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NUTRITIONAL QUALITY ENRICHMENT OF RICE STRAW USING *PLEUROTUS SAJOR-CAJU* (FR.) SINGER AND MICRO-FILAMENTOUS FUNGI

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Abstract

Solid state fermentation was carried out by *Pleurotus sajor-caju*, micro-filamentous fungi and culture filtrate of *P. sajor-caju* with different combinations to improve nutritional qualities of rice straw. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents of the fermented rice straw were significantly reduced in mixed culture with filamentous fungi and *P. sajor-caju.* The cellulose and hemicellulose contents also reduced in similar manner. *P. sajor-caju* showed strong lignolytic activity resulting cellulose/lignin ratio of 5:1 when rice straw and PKC were supplemented. The protein content increased from 5.86 - 11.12, 12.98 and 13.14%, respectively. The maximum and significant amount of soluble protein 11.84 mg/g and glucosamine 18.50 mg/g was recorded in mixed culture at 30% palm oil kernel cake (PKC) supplementation. The maximum reducing sugar of 18.76 mg/g and endoglucanase activity 11.54 U/ml was recorded in same treatment. The mineral contents K, P, Ca, Mg and Fe were significantly higher in fermented sample while Cu and Zn were non-significant. The antioxidant activity improved by 45% and significantly varied between fermented and unfermented samples.

Introduction

Rice straw is one of the abundant lignocellulosic renewable waste materials in the world which contains 32 - 47% cellulose, 19 - 27% hemicellulose and 5 - 24% lignin (Saha 2003, Karimi *et al.* 2006). This lignocellulosic residue is a potential source of dietary energy for ruminants but is poor in protein content and digestibility. Biological treatment of lignocellulose by solid-state fermentation (SSF) with white-rot fungi can potentially generate a product with an improved digestibility and nitrogen content (Villas-Boas *et al*. 2002, Vadiveloo 2003). Microbes, further growing microbes on lignocellulosic wastes excretes all the hydrolytic enzymes and makes the minerals more available for absorption by the animal (Villas-Boas *et al.* 2002). Although cellulolytic fungi occur in all major fungal taxa and can degrade complex carbohydrate to simple monomer but there are relatively a few groups of microorganisms can produce the lignolytic enzymes. The most efficient lignin degrading fungi is white rot fungus (*Pleurotus* sp.) which is predominantly used in the bioconversion processes for upgrading the straw. Nitrogenous substrates are usually supplemented to lead to better proliferation of fungi that is the key need to accelerate the fermentation process properly. So, it would be meaningful to use *Pleurotus* sp. in combination with potential cellulolytic fungi for effective SSF. Therefore, the present study was undertaken to improve the nutritive qualities of rice straw through SSF using *Pleurotus sajor-caju* (Fr.) Singer and micro-filamentous fungi.

Materials and Methods

Pleurotus sajor-caju and *Rhizopus oligosporus* were collected from Animal Production Laboratory, Institute of Tropical Agriculture, University Putra Malaysia (UPM), Malaysia and *Aspergillus niger* S/5 a local strain was obtained from Mycology and Plant Pathology Laboratory of Science Faculty, UPM, Malaysia.

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There were three sets of experiments. In the first set, rice straw was supplemented with urea, molasses and palm oil kernel cake (PKC) in different combinations and inoculated with *P. sajor-caju*. In the second set, only PKC was supplemented with rice straw in different increments and cellulolytic fungi *Aspergillus niger* and *R. oligosporus* were combindley inoculated with *P. sajor-caju.* In the third set, culture filtrate of *P. sajor-caju* was directly added to the autoclaved substrate at the ratio of 1 : 1 and ensured 80% moisture content by adding autoclaved distilled water and subsequently inoulated with cellulolytic fungi *A. niger* and *R. oligosporus.*

P. sajor-caju was grown in Erlenmeyer flask (250 ml) containing 100 ml potato dextrose broth with 1% rice straw. The medium was inoculated with three culture discs (8 mm) and allowed to grow at 30ºC in incubator (Memmert INE 700) for 15 days. Fungal mat was separated by using sterilized glass wool under laminar air hood and the filtrate was fresh used.

SSF was carried out in Erlenmeyer flask (500 ml) with 15 g of substrate. The substrate was mixed with 65 ml mineral solution (KH_2PO_4 2.0 g, $MgSO_4$ 200 mg, $FeSO_4$ 50 mg, $CuSO_4$ 0.14 mg/l and pH 5.6) and ensured a moisture content of 80%. The organisms were grown on PDA and the culture discs (8 mm diam.) were added at the rate of 6 discs per flask and kept in an incubator at 30ºC for 21 days. However, in the treatment of culture filtrate and micro-filamentous fingi the incubation period was control treatments were performed without inoculation. After fermentation, the products were dried to constant weight at 60ºC.

Unfermented and fermented dried samples were analyzed in triplicate for dry matter (DM) content, ash by complete combustion in a muffle furnace at 550°C (AOAC 1990) while crude protein (CP) was determined by micro-Kjeldahl technique (AOAC 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the detergent system (Van Soest *et al.* 1991) and acid detergent lignin (ADL) was determined after AOAC (1990). Hemicellulose content was estimated as the difference between NDF and ADF while cellulose content was estimated as the difference between ADF and ADL. The mineral P and K were analyzed with auto analyzer while Ca, Mg, Fe, Cu, Zn and Mn were determined using an atomic absorption spectrophotometer (Perkin-Elmer M-5100) from aliquots of the solutions of ash. Soluble protein was measured according to Lowery *et al.* (1951) with bovine serum albumin as standard. Glucosamine was analyzed after Zheng and Shetty (1988) using glucosamine as standard. Reducing sugar was determined according to Miller (1959) and endoglucanase activity was measured as described by Ghose (1987).

The antioxidant activity was measured following Pérez-Jiménez *et al.* (2008) based on the scavenging of the stable free radical 1, 1-diphenyl-2-pirylhydrzyl (DPPH). The scavenging activity on hydroxyl radicals was calculated by the following equation:

$$\text{Scavenging activity (\%)} = [(A_{\text{control517nm}} - A_{\text{sample 517nm}})/ A_{\text{control517 nm}}]\times 100$$

The experiment was conducted by using a completely randomized design with three replications. Data were statistically analyzed for F test using the general linear model (GLM) procedure of SAS 9.1. Multiple comparisons among means were performed using Tukey tests at 5% level.

Results and Discussion

The SSF with *Pleurotus sajor-caju* improved the nutritional qualities of the rice straw and significantly ($p < 0.01$) varied among the treatments (Table 1). The ADF, NDF, ADL were significantly ($p < 0.01$) reduced with different supplementations and ranged from 55.12 - 47.19, 82.53 - 63.99 and 11.52 - 7.69%, respectively. Cellulose and hemicellulose were reduced 43.60 - 39.50% and 27.41 - 16.59%. The maximum cellulose/lignin ratio was 5.1 : 1. Ortega *et al.* (1992) reported that white rot fungi *Pleurotus ostreatus* and *P. sajor-caju* are potent to degrade lignin,

cellulose and hemicellulose contents of agro-residues and having strong ligninolytic activity together with variable cellulolytic and xylanolytic action. The present results are in conformity with the findings of Ortega *et al.* (1992). The crude protein content of the fermented substrates was significantly ($p < 0.01$) increased from 5.86 to 11.12% when PKC was supplemented. The rapid increase in growth of the fungus may account for the increase in the protein contents of fermented substrate where the fungal hyphae serving as single cell protein (Lateef *et al.* 2008). Among the supplementations PKC showed the best result in terms of fiber reduction and protein improvement, and the order of supplementations were molasses < urea < molasses + urea < PKC + molasses < PKC + molasses + urea < PKC. In the present study *P. sajor-caju* showed large amount of lignin degradation and as well as cellulose and hemicelluloses and at the same time increased protein about 89.42 % when PKC was supplemented. So, *P. sajor-caju* may be used in solid state fermentation to improve nutritional qualities of rice straw with PKC supplementation.

The mixed culture had significant ($p < 0.01$) effect on ADF and NDF and reduced by 23 and 32%, respectively when *P. sajor-caju* and mixed culture of micro-filamentous fungi were used at 30% PKC supplementation (Table 2). The maximum lignin reduction was exhibited by single culture while highest lignonolytic activity as shown by the cellulose/lignin ratio of 5.2 : 1. The crude protein content increased significantly ($p < 0.01$) and the maximum was obtained in mixed culture at 30% PKC supplementation which improved by 121.09% (5.86 - 12.98%) and comparatively 32% greater than single culture. Ofuya and Nwajiuba (1990) reported an increase of 185% (5.6 - 16%) in the protein content of cassava peels when fermented with *Rhizopus* spp. The maximum cellulose and hemicellulose reduction was exhibited in mixed culture compare to single culture of *P. sajor-caju.* It has been reported that several fungi including *A. niger* and *Trichoderma viride* can degrade cellulose/hemicelluloses in same manner by the activities of cellulase, beta-glucosidase and xylanase enzymes (Pothiraj *et al.* 2006, Jahromi *et al.* 2010).

White rot fungi *Pleurotus* sp. has powerful ligninolytic enzyme aryloxidase and produces H_2O_2 in SSF and oxidizes polyunsaturated primary alcohols (Guillen *et al.* 1990). But the fungus is slow grower and in mixed culture it cannot compete with faster micro-filamentous fungi consequently had low lignolytic activity. For this reason, in current experiment, culture filtrate of *P. sajor-caju* was used for lignin degradation before inoculation of micro-filamentous fungi. It was observed that culture filtrate with filamentous fungi had significant ($p < 0.01$) effect on ADF, NDF and ADL reduction and reduced by 24, 34 and 22%, respectively at 30% PKC (Table 3). The reduction of cellulose and hemicelluloses contents also followed this result. Yang *et al.* (2012) reported that the cellulose and hemicellulose degradation was greatly increased when soybean fiber was inoculated by *Aspergillus oryzae, Trichoderma reesei* and *P. chrysosporium* in SSF. In nature, it is well known that lignin physically encrusts with cellulose that makes it resistant to enzymatic degradation. The cellulolytic micro-filamentous fungi cannot dipolymerise lignin easily but the used culture filtrate contain lignolytic enzyme as shown in cellulose lignin ratio of 4 : 1 which can degrade lignin, consequently, the higher amount of cellulose and hemicellulose reduction occurred in mixed culture with culture filtrate treatment. The crude protein content significantly ($p < 0.01$) increased by 125% in culture filtrate and filamentous fungi treatment at 30% PKC supplementation.

In the present study, soluble protein and glucosamine increased significantly ($p < 0.01$) by fungal treatment (Table 4). The maximum soluble protein and glucosamine was obtained about 11.84 and 18.50 mg/g, respectively by mixed culture at 30% PKC. Zheng and Shetty (1998) reported that fungal biomass production is positively correlated to soluble protein production in SSF. Molla *et al.* (2004) reported glucosamine an essential component of chitin of fungal cell wall, can be considered as a good parameter for estimation of mycelia growth in solid substrate.

Table 1. Proximate composition (% DM) of solid state fermented rice straw by *P. sajor-caju* with different supplementations.

Treatment	ADF	NDF	ADL	Cellulose	Hemicellulose	Cellulose / lignin ratio	Crude protein	Dry matter	Ash
Rice straw									
Fermented	52.36^{d}	76.91^{d}	8.84^{d}	43.52^{b}	24.55^{b}	4.9	6.88^{e}	95.07^{f}	12.69^{d}
Un-fermented	55.12^{a}	82.53^{a}	11.52^{a}	43.60^{a}	27.41^{a}	3.7	5.86^{g}	97.12^{c}	12.05^{g}
RS + urea									
Fermented	51.64^{f}	71..92^{g}	8.55^{e}	43.09^{b}	20.28^{f}	5.0	8.84^{bc}	97.51^{b}	12.96^{c}
Un-fermented	54.59^{b}	78.67^{c}	11.10^{bc}	43.49^{a}	24.08^{c}	3.9	6.92^{e}	95.47^{d}	12.19f^{g}
RS+ molasses									
Fermented	51.21^{g}	75.27^{e}	8.58^{ed}	42.63^{c}	24.06^{d}	4.9	7.95^{d}	97.28^{c}	12.95^{c}
Un-fermented	54.49^{b}	81.90^{b}	11.35^{ab}	43.14^{b}	27.41^{a}	3.8	6.70^{ef}	95.25^{e}	12.05^{g}
RS + urea + molasses									
Fermented	50.25^{h}	68.45^{j}	8.32^{ef}	41.93^{d}	18.10^{h}	5.0	8.93^{bc}	97.78^{a}	13.28^{b}
Un-fermented	53.92^{c}	75.23^{e}	11.18^{bc}	42.74^{c}	21.31^{e}	3.8	6.38^{f}	95.57^{d}	12.38^{ef}
RS+ 10%PKC									
Fermented	47.19^{k}	63.99^{l}	7.69^{h}	39.50^{i}	16.80^{j}	5.1	11.12^{a}	97.90^{a}	13.98^{a}
Un-fermented	52.13^{e}	71.55^{h}	11.52bc	40.61^{f}	20.42^{ef}	3.5	8.07^{d}	94..62^{g}	12.95^{c}
RS+10%PKC+molasses									
Fermented	48.45^{i}	65.57^{j}	8.16^{gf}	40.29^{g}	17.32^{i}	4.9	10.88^{b}	97.54^{b}	13.05^{c}
Un-fermented	52.10^{e}	72.67^{f}	11.05^{c}	41.05^{e}	20.57^{f}	3.7	8.55^{d}	94.96^{f}	12.12^{g}
RS+0%PKC + mola + urea									
Fermented	47.85^{j}	64.44^{k}	8.00^{g}	39.85^{h}	16.59^{k}	5.0	10.96^{b}	97.48^{b}	13.42^{b}
Un-fermented	52.01^{e}	71.94^{g}	11..21^{bc}	40.80^{f}	19.93^{g}	3.6	8.73^{c}	94.53^{g}	12.47^{c}
SE diff	0.058*	0.084*	0.074*	0.054*	0.061*		0.225*	0.197*	0.397*

Means with different superscripts in the same column are significantly different at $p < 0.05$. *Significantly different at $p<0.01$.

Table 2. Proximate composition (%DM) of solid state fermented rice straw by mixed culture of *P. sajor-caju* and micro-filamentous fungi with PKC supplementation.

Treatment	ADF	NDF	ADL	Cellulose	Hemi cellulose	Cellulose/ lignin	CP	DM	Ash
RS+PSC	52.32^{b}	76.98^{b}	8.86^{g}	43.46^{a}	24.66^{b}	4.9	7.88^{m}	97.12^{h}	12.69^{l}
RS+PSC+AN	51.95^{d}	76.44^{c}	9.61^{d}	42.34^{b}	24.49^{c}	4.4	8.21^{jk}	97.46^{g}	12.76^{kl}
RS+PSC+RO	52.24^{b}	76.81^{b}	9.87^{c}	42.37^{b}	24.57^{b}	4.3	8.04^{l}	97.23^{h}	12.72^{l}
RS+PSC+AN+RO	51.26^{d}	75.61^{d}	9.57^{d}	41.69^{c}	24.35^{c}	4.4	8.32^{ij}	97.52^{g}	12.91^{k}
Control	55.12^{a}	82.53^{a}	11.52^{a}	43.60^{a}	27.41^{a}	3.7	5.86^{n}	95.07^{i}	12.05^{m}
RS+10% PKC+PSC	47.19f	65.99^{h}	7.69^{i}	39.50^{f}	18.80^{f}	5.1	10.12^{i}	97.90^{f}	13.28^{gh}
RS+10%PKC+PSC+AN	46.38^{g}	65.49^{j}	9.28^{f}	37.50^{g}	18.71^{fg}	4.0	$10.\ 68^{f}$	98.16^{e}	13.44^{efg}
RS+10%PKC+PSC+RO	46.86^{g}	65.73^{i}	9.35^{f}	37.52^{g}	18.87^{f}	4.0	10.31^{g}	98.01^{f}	13.32^{gh}
RS+10%PKC+PSC+AN+RO	46.25^{g}	64.26^{l}	9.06^{f}	37.19^{i}	18.01^{g}	4.1	10.91^{e}	98.20^{e}	13.48^{def}
Control	51.13^{e}	71.55^{e}	11.52^{a}	39.61^{e}	19.42^{e}	3.5	8.07^{ek}	94.62^{j}	12.95^{j}
RS+20% PKC+PSC	46.62^{h}	65.43^{k}	7.68^{j}	38.94^{h}	18.81^{h}	5.1	12.16^{d}	98.47^{d}	13.4^{efg}
RS+20%PKC+PSC+AN	46.46^{gh}	65.24^{jk}	9.22^{de}	36.94^{i}	18.78^{h}	4.0	12.28^{d}	98.58^{cd}	13.52^{def}
RS+20%PKC+PSC+RO	46.59^{g}	65.42^{k}	9.48^{c}	36.91^{i}	18.83^{h}	4.0	12.20^{cd}	98.46^{d}	13.48^{def}
RS+20%PKC+PSC+AN+RO	45.98^{j}	64.12^{m}	9.18^{ef}	36.60^{j}	18.14^{i}	4.0	12.41^{c}	98.66^{bc}	13.62^{cde}
Control	51.10^{e}	71.24^{f}	11.43^{a}	39.67^{e}	20.14^{e}	3.5	8.11^{jkl}	94.65^{j}	13.06^{ij}
RS+30% PKC+PSC	45.62^{i}	63.72^{m}	7.32^{k}	38.70^{h}	17.90_{j}	5.2	12.67^{b}	98.73^{bc}	13.68^{cd}
RS+30%PKC+PSC+AN	45.25^{j}	62.98^{o}	8.67^{g}	36.58^{i}	17.73^{lk}	4.2	12.93^{a}	98.82^{b}	13.82^{ab}
RS+30%PKC+PSC+RO	45.4^{j}	63.26^{n}	8.85^{g}	36.65^{i}	17.86^{k}	4.1	12.84^{a}	98.76_{b}	13.68^{bc}
RS+30%PKC+PSC+AN+RO	44.86^{k}	62.48^{p}	8.32^{h}	36.54^{j}	17.62^{l}	4.3	12.98^{a}	99.91^{a}	13.98^{a}
Control	51.00^{e}	71.10^{g}	11.23^{b}	39.77^{d}	20.10^{e}	3.5	8.24^{jk}	94.70^{j}	13.18^{i}
SE	0.414*	0.733*	0.158*	0.326*	0.467*		0.283*	0.201*	0.621*

Means with different superscripts in the same column are significantly different at $p < 0.05$. *Significantly different at $p < 0.01$.
PSC = *Pleurotus sajor-caju*, RO = *Rhizopus oligosporus,* AN= *Aspergillus niger,* RS = Rice straw.

Table 3. Proximate composition (%DM) of solid state fermented rice straw by mixed culture with culture filtrate of *P. sajor-caju* and micro-filamentous fungi.

Treatment	ADF	NDF	ADL	Cellulose	Hemicellulose	Cellulose/ lignin	CP	DM	Ash
RS+P-CF	52.65^{b}	80.26^{b}	10.36^{h}	42.29^{b}	27.61^{b}	4.1	6.08^{o}	95.14^{gh}	12.39^{k}
RS+P-CF+AN	51.55^{d}	78.52^{d}	10.98^{de}	40.57^{d}	26.97^{c}	3.7	7.60^{m}	96.54^{b}	12.58^{j}
RS+P-CF+RO	51.94^{c}	78.84^{c}	11.32^{b}	40.62^{c}	26.90^{c}	3.6	7.41^{n}	96.52^{b}	12.46^{jk}
RS+P-CF+AN+RO	51.21^{f}	77.85^{e}	10.89^{de}	40.32^{i}	26.64^{c}	3.7	7.85^{l}	96.05^{d}	12.81^{i}
Control	55.12^{a}	82.99^{a}	11.92^{a}	42320^{a}	27.87^{a}	3.6	5.86^{p}	95.07^{ih}	12.05^{l}
RS+10% PKC+P-CF	48.28^{h}	70.33^{h}	9.74^{j}	38.54^{h}	22.05^{e}	4.0	8.29^{j}	94.74^{jk}	13.06^{g}
RS+10%PKC+P-CF +AN	47.65^{j}	66.53^{j}	10.53^{ef}	37.12^{k}	18.88^{i}	3.5	10.52^{f}	95.70^{f}	13.17^{fg}
RS+10%PKC+P-CF +RO	47.94^{hi}	66.90^{i}	10.77^{e}	37.17^{j}	18.96^{h}	3.5	10.22^{g}	95.64^{g}	13.09^{g}
RS+10%PKC+P-CF +AN+RO	47.14^{j}	65.82^{k}	10.41^{fhg}	36.73^{k}	18.68^{k}	3.5	10.81^{e}	95.88^{de}	13.24^{ef}
Control	51.13^{e}	71.55^{f}	11.52^{b}	39.71^{fg}	20.32^{d}	3.4	8.07^{k}	94.62^{k}	12.95^{h}
RS+20% PKC+P-CF	47.12^{g}	69.10^{h}	9.42^{ij}	37.77^{gh}	21.98^{f}	4.0	8.79^{h}	94.82^{ij}	13.25^{ef}
RS+20%PKC+P-CF +AN	46.42^{k}	64.72^{l}	10.32^{fg}	36.10^{l}	18.30^{j}	3.5	12.34^{c}	95.91^{d}	13.32^{de}
RS+20%PKC+P-CF +RO	46.85^{j}	65.23^{i}	10.51^{fg}	36.34^{kl}	18.38^{g}	3.5	12.11^{l}	95.84^{ef}	13.38^{cd}
RS+20%PKC+P-CF +AN+RO	45.84^{k}	64.03^{m}	10.24^{gh}	35.60^{m}	18.19^{l}	3.5	12.96^{b}	95.99^{d}	13.42^{cd}
Control	51.02^{l}	71.24^{g}	11.48^{c}	39.54^{f}	20.22^{e}	3.4	8.14^{k}	94.65^{k}	13.06^{gh}
RS+30% PKC+P-CF	45.62^{i}	68.84^{j}	9.22^{k}	37.69^{i}	21.93^{j}	4.1	8.63^{i}	95.14^{g}	13.33^{de}
RS+30%PKC+P-CF +AN	45.11^{l}	62.78^{o}	10.12^{i}	34.59^{n}	17.87^{m}	3.5	12.92^{b}	95.92^{d}	13.55^{b}
RS+30%PKC+P-CF +RO	45.62^{l}	63.63^{n}	10.28^{ij}	35.25^{n}	18.1^{m}	3.5	12.85^{b}	95.89^{d}	13.46^{c}
RS+30%PKC+P-CF +AN+RO	44.58^{m}	62.01^{p}	10.04^{i}	34.24^{o}	17.73^{n}	3.5	13.14^{a}	96.02^{a}	13.68^{a}
Control	50.74^{e}	71.10^{g}	11.38^{d}	39.36^{ef}	20.36^{l}	3.4	8.54^{i}	94.91^{i}	13.18^{fg}
SE	0.282*	0.782*	0.105*	0.248*	0.583*		0.303*	0.651*	0.454*

Means with different superscripts in the same column are significantly different at $p < 0.05$. *Significantly different at $p<0.01$. P-CF= Culture filtrate of *Pleurotus sajor caju*, RO= *Rhizopus oligosporus,* AN= *Aspergillus niger,* RS = Rice straw.

The maximum endoglucanse activity 11.54 U/ml was exhibited at 30% PKC supplementation in *P. sajor-caju* and mixed culture treatment. The increased reducing sugar with a decreased fiber level obtained in this study support the ability of cellulase production of the fungi.

Table 4. The amount of soluble protein, glucosamine, reducing sugar and endoglucanase activity of fermented rice straw by mixed culture of *P. sajor-caju* and mico-filamentous fungi.

Treatment	Soluble protein (mg/g)	Glucosamine (mg/g)	Reducing sugar (mg/g)	Endoglucanase (IU/g)
RS+PSC	7.20^{j}	8.16^{l}	7.51^{k}	6.41^{k}
RS+PSC+AN	7.96^{h}	9.79^{j}	9.58^{i}	7.48^{h}
RS+PSC+RO	7.54^{i}	9.65^{jk}	9.22^{j}	7.31^{i}
RS+PSC+AN+RO	8.05^{h}	9.82^{j}	9.79^{i}	7.83^{g}
Control	6.14^{l}	4.55^{o}	5.61^{l}	-
RS+10% PKC+PSC	8.40^{g}	9.61^{k}	8.91^{j}	6.83^{j}
RS+10%PKC+PSC+AN	9.18^{f}	10.29^{i}	11.22^{h}	8.16^{f}
RS+10%PKC+PSC+RO	9.04^{f}	10.17^{i}	11.14^{h}	8.02^{gh}
RS+10%PKC+PSC+AN+RO	9.56^{e}	11.75^{h}	12.25^{g}	8.50^{e}
Control	6.20^{l}	5.28^{n}	5.60^{l}	-
RS+20% PKC+PSC	9.62^{e}	12.40^{g}	11.30^{h}	7.56^{h}
RS+20%PKC+PSC+AN	9.88^{d}	14.29^{f}	14.78^{l}	9.41^{d}
RS+20%PKC+PSC+RO	9.75d^{e}	14.79^{e}	14.63^{e}	9.34^{d}
RS+20%PKC+PSC+AN+RO	10.44^{c}	16.11^{c}	16.90^{d}	10.18^{c}
Control	6.90^{k}	6.71^{m}	5.57^{l}	-
RS+30% PKC+PSC	10.56^{bc}	15.90^{d}	12.91f	8.16^{f}
RS+30%PKC+PSC+AN	10.72^{b}	16.14^{c}	17.59b	10.42^{b}
RS+30%PKC+PSC+RO	10.61^{bc}	16.86^{b}	17.22c	10.21^{c}
RS+30%PKC+PSC+AN+RO	11.84^{a}	18.50^{a}	18.76a	11.54^{a}
Control	7.44^{i}	6.85^{m}	5.55^{l}	-
SE diff	0.205*	0.518*	0.543*	0.204*

Means with different superscripts in the same column are significantly different at $p < 0.05$**.** *Significantly different at $p < 0.01$. PSC=*Pleurotus sajor-caju*, RO=*Rhizopus oligosporus*, AN=*Aspergillus niger*.

The mineral contents of the fermented rice straw contained significantly ($p < 0.01$) higher amount of K, P, Ca, Mg, Fe and Mn while Cu, and Zn were non-significant (Table 5). Akindahunsi *et al.* (1999) reported that the fermented sample was very rich in some essential minerals. The obtained mineral contents of the fermented RS were higher than previously reported fermented cassava peels (Oboh 2006).

The antioxidant activity significantly ($p < 0.01$) varied between fermented and unfermented samples and significantly increased with increase of PKC concentration (Table 6). The maximum activity was obtained 68.87% by mixed culture of *P. sajor-caju*, *A. niger* and *R. oligosporus* at 30% PKC supplementation when original extraction was concentrated by 75%. However, in the present investigation, improved antioxidant activity by 45% was exhibited in fermented sample that was higher than fermented maize reported by Daker *et al.* 2009.

Table 5. Mineral composition of PKC (30%) supplemented fermented rice straw by mixed culture of *P. sajor -caju* and micro-filamentous fungi in percentage of dry weight.

Element	Unfermented	Fermented				SE diff
		P. sajor-caju	*P. sajor-caju +A. niger*	*P. sajor-caju +R. oligosporus*	*P. sajor-caju +A. niger+R. oligosporus*	
K	1.21^{c}	1.51^{b}	1.58^{b}	1.62^{b}	1.71^{a}	0.109*
p	0.93^{c}	1.16^{b}	1.21^{b}	1.34^{ab}	1.38^{a}	0.107*
Ca	0.29^{c}	0.68^{b}	0.82^{a}	0.82^{a}	0.91^{a}	0.149*
Mg	0.24^{b}	0.72^{a}	0.78^{a}	0.81^{a}	0.88^{a}	0.196*
Fe	0.41^{c}	0.69^{b}	0.71^{b}	0.71^{b}	0.78^{a}	0.160*
Cu	0.006	0.008	0.008	0.009	0.011	0.0001ns
Zn	0.07	0.08	0.08	0.08	0.09	0.005ns

Means with different letter within a row differed significantly at 5% level. ns: not significantly different at $p > 0.05$, *significantly different at $p < 0.01$.

Table 6. Antioxidant activity (%) of PKC (30) supplemented fermented rice straw by mixed culture of *P. sajor-caju* and micro-filamentous fungi.

Treatment	Unfermented	Fermented				SE diff
		P. sajor-caju	*P. sajor-caju + A. niger*	*P. sajor-caju + R. oligosporus*	*P. sajor-caju + A. niger + R. oligosporus*	
Rice straw	47.56^{ez}	55.14^{dz}	55.68^{cz}	57.90^{bz}	58.14az	1.02*
RS+ 10%PKC	48.87^{dy}	59.36^{cy}	59.92^{by}	60.01by	62.25^{ay}	1.25*
RS+ 20%PKC	49.74^{ex}	60.19^{dx}	61.54^{cx}	64.96^{bx}	65.31^{ax}	1.51*
RS+ 30%PKC	50.85^{ew}	63.74^{dw}	66.30^{cw}	68.42^{bw}	68.87^{aw}	1.77*
SE diff	0.363*	0.541*	0.922*	0.221*	0.756*	

*Significantly different at $p < 0.01$. ^{a,b,c,d,e,f}Means with different superscripts in the same column are significantly different at $p < 0.05$. ^{w,x,y,z}Means with different superscripts in the same row are significantly different at $p < 0.05$.

So, use of *P. sajor-caju* with micro-filamentous fungi may be helpful in the creation of healthy and functional feeds for animal and also can prevent the deleterious effects of free radicals. Further this treatment caused maximum fiber reduction, improved crude protein and mineral contents.

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