented in Table 1. *Pleurotus ostreatus* significantly increased ($p<0.05$) the CP contents of NRH while the DM and ash values were not affected ($p>0.05$). The highest CP was obtained in the NRH fermented for 7days (12.37 %), while the value for 21 days was the lowest (7.94%). The solid state fermentation decreased the NDF value of the NRH from 13.42% in the control to 12.00% for the 7 days fermentation period but no significant difference ($p>0.05$) was obtained in the control and 21 days fermentation. The ADF values for the 7 days (12.23%) and 14 days (12.27%) fermentation periods were similar. However, the ADF in the untreated NRH (17.45%) was significantly higher ($P<0.05$) than the 21 days fermentation period (12.57%). The fermentation also significantly ($p<0.05$) decreased the crude fibre content of NRH (13.37% in the control) as the treatment days increased to 14 days, that is 12.58% and 12.14% for 7 and 14 days, respectively. Fermentation of NRH with *Pleurotus ostreatus* also significantly ($p< 0.05$) decreased the ether extract (EE) with the lowest value of 4.87% recorded for the 7 days period while 14 days had the highest (8.53%). The calculated metabolizable energy (ME) was similar for the fermented NRH at 7, 14 and 21 days with values 2289.19; 2308.77 and 2328.59 Kcal/kg, respectively. These values were significantly higher ($p<0.05$) than in the untreated NRH sample (2269.29 Kcal/kg).

The nitrogen free extract (NFE) was significantly lowest in the 14 days sample of fermented rice husk highest ($p<0.05$) while the values obtained for the untreated NRH

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(42.93%), 7 and 21 days fermented rice husk (40.37% and 39.53%, respectively) were similar.

The different periods of fermentation had no significant difference ($p>0.05$) on the values of calcium and phosphorus contents recorded.

Amino acid profile

The quantitative composition of amino acids profile of NRH fermented with *Pleurotus ostreatus* for different periods is shown in Table 2. The duration of fermentation significantly ($p<0.05$) affected the values of the amino acids. Methionine, aspartate, histidine and cystine had significantly higher values ($p<0.05$) of 2.69, 4.20, 8.56 and 2.37 g/100g, respectively than other amino acids in samples fermented for 7 days. Lysine, alanine, valine, threonine, isoleucine and phenylalanine had highest values in the 14 days fermentation of native rice husk with values of 4.94, 7.06, 7.34, 4.81, 4.83, 2.09 and 5.59 g/100g, respectively, while glycine (6.02g/100g), serine (5.18g/100g), proline (4.85g/100g), leucine (7.44g/100g) and glutamate (14.54g/100g) were significantly higher ($p<0.05$) in the 21 days fermentation period. Total amino acids were also significantly influenced ($p<0.05$) by the fermentation (Table 3). The values recorded in the native rice husk fermented for the various days of 7, 14 and 21 were similar (95.43, 93.80 and 95.06 g/10g, respectively) but higher than the value obtained from the untreated native rice husk (80.47 g/100g). The total essential amino acids (TEAA) increased significantly and highest ($p<0.05$) in days 7 and 14 (45.01 and 45.31g/100g, respectively) than in day 21 while the lowest value was obtained in the control or untreated NRH (35.87 g/100g). The nonessential amino acids (NEAA g/100g) values were highest and similar in the 21 and 7 days fermentation periods while the unfermented NRH had the lowest (44.60 g/100g). Percent EAA followed the same trend as the TEAA while % NEAA was highest ($p<0.05$) in the untreated NRH but lowest in the 14 days fermented samples (51.70%).

DISCUSSION

The microbial action of *Pleurotus ostreatus* no doubt resulted in the release of the trapped nutrients in the fibre matrix and led to an increment of 44.17% in the crude protein content of the fermented NRH. This result differs from the findings of Fasuyi and Olumuyiwa [19] who reported 4.8%, 4.9%, 5.1% and 5.3% CP values for rice husk fermented with molasses for 7, 14, 21, and 28 days period. However, it compared favourably with the report of Aderolu and Onilude [3] who obtained an increase in CP especially during the first 10 days when *Trichoderma viride* was used to degrade rice husk over a period of 40 days. This huge increase in CP may be due to favourable environmental factors such as pH and temperature which supported the inoculum activities during the fermentation period. These factors have been implicated in the release of xylanase by *Pleurotus ostreatus,* which activity had been reported to be optimal at 7 days of fermentation [20]. The xylanase and other hemicellulolytic enzymes of *Pleurotus ostreatus* have the capacity to biodegrade the cell wall structure and their residue after expiration of life span or termination of fermentation added as cell protein to the substrate. In addition, antifungal properties of some bacteria might have inhibited the potential of *Pleurotus ostreatus* as the fermentation time increased

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[21, 22]. The increment of 44.17% in CP for the 7 days fermentation period compared favourably with the results obtained by other workers [4, 5, 6,22]. This is an indication of the potential biodegradable effects of *Pleurotus ostreatus* on cheap ligno-cellulosics and low-grade agro-wastes, with bioconversion to protein-rich products.

The NDF and ADF decreased by 10.58% and 29.91%, respectively after 7 days of fermentation. This shows that *Pleurotus ostreatus* have the capacity to biodegrade fibre and possibly the non-soluble polysaccharides (NSP) that may be present in the NRH. This perhaps may be used as a source of energy during growth and other activities of lignin degrading enzymes [11]. This is supported by the study that *Pleurotus ostreatus* produce hemicellulolytic enzymes, lignin peroxidase and manganese peroxidase with the ability to degrade lignin and a variety of β-(1, 4) linked glucan substrates as well as glycosides [8]. Therefore, the ADF and NDF could have served as a source of energy for *Pleurotus ostreatus* or other microbes in the fermentation medium which agrees with previous studies on cocoa pod husk and wheat straw where 20 – 45 % reduction in hemicelluloses were reported [23, 24, 25]. The increased values of the ether extract with the fermentation period explained why the calculated values of the metabolizable energy increased correspondingly using Pauzenga prediction equation [17]. These values were similar and higher in the entire fermented samples than the NRH (Table 2). The decline in the values of crude fibre was as a result of the ability of *Pleurotus ostreatus* to produce cell wall degrading enzymes acting on fibrous agro wastes as earlier explained. This breaks down the polysaccharides into less complex carbohydrates for optimum utilization. The decline in crude fibre values agrees with previous studies where rice husk and wheat straw were fermented with *Pleurotus spp* [22, 26]. The decrease in the values of crude fibre may also be as a result of the utilization of carbohydrates by the fungus as an energy source for mycelia growth as explained earlier for NSP [11].

The calcium and phosphorous content of the fermented and the unfermented NRH appeared inadequate for the requirements of most farm animals. This implies that native NRH degraded with *Pleurotus ostreatus* may have to be fortified with good calcium and phosphorous sources when used as a component of farm animal diets.

The levels of essential amino acids in the fermented samples were substantially higher than that of the NRH. The additive effect of the *Pleurotus ostreatus* after fermentation as cell protein and those of other microbial organisms must have contributed to the observed increase because they are richer in essential amino acids particularly lysine and methionine. The results of this study indicated that the duration of fermentation has effect on the amino acid composition of rice husk when fermented with *Pleurotus ostreatus*. The TEAA of the fermented NRH samples show that *Pleurotus ostreatus* may both enhance the protein quantity and improve the quality. This agrees with the findings of Kutlu *et al. and* Muhammad and Oloyede [26, 27] that *Pleurotus species* are a rich source of proteins and also contain all the essential amino acids.

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CONCLUSION

The use of *Pleurotus ostreatus* as an inoculant for fermentation of native rice husk (NRH) influenced the proximate composition and amino acid profile of the waste material studied in this trial. The crude protein of the NRH that is very low was improved by 44.17% in the 7 days fermentation trial with 2328.59 kcal/kg metabolizable energy than 14 and 21 days. The nutritive value of NRH that is abundant in rice growing communities or states could be improved through the use of fungi or microbial fermentation which may reduce the cost of feed in livestock production. The productive utilization of the rice husk will also reduce the environmental nuisance which this agro waste often constitute both in towns and villages of most of the developing countries and reduce government and rice processors expenditure on disposal of the agro waste.

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Table 1: Proximate composition, fibre fractions, calcium and phosphorous contents of native rice husk fermented with *Plearotus ostreatus* for different period (n=3)

Parameters	Native RH	7 day Fermentation	14 day Fermentation	21 day Fermentation
Dry Matter	90.4 ± 0.3	88.90 ±0.1	86.71 ±0.2	89.50± 0.3
Crude Protein	8.58±0.16^{c}	12.37±0.30^{a}	10.67±0.02^{b}	7.94±0.04^{d}
Crude Fibre	13.37±0.03^{a}	12.58±0.02^{b}	12.14±0.02^{bc}	12.48±0.02^{b}
Ether Extract	5.23±0.01^{c}	4.87±0.02^{d}	8.53±0.02^{a}	7.72±0.02^{b}
Nitrogen Free Extract	42.93± 0.5^{a}	40.37±0.2^{a}	34.26±0.15^{b}	39.53±0.01^{a}
Ash	20.30±0.03	18.71±0.01	21.10±0.1	21.83± 0.2
Acid Detergent Fibre	17.45±0.03^{a}	12.23±0.03^{ab}	12 .27±0.02^{ab}	12.57±0.03^{b}
Neutral Detergent Fibre	13.42±0.02^{ab}	12.00±0.02^{c}	12.41±0.02^{b}	13.78±0.14^{a}
Calcium	0.05±0.00	0.06±0.057	0.65±0.057	0.06±0.057
Phosphorous	0.51±0.02	0.60±0.10	0.55±0.10	0.46±0.01
Metabolizable Energy (kcal/kg)	2269.29 ±10.2^{c}	2289.19± 13.4^{ab}	2308.77±11.5^{ab}	2328.59±9.3^{a}

a,b,c,d, means within the same row with different superscripts differ significantly ($p<0.05$). Values are represented as mean ± standard deviation (n=3)

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Table 2: Amino acids of native rice husk fermented for different periods (g/100g)

Amino acids	*fermentation in days*			
	NRH	7days	14 days	21 days
Aspartate	8.60±0.06^{a}	8.70±0.08^{a}	8.03±0.02^{b}	8.56±0.03^{a}
Lysine	3.46±0.01^{d}	4.77±0.04^{b}	4.94±0.04^{a}	4.35±0.05^{c}
Histidine	1.64±0.01^{c}	2.37±0.05^{a}	2.08±0.04^{b}	2.18±0.04^{b}
Glycine	5.48±0.03^{c}	5.93±0.03^{b}	4.93±0.02^{d}	6.02±0.02^{a}
Alanine	6.10±0.04^{c}	6.25±0.05^{b}	7.06±0.06^{a}	5.90±0.06^{d}
Serine	4.35±0.03^{c}	4.95±0.05^{b}	4.91±0.03^{b}	5.18±0.03^{a}
Proline	4.28±0.04^{b}	4.10±0.05^{c}	3.43±0.05^{d}	4.85±0.05^{a}
Valine	5.41±0.03^{d}	6.77±0.06^{b}	7.34±0.04^{a}	6.35±0.04^{c}
Threonine	3.22±0.06^{d}	4.07±0.07^{b}	4.81±0.01^{a}	3.65±0.05^{c}
Isoleucine	3.38±0.04^{d}	4.36±0.04^{b}	4.83±0.03^{a}	4.22±0.04^{c}
Leucine	6.82±0.06^{c}	7.24±0.04^{b}	7.26±0.06^{b}	7.44±0.08^{a}
Methionine	1.66±0.07^{d}	2.69±0.05^{a}	1.94±0.04^{c}	2.54±0.04^{b}
Glutamate	11.51±0.05^{c}	14.20±0.01^{b}	14.26±0.06^{b}	14.54±0.04^{a}
Phenylalanine	4.90±0.01^{d}	5.22±0.02^{b}	5.59±0.02^{a}	5.03±0.03^{c}
Arginine	5.38±0.03^{d}	7.52±0.08^{b}	6.52±0.04^{c}	8.51±0.05^{a}
Tryptophan	ND	ND	ND	ND
Tyrosine	2.33±0.02^{c}	4.20±0.04^{a}	4.12±0.06^{a}	3.88±0.06^{b}
Cystine	1.95±0.04^{b}	2.09±0.04^{a}	1.75±0.05^{d}	1.86±0.06^{c}

a, b, c, d, Means with different superscripts within the rows differ significantly ($p<0.05$)
ND= Not determined

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Table 3: Sum of total amino acids, essential and non-essential amino acids (g/100g) and their percentages in fermented native rice husk

Amino Acids	*Fermentation duration in days*			
	Native RH	7 days	14 days	21 days
TAA	80.47±0.02[b]	95.43±0.05[a]	93.80±0.07[a]	95.06±0.04[a]
EAA	35.87±0.01[c]	45.01±0.05[a]	45.31±0.03[a]	44.27±0.05[ab]
% EAA	44.58[c]	47.17[a]	48.30[a]	46.57[ab]
NEAA	44.60±0.03[c]	50.42±0.04[a]	48.49±0.04[b]	50.79±0.04[a]
% NEAA	55.42[a]	52.83[b]	51.70[c]	53.43[b]

a, b, c, Means with different superscripts within the rows differ significantly ($p<0.05$)

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