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New York Science Journal

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Nutritional composition of *Rhizopus oligosporus*-treated rice (*Oryza sativa*) husk (*RoTRH*)

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Abstract: A study was carried out to determine the effect of fungal treatment of rice (*Oryza sativa*) husk on its nutrient content using *Rhizopus oligosporus* fungus. The rice husk was divided into two (2) treatments each of which was replicated thrice. One treatment was treated with *R. oligosporus* fungus using Solid State Fermentation (SSF) method while the other treatment was left untreated. The two treatments were analysed for proximate composition and mineral contents. Data collected was subjected to student T-test. The findings of the study revealed that there were variations between the untreated and treated rice husk. It was concluded that fungal treatment of rice husk using *R. oligosporus* improved its nutritional value thus making *RoTRH* a potential valuable feedstuff.
[Ogunbajo, Shakirudeen Adetokunbo. and Belewu, Moshood Adewale. **Nutritional composition of Rhizopus oligosporus-treated rice (Oryza sativa) husk (RoTRH).** *N Y Sci J* 2021;14(3):5-9]. ISSN 1554-0200 (print); ISSN 2375-723X (online). http://www.sciencepub.net/newyork. 2. doi:10.7537/marsnys140321.02.

Keywords: *Rhizopus oligosporus,* water, nutrients, treatment.

1. Introduction

Rice husk is an agricultural by-product which is produced at 20 % of the about 2 million tons of rice produced in Nigeria annually (Oyetola and Abdullahi, 2006). Rice husk is the dry outer covering of rice grain, which is always removed during the milling of rice. Belewu and Adenuga (2003) reported that Rice husk contains 71.4 % Acid Detergent Fibre and 15.2 % Acid Detergent Lignin. Rice husk is considered a poor quality feed material for all animals; monogastrics and ruminants alike but it is produced abundantly throughout the rice growing areas of the tropics and subtropics. According to Adeoye *et al.* (2011), it is believed to have no direct nutritional value to man and in most mills it is often discarded or allowed to rot away. Parimala *et al.* (2007) noted that lignocellulosic biomass can serve as low cost feed stocks for production of fuel, ethanol and other value-added commodity chemicals but are of poor nutritive value and low digestibility because of their higher lignin content. Ash constitutes the inorganic matter mainly containing minerals and is also difficult for animal digestion but required in trace amounts. Minerals also act as a barrier against the attack of rumen microbes to structural carbohydrates. According to Arora and Sharma (2011), degradation of lignin has got the potential to improve the digestibility of such residues. Attempts at increasing the utilization of fibrous feed ingredients like rice husk include supplementation with high quality protein and amino acids (Delorme and Wojak, 1982), adequate fortification with micro nutrients (Onifade, 1993), physical and chemical pre-treatments and the use of microbial enzymes and antibiotics (Onilude, 1994; Wing-Keong *et al*., 2002; Akinyele and Akinyosoye, 2005). Natural degradation of plant biomass is mainly caused by different fungal and bacterial species (Bugg *et al*., 2011). In the opinion of Agosin *et al*. (2006), fungal bioconversion of lignocellulosic by-products is a practical and promising alternative for increasing their nutritional value, transforming them into animal feed and thus producing a value-added product. Fungi are usually better degraders of plant cell wall constituents because of their extracellular enzymatic system and hyphal penetration power (Sharma and Arora, 2013). This study therefore seeks to evaluate the nutritional composition of *RoTRH*.

2. Materials and Methods

Rice husk was collected from rice millers in Minna metropolis, Niger State. It was soaked in water for twenty-four hours after which the excess water was strained using a muslin cloth. The soaked rice husk was then packaged in polythene bags at 1kg per bag ready for autoclaving at 121^{0}C, 15psi for 30 minutes so as to get rid of any microbes that could be present in the husk. The *Rhizopus oligosporus* which was obtained from the Department of Microbiology, University of Ilorin, Kwara State, Nigeria was sub-cultured on Potato Dextrose Agar (PDA) by transferring the spores aseptically from the cultures to freshly prepared PDA-containing Petri-dishes. The

PDA was amended with streptomycin[R] to suppress any bacterial growth and later autoclaved at 121^0C, 15psi for 15 minutes to sterilize it. The Petri-dishes were later incubated at ambient temperature for four (4) days to stimulate the fungal growth. The cooled autoclaved rice husk was divided into two Treatments thus: A and B each of which was replicated thrice. Suspension of actively growing mid-log phased culture of *R. oligosporus* was individually adjusted to 5 x 10^4 spores/ml with distilled water in line with the methods of Sani *et al*. (1992). Twenty (20) ml from the suspension was used to inoculate one (1) kg of cooled autoclaved rice husk in layers in a container, covered and incubated at room temperature for eight (8) days when the fungus had enveloped the substrate. Growth was terminated by oven-drying at 80^0C for twenty four (24) hours. Treatment A was left untreated and equally incubated at room temperature. The rice husk was analysed before and after treatment for its proximate compositions and energy values. The proximate composition was determined according to the methods of AOAC (1990). The fiber fractions: Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were determined according to the methods of Goering and Van Soest (1970) while the energy value was determined by calculation using the formula proposed by Pauzenga (1985).

Metabolizable Energy = (37 x Crude Protein) + (81.8 x Ether Extract) + (35.5 x Nitrogen Free Extract)

Hemicellulose was determined by calculation using the formula

Hemicellulose = NDF – ADF

The cellulose was also determined by calculation using the formula

Cellulose = ADF – ADL

Two macro minerals namely potassium and sodium were determined by Flame Photometer method using Sherwood 410 Flame Photometer while another two macro minerals namely calcium and magnesium and three micro minerals namely iron, copper and zinc were determined by Atomic Absorption Spectrometer (AAS) method using Buck Scientific ACCUSYS 211 Atomic Absorption Spectrometer. Phosphorus was determined using the ascorbic acid method. All data collected were subjected to student 'T' Test analysis using the statistical package SAS 9.2.

3. Results and Discussion

The results of the Proximate Composition and Energy values of the untreated rice husk and *RoTRH* are presented in Table 1. The results showed a significant ($p < 0.05$) difference in the Dry Matter content of 94.36 % in the untreated rice husk and 92.21 % in the *RoTRH*. The Crude protein and ash contents of the untreated rice husk (6.34 % and 19.25 %) differed significantly ($p < 0.05$) from that of the *RoTRH* (8.75 % and 24.09 %). The higher crude protein content in the *RoTRH* when compared to the untreated rice husk could be attributed to the microbial protein synthesized by the fungus used. Also, the enzymes secreted by the *R. oligosporus* equally contributed to the higher protein content of the *RoTRH* as enzymes are naturally known to be proteinous in nature. It has also been reported by Bhatnagar (2004), Ekpe *et al*. (2007) and Belewu (2008) that there was a higher crude protein content of fermented substrates which the authors attributed to single cell microbial protein addition during the fermentation process. According to Palvis and Walia (2001), higher nitrogen and protein contents and lower contents of crude fiber of *R. oligosporus*-Treated rape seed could be probably due to the action of the fermentative enzymes. The Nitrogen Free Extract content varied significantly ($p < 0.05$) between 39.90 % for the untreated rice husk and 34.58 % for the *RoTRH*. Equally, energy content of the untreated rice husk (1865.35 Kcal/kg) differed significantly ($p < 0.05$) from 1791.83 Kcal/kg for the *RoTRH*. The lower energy and Nitrogen Free Extract contents of the *RoTRH* when compared to the untreated rice husk might be attributed to the metabolism of the micro-organisms in the fermentation medium which corroborates the findings of Difo *et al*. (2014). The reported lower energy content in the *RoTRH* could also be due to the secretion of hydrolytic enzymes which hydrolyze the carbohydrates into simple sugars to be utilized as carbon source by the microbes and transform it to other metabolites such as protein and fat resulting in higher levels of these metabolites in the treated substrates (Oboh and Akindahunsi, 2003).

The Crude fiber contents of the untreated rice husk (26.25 %) differed significantly ($P < 0.05$) from that of the *RoTRH* (21.85 %). The Ether extract content of the untreated rice husk (2.62 %) and that of the *RoTRH* (2.94 %) differed significant ($p < 0.05$) from each other. The lower fiber fractions of the *RoTRH* reported in this study can be attributed to the degradation of the cell wall component of the substrates by the action of the enzymes produced by the extra-cellular enzymatic systems of the fungus. These extra-cellular enzymatic systems according to Sanchez (2009) are the hydrolytic system which produces hydrolases that are responsible for polysaccharides degradation and a unique oxidative ligninolytic system which degrades lignin and opens phenyl rings. According to Belewu and Babalola (2009), a reduction in the fiber fractions of *R. oligosporus*-Treated agricultural residues may be an indication that fungi have the enzymatic potential to use ligninocellulose components as carbon and energy sources. The lower fiber fractions might also be associated with the available fermentable sugars released as a result of the hydrolyzing enzymes of the substrate which led to the stimulation of the fungus to produce high levels of activated enzymes to degrade the fiber fractions. This assertion corroborates the findings of Abo-Eid (2007). The higher lipogenic activity than lipolytic activity of the lipase enzymes present in the *R. oligosporus* could also be responsible for the higher ether extract content of the *RoTRH* in the experiment. This was in line with the reports of Belewu and Popoola (2007) that fermentation of substrates using fungi that have more lipogenic ability than lipolytic activity resulted in a higher Ether extract content of the substrate. Also, Belewu and Sam (2010) reported a higher Ether extract content of *R. oligosporus*-Treated *Jatropha curcas* cake. It has been reported by Oboh and Akindahunsi (2003) that higher ether extract content of RoTRH could be due to the possibility of microbial oil production during the fermentation process. The Cellulose and Hemicellulose contents of the untreated rice husk (10.47 % and 3.20 %) and the *RoTRH* (8.53 % and 5.07 %) were similar.

Table 1: Proximate Composition and Energy values of untreated rice husk and *RoTRH*

Parameters (%)	URH	*RoTRH*	+SEM	Remark
Dry matter	94.36	92.21	0.53	*
Crude protein	6.34	8.75	0.06	*
Ash	19.25	24.09	0.14	*
Ether extract	2.62	2.94	0.09	*
NFE	39.90	34.58	0.77	*
Crude fiber	26.25	21.85	0.17	*
TDN	64.06	70.46	0.76	*
NDF	41.30	33.30	0.17	*
ADF	38.10	28.30	0.13	*
ADL	30.83	24.77	0.19	*
Hemicellulose	3.20	5.07	0.06	NS
Cellulose	10.47	8.53	0.08	NS

Key: URH – Untreated rice husk, *RoTRH* - *Rhizopus oligosporus* – Treated Rice husk, NFE - Nitrogen Free Extract, TDN - Total Digestible Nutrients, NDF - Neutral Detergent Fiber, ADF - Acid Detergent Fiber, ADL - Acid detergent Lignin, NS = Not Significant ($p > 0.05$), * = Significant ($p < 0.05$) SEM = Standard Error of Means

Table 2: Mineral composition of untreated rice husk and *RoTRH*

Parameters (%)	URH	*RoTRH*	+ SEM	Remarks
Magnesium	0.84	1.29	0.005	*
Copper	0.07	0.08	0.003	NS
Zinc	0.12	0.15	0.003	*
Iron	4.83	1.47	0.203	*
Sodium	2.23	2.20	0.067	NS
Potassium	5.23	6.53	0.047	*
Calcium	0.14	0.12	0.004	*
Phosphorus	0.05	0.03	0.002	*

Key: URH – Untreated rice husk, *RoTRH* - *Rhizopus oligosporus* – Treated Rice husk, NS = Not Significant ($p > 0.05$), * = Significant ($p < 0.05$), SEM = Standard Error of Means

The results of the mineral composition of untreated rice husk and *RoTRH* is presented in Table 2. From the results, while the Copper (Cu) and the Sodium (Na) contents of the untreated rice husk (0.07 % and 2.23 % respectively) and the *RoTRH* (0.08 % and 2.20 %) did not differ from each other, the Magnesium (Mg), Zinc (Zn) and Iron (Fe) contents of the untreated rice husk (0.84 %, 0.12 % and 4.83 %) differed significantly ($p < 0.05$) from that of the *RoTRH* (1.29 %, 0.15 % and 1.47 % respectively). Equally, the Potassium (K), Calcium (Ca) and phosphorus (P) contents of the untreated rice husk (5.23%, 0.14% and 0.05% respectively) differed significantly ($p < 0.05$) but slightly from 6.53%, 0.12% and 0.03% respectively in the *RoTRH*. The reported significantly higher ($p < 0.05$) contents of Magnesium (Mg), Zinc (Zn) and Potassium (K) in the *RoTRH* in this study might probably be as a result of their being tightly bound to the dietary fibre of the rice husk prior to fermentation and partial release when the influence of phytic acid was lowered by degradation. This was in line with the reports of Ekholm *et al.*

(2003). However, the lower contents of Sodium (Na), Iron (Fe), Calcium (Ca) and Phosphorus (P) of the *RoTRH* might be due to their being utilized by the *R. oligosporus* organism for its growth and metabolism which was in line with the reports of Bello and Akinyele (2007) and Oladele and Oshodi (2008). Generally, the variations in the nutrient values of the untreated rice husk and *RoTRH* when compared to the reports of Belewu *et al*. (2007) might be due to factors such as age of harvest, climate, edaphic and methods of processing (Odetola *et al*., 2012).

4. Conclusion and Recommendation

The findings of this study supports the fact that fermentation improves the nutritive quality of feedstuffs by degradation and lowering of fiber content of the *RoTRH* From these suggestive findings, it can be concluded that the treatment of rice husk with *R. oligosporus* fungus is a means to improving the nutritive value of rice husk and thereby reducing the level of dependence on and competition for conventional feedstuffs between man and livestock. This conclusion is therefore recommended for cheaply improving the nutritive quality of rice husk.

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3/10/2021