

BIOCONVERSION OF ORANGE PEELS INTO A NUTRITIONALLY ENRICHED SUBSTRATE BY *Trichosporonoides oedocephalis*, *Penicillium italicum* AND *Aspergillus flavus*

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Abstract: This study was conducted to investigate the effects of fungal based fermentation on the nutritional composition of orange peels. The effect of solid state fermentation (SSF) on the nutrients and anti-nutritional factors (ANF) of orange peels was achieved by *Trichosporonoides oedocephalis*, *Penicillium italicum* and *Aspergillus flavus* with two inocula concentrations (10^3 and 10^5 spores/ml). The protein content of *T. oedocephalis* and *A. flavus* fermented orange peels increased significantly ($P < 0.05$) with the highest values of 13.25 and 13.42% respectively obtained with 10^3 spores/ml. With an increase in the inocula concentrations of *P. italicum* from 10^3 to 10^5 spores/ml, the crude protein increased when compared with control treatments. The contents of alkaloid, saponin and phytate of *T. oedocephalis* fermented orange peels decreased significantly ($P < 0.05$) by 2.89% (10^3 spores/ml), 41.04% (10^5 spores/ml), 7.09% (10^3 spores/ml), 24.41% (10^5 spores/ml), 68.93% (10^3 spores/ml) and 53.40% (10^3 spores/ml) respectively in comparison with control treatments. The oxalate, phytate and tannin contents of *P. italicum* treated orange peels with 10^5 spores/ml decreased by 21.66, 63.84 and 66.67% respectively in comparison with control treatments. This result suggests that fungal treatment of orange peels with appropriate inocula concentrations resulted in the enhancement of its nutritional status; hence it's potential in animal rations.

Keywords: Orange peels, nutritional status, anti-nutrient, inoculum concentration, fermentation

Introduction

The utilization of agricultural wastes as feedstuff is one of the strategies involved in the reduction of cost of livestock production. Agricultural by-products in Nigeria vary from primary processing of farm produce wastes to wastes from agro allied industries. Some of these wastes are left unutilized, often causing environmental pollution and hazard.

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Those that are utilized do not have their full potentials harnessed. Agro-industrial wastes can be of tremendous use in the livestock industry for feeding animals include brewers dried grain, palm kernel cake, maize offal, wheat offal, rice bran and cassava peels just to mention few. Microbial bioconversion and associated enzymes, especially fungal bioconversion of wastes seems to be a practical and promising alternative for increasing their nutritional value, transforming them into animal feed and thus producing a value-added product

(Villas-Boas *et al.*, 2003; Agosin *et al.*, 2006; Olaniyi, 2014). Fungal bioconversion of agro-industrial by-products is an environmentally friendly biotechnological process (Karunanandaa *et al.*, 1995; Zhang *et al.*, 2002; Mukherjee and Nandi, 2004). From an animal nutrition point of view, agro-industrial wastes are not suitable feed ingredients as they are deficient in digestible protein (Song *et al.*, 2009), rich in complex fiber fractions and anti-nutrient compounds (Khanongnuch *et al.*, 2006).

Elimination or reduction of ANFs contents and improve nutritive value, taste and smell of plant origin ingredients was performed mainly through fungal species (*Rhizopus oligosporus*, *Aspergillus oryzae*, *Neurospora sitophila*, *Penicillium italicum*, etc) via consumption of substrate carbohydrates, excretion of effective enzymes in this regard and the production of protein biomass in SSF and recently it has found spread applications in the food production industry (Gowthaman *et al.*, 2001; Amadou *et al.*, 2010; Omid *et al.*, 2012). Using fermentation technology has attained higher significance as a new option (biotechnology) in the food processing compared to recently common methods (Khalil, 2006). The fermentation efficiency depends on various parameters including the type of the used microorganism, inoculation density, age of culture, incubation time, suitable conditions of culture (temperature, pH, moisture, scale of culture, etc), type and particle size of substrate, previous processing history and carbon to nitrogen ratio in the substrate (Krishna, 2005; Khalil, 2006). To monitor fermentation technology efficiency, the indirect parameters

including crude protein content, glucoseamine and dry matter loss of the produced products were measured (Singhania *et al.*, 2009). Fermentation process leads to offer new fermented products (Kim *et al.*, 2010) to the food industry in the near future with reduction of peptide sizes of protein sources (Hong *et al.*, 2004), lowering excretion capacity of digestion enzymes via production of more bioavailable products and consequently, reduction of energy expenditure needed for metabolism (Kiers *et al.*, 2003), lowering food allergy (Frias *et al.*, 2008; Amadou *et al.*, 2010), production of growth promotor factors (essential amino acids and vitamin generated with fungi) and immune stimulator compounds (Yamamoto *et al.*, 2007).

In a study conducted by Olaniyi (2014), β -mannanase produced by *P. italicum* was reported to improve the nutritive quality of palm kernel meal; although, there is no study on the usability of the mentioned microorganism, *Trichosporonoides oedocephalis* and *Aspergillus flavus* in bioconversion of orange peels to protein enriched substrate. The purpose of this study is to evaluate the effect of different inocula concentrations of *P. italicum*, *T. oedocephalis* and *Aspergillus flavus* on orange peels with the aim of increasing its nutrient contents and reducing ANFs contents.

Materials and Methods

Microorganisms

Penicillium italicum, *Trichosporonoides oedocephalis* and *Aspergillus flavus* (Akinyele *et al.*, 2013) previously confirmed to possess mannolytic property was obtained from the Research Laboratory, Microbiology

Department, Federal University of Technology Akure (FUTA), Ondo State, Nigeria. The authenticity of the culture was confirmed by the method of Pitt and Hocking (1997) on the bases of cultural characters (colour, shape of colony, surface and reverse pigmentation and texture of the colony) as well as microscopic structure (septate or nonseptate hyphae, structure of hyphae and conidia). The fungal isolate was maintained on Malt Extract Agar (MEA) and sub-cultured at regular intervals and stored at 4 °C in refrigerator on agar slant.

Inoculum preparation

The fungal cultures were grown on MEA slants until sporulation for 96 h at 30°C. The inoculum was prepared by adding 10 ml of sterile distilled water which contained 0.1% (v/v) Tween 80 to the agar slant and shook vigorously. The spore suspension was adjusted to the spore concentration of 10^3 and 10^5 spores/ml (as the initial inoculum size) (Ibrahim *et al.*, 2012).

Substrate collection

Orange peels were procured as household waste in Akure, Ondo State, Nigeria and it was utilized as substrate for solid state fermentation. The substrate was oven-dried at 70°C for 2 h with Model DHG Heating Drying Oven for a period of 2 h, stored in air tight transparent plastic containers to keep it moisture free.

Fungal solid state fermentation of orange peels

For the production of fermented orange peels in solid state fermentation, 10 g of the coarsely ground orange peels was suspended in 33 ml Mandels and Weber's medium modified by El-

Naggar *et al.* (2006) and inoculated with different inocula concentrations (10^3 and 10^5 spores/ml). This medium (moistening agent) contained the following ingredients (g/L): Peptone, 2; yeast extract, 2; NaNO_3 , 2; K_2HPO_4 , 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; KCl, 0.5 and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ traces. After sterilization at 121°C for 15 min, it was cooled and inoculated with different inocula concentrations of the test organisms. The conical flasks were incubated at 30°C for a period of 20 days in culture room. After fungal treatment, samples were exposed to free air and kept in shadow for 24 h to let out excessive moisture. The residual samples were oven-dried at 55°C for 48 h until they reach constant weight and then kept in air tight containers for further proximate evaluation.

Determination of proximate composition of fungal treated orange peels

The proximate composition of fungal treated orange peels was determined by standard methods according to AOAC (2005). Phytate was determined through the extraction of the samples with hydrochloric acid and sodium sulphate and absorbance measured at 660 nm (De Boland *et al.*, 1975). Tannin was determined using the method of vanillin hydrochloric acid and absorbance was measured at 500 nm (Price *et al.*, 1978). Oxalate determination was done according to the standard method of Day and Underwood (1986), while cyanide content was evaluated by the method of Obadeni and Ochuko (2001).

Statistical analyses

The statistical analysis was performed using the general linear

model function of Statistical Package for Social Science (SPSS), version 16.0. All data generated was subjected to one-way ANOVA while statistical differences of treatment were determined using Duncan's Multiple Range.

Results

Fermentation has been reported to be one of the major biotechnological tools involved in the nutritionally enrichment of agricultural wastes intended for animal feed formulation. In the present study, orange peels considered to be agricultural wastes were subjected to solid state fermentation using different inocula concentrations.

The proximate compositions of fungal treated orange peels are presented in Table 1, 2 and 3. Fermentation of orange peels with *T. oedocephalis* (10^3 spores/ml) caused a significant increase in its protein content from $11.55^a \pm 0.17\%$ in untreated sample to $13.25^b \pm 0.78\%$ treated sample. Although, there was no significantly differences in protein contents of fungal-treated orange peels with an increase in inoculum concentration (from 10^3 to 10^5 spores/ml) in comparison to the control treatments. The protein contents of *P. italicum* treated orange peels increased significantly with different inocula concentrations (10^3 and 10^5 spores/ml) compared to control treatment. However, there was no significant difference in protein content of *P. italicum* treated orange peels with increase in inoculum concentration (from 10^3 to 10^5 spores/ml). The protein content of *A. flavus* fermented orange peels varied significantly with inocula concentrations. The fermentation of

orange peels with 10^3 and 10^5 spores/ml of *A. flavus* caused approximately 10 and 16 % respectively increase in protein content.

With an increase in inoculum concentration from 10^3 to 10^5 spores/ml, significant ($P < 0.05$) reduction in fat content was achieved in *T. oedocephalis* treated orange peels from $12.73^b \pm 0.41\%$ in untreated sample (control) to $7.32^a \pm 0.16\%$ with 10^3 spores/ml and $6.86^a \pm 0.07$ with 10^5 spores/ml respectively. The fat contents in *P. italicum* treated orange peels decreased significantly ($P < 0.05$) with increase in the inoculum concentration. The treatment of orange peels with 10^3 and 10^5 spores/ml of *P. italicum* caused approximately 32 and 42% reduction respectively in its fat content. Treatment of orange peels with 10^3 and 10^5 spores/ml of *A. flavus* caused significant reduction in fat content when compared with control experiment. However, there was no significant difference between the fat content of 10^3 and 10^5 spores/ml of *A. flavus* fermented orange peels.

With an increase in inoculum concentration (from 10^3 to 10^5 spores/ml), the amounts of ash in *T. oedocephalis* treated orange peels increased significantly from $5.64^a \pm 0.08\%$ in the control treatment to $7.91^b \pm 0.00\%$ (with 10^3 spores/ml) and $9.03^c \pm 0.12$ (with 10^5 spores/ml) respectively. Similarly, an increase in ash content was achieved for *P. italicum* treated orange peels. However, the increment varied with inoculum concentration. Increase in inoculum concentration of *A. flavus* from 10^3 to 10^5 spores/ml led to an increase in ash content of fungal-treated wastes with the highest increase lied on 10^5 spores/ml.

The anti-nutrient composition of *T. oedocephalis* and *P. italicum* treated orange peels revealed a significant difference ($P<0.05$) between treatments (Table 4, 5 and 6). Alkaloid and saponin content of *T. oedocephalis* treated orange peels decreased significantly with increase in inoculum concentration. The treatment of orange peels with 10^3 and 10^5 spores/ml of *T. oedocephalis* caused approximately 69 and 53% reduction respectively in the phytate content. Similarly, increase in inoculum concentration (from 10^3 to 10^5

spores/ml) of *P. italicum* on orange peels caused significant ($P<0.05$) reduction in the oxalate, phytate and tannin content. There was reduction in alkaloid and saponin content of *P. italicum* treated sample. However, the reduction varied significantly with the inoculum concentration. The fermentation of orange peels with different inocula concentrations of *A. flavus* caused varied degrees of reduction in phytate, tannin and saponin contents.

Table 1: Comparison of different treatments on the proximate composition of *T. oedocephalis* fermented orange peels (% dry weight)

Parameters (%)	<i>T. oedocephalis</i> (Spores/ml)	
	Control (unfermented)	10 ³ 10 ⁵
Moisture	7.76 ^a ±0.34	5.43 ^a ±0.10 6.29 ^b ±0.35
Fat	12.73 ^b ±0.41	7.32 ^a ±0.16 6.86 ^a ±0.07
Ash	5.64 ^a ±0.08	7.91 ^b ±0.00 9.03 ^c ±0.12
Protein	11.55 ^a ±0.17	13.25 ^b ±0.78 11.94 ^a ±0.80

Means with the same superscript letters in the same row are not significantly different (P>0.05).

Table 2: Comparison of different treatments on the proximate composition of *P. italicum* fermented orange peels (% dry weight)

Parameters (%)	<i>P. italicum</i> (Spores/ml)	
	Control (unfermented)	10 ³ 10 ⁵
Moisture	7.76 ^b ±0.34	5.59 ^a ±0.25 5.99 ^a ±0.13
Fat	12.73 ^c ±0.41	8.69 ^b ±0.01 7.41 ^a ±0.03
Ash	5.64 ^a ±0.08	10.01 ^c ±0.19 9.33 ^b ±0.12
Protein	11.55 ^a ±0.17	13.57 ^b ±0.01 13.37 ^b ±0.26

Means with the same superscript letters in the same row are not significantly different (P>0.05).

Table 3: Comparison of different treatments on the proximate composition of *A. flavus* fermented orange peels (% dry weight)

Parameters (%)	<i>A. flavus</i> (Spores/ml)		
	Control (unfermented)	10 ³	10 ⁵
Moisture	7.76 ^a ±0.33	7.11 ^b ±0.12	5.35 ^c ±0.10
Fat	12.73 ^b ±0.41	7.37 ^a ±0.44	7.25 ^a ±0.22
Ash	5.64 ^a ±0.08	7.65 ^b ±0.29	8.51 ^c ±0.29
Protein	11.55 ^a ±0.17	13.42 ^c ±0.80	12.68 ^b ±0.00

Means with the same superscript letters in the same row are not significantly different (P>0.05).

Table 4: Comparison of different treatments on the anti-nutrient composition of *T. oedocephalis* fermented orange peels (% & mg/g dry weight)

Parameters	<i>T. oedocephalis</i> (Spores/ml)		
	Control (unfermented)	10 ³	10 ⁵
Alkaloid (%)	1.73 ^b ±0.18	1.68 ^b ±0.16	1.02 ^a ±0.03
Oxalate (mg/g)	1.57 ^a ±0.05	1.52 ^a ±0.00	1.56 ^a ±0.05

Phytate (mg/g)	12.36±0.00	3.84±0.48	5.76±0.00
Tannin (mg/g)	0.06±0.00	1.96±0.00	2.13±0.31
Saponin (%)	1.27±0.03	1.18±0.02	0.96±0.06

Means with the same superscript letters in the same row are not significantly different (P>0.05).

Table 5: Comparison of different treatments on the anti-nutrient composition of *P. italicum* fermented orange peels (% & mg/g dry weight)

Parameters	Control	<i>P. italicum</i> (Spores/ml)	
		10 ³	10 ⁵
Alkaloid (%)	1.73±0.18	0.79±0.01	1.09±0.02
Oxalate (mg/g)	1.57±0.05	1.50±0.01	1.23±0.10
Phytate (mg/g)	12.36±0.00	6.25±0.44	4.47±0.42
Tannin (mg/g)	0.06±0.01	0.03±0.00	0.02±0.00
Saponin (%)	1.27±0.03	1.16±0.06	1.17±0.01

Means with the same superscript letters in the same row are not significantly different (P>0.05).

Table 6: Comparison of different treatments on the anti-nutrient composition of *A. flavus* fermented orange peels (% & mg/g dry weight)

Parameters (%)	<i>A. flavus</i> (Spores/ml)		
	Control (unfermented)	10 ³	10 ⁵
Alkaloid (%)	1.73 ^b ±0.18	0.87 ^a ±0.04	0.75 ^a ±0.02
Oxalate (mg/g)	1.57 ^b ±0.05	1.36 ^a ±0.06	1.39 ^{ab} ±0.15
Phytate (mg/g)	12.36 ^c ±0.00	6.57 ^b ±0.04	3.66 ^a ±0.42
Tannin (mg/g)	0.06 ^b ±0.01	0.02 ^a ±0.00	0.02 ^a ±0.00
Saponin (%)	1.27 ^b ±0.01	0.74 ^a ±0.12	0.76 ^a ±0.22

Means with the same superscript letters in the same row are not significantly different (P>0.05).

Discussion

Fungal biodegradation of agricultural-wastes had been achieved through extracellular enzymes (pectinase, cellulase, hemicellulase, glucanase, xylanase, protease, lipase, tannase, phytase, etc) (Hanson, 2008; Omid *et al.*, 2012; Olaniyi, 2014). Predigesting fiber compounds via secreting carbohydrases increases bioavailability of these compounds for target microorganism and consequently causes to produce nutritive protein biomass. Improvement in the nutritive quality of fungal treated samples produces unique products with the functional and nutraceutical properties. The application of fungal base treatment of agricultural wastes has led to the production of different kinds of products to solve challenges faced in animal feed formulation. In this study, the effect of different inocula concentrations on bioconversion of orange peels into a nutritionally enriched substrate by *P. italicum*, *T. oedocephalis* and *A. flavus* was evaluated. Increasing in crude protein content of *P. italicum*, *T. oedocephalis* and *A. flavus* treated orange peels with different inocula concentrations was an indication of effective bioconversion of this waste into nutritionally enriched substrate. Solid state fermentation of biomass has been attempted as a means of elevating the total protein content by many workers (Iyayi, 2004; Akinfemi *et al.*, 2010; Akinyele *et al.*, 2011). The increase in the crude protein contents may be due to secretion of certain extracellular enzymes which are proteineous in nature into the wastes during their breakdown (Akinfemi *et al.*, 2010; Akinyele *et al.*, 2011). Crude protein increase could also be due to the capture

of excess nitrogen by aerobic fermentation (Sallam *et al.*, 2008) suggesting that the treated substrates are good source of protein for livestock. This agrees with the findings of Iyayi and Aderolu (2004) and Iyayi (2004). Apart from the afore-mentioned factors that might be responsible for protein increment in fungal treated wastes, hydrolysis of starch to glucose and its subsequent use by the organisms as carbon sources to synthesis fungal biomass rich in protein might be linked with increase in protein content (Akinyele *et al.*, 2011). According to Akinyele *et al.* (2011), the differences in crude protein content between the treatments may be due to physical and environmental factors which are known to induce differences in the physiology of the organisms involved. Omid *et al.* (2012) reported 54.93% increase in crude protein when *A. niger* was utilized for nutrient enrichment of canola meal with inoculum density of 10^7 spores/g. A 65% increase in crude protein of rapeseed meal was reported by Pal vig and Walia (2001) with five-day-age culture of *Rhizopus microsporus* after 10 days of fermentation. Rakariyatham and Sakorn (2002) reported 48% increase in crude protein for fermented mustard meal with an inoculation density 10^6 spores/g *Aspergillus* sp. after 48 hours. An increment of 10% crude protein for fermented soybean meal with 10^4 spores/g *A. oryzae* after 2 days of fermentation was reported by Kim *et al.* (2010).

The anti-nutrient contents of fungal treated orange peels varied with the treatment type and the concentration of the inoculum. However, almost all the anti-nutrient parameters evaluated decreased in fungal treated samples.

The decrease in anti-nutrient compounds by fermentation biotechnology had been reported by many researchers. Phytate is capable of chelating divalent cationic minerals like Ca, Fe, Mg and Zn, thereby reducing dietary deficiency. The decrease of phytate in treated samples could be attributed to the secretion of hydrolytic enzyme (phytase) by the organisms involved. This enzyme is capable of hydrolyzing phytate content in the fermented mango peels (Ojokoh *et al.*, 2005), fermented sorghum cultivars (Abdelhaleem *et al.*, 2008) and fermented mango kernel cake (Kayode and Sani, 2008). Phytate contents in the fermented canola meal with *A. niger* decreased by 1.57% when compared with control treatment (Omid *et al.*, 2012). The use of *R. microsporus* for the fermentation of soybean for soybean tempe production caused 30-33% reduction in its phytate content (Egounlety and Aworth, 2003). Similar findings have also been reported, Fardiaz and Markakis (1981) reported that 7-day culture of *R. oligosporus* and *Neurospora sitophila* with 1.3×10^6 spores per gram on peanut meal caused 95 and 58% reduction in its phytate contents. The detoxification of tannin, alkaloid, saponin and oxalate in cassava wastes by fermentation was reported by Aro *et al.* (2008). Tannin affects the nutritive value of food products by forming complex with protein (both substrate and enzyme) thereby inhibiting digestion and absorption (Aro *et al.*, 2008). They also bind iron (Fe) ion making it unavailable (Aletor and Adeogun, 1995) and other evidence suggests that condensed tannins may cleave DNA in the presence of copper ions (Ojokoh *et al.*, 2005). The decrease in tannin contents in some treatment

may be as a result of the processing that the samples were subjected to couple with the activities of microbial enzymes involved in the fermentation (Abdelhaleem *et al.*, 2008). Oxalate content of the cocoyam flour samples also showed a significant decrease with increase in fermentation time (Igbabul *et al.*, 2014). It is known that oxalate forms insoluble complex with calcium ions, and it is often anticipated that oxalate containing foods when consumed may interfere with calcium metabolism (Igbabul *et al.*, 2014).

In conclusion, bioconversion of orange peels with different inocula concentrations of *T. oedocephalis* and *P. italicum* led to an improvement in its nutritional status. Nutritive values of fungal treated orange peels vary with the inoculum concentrations. Fermented orange peels might be used as feed ingredient and substitute to essential ingredients known to be expensive in animal feed formulation. The fungal treated orange peels might be fed to experimental animals to evaluate its effect on blood parameters and organs.

References

- Abdelhaleem, W. H., Eltinay, A. H., Mustafa, A. I. and Babiker, E.E. (2008). Effect of fermentation, malt-pretreatment and cooking on anti-nutritional factors and protein digestibility of sorghum cultivars. *Pakistan Journal of Nutrition*. 7 (2): 335-341
- Agosin, E., Monties, B. and Odier, E. (2006). Structural changes in wheat straw components during decay by lignin-degrading white-rot fungi in relation to improvement of digestibility for ruminants. *Journal of Science, Food and Agriculture*. 36(10): 925-935.
- Akinfemi, A., Adu, O.A. and Doherty, F. (2010). Conversion of sorghum

- stover into annual feed with white-rot fungi: *Pleurotus ostreatus* and *Pleurotus pulmonarius*. *African Journal of Biotechnology*. 9 (1): 1706-1712.
- Akinyele, B.J., Olaniyi, O.O. and Adetunji, C.O. (2013). Screening and optimization of nutritional conditions for mannanase production by *Penicillium italicum* LAD-A5 in solid state cultivation. *E3 Journal of Biotechnology and Pharmaceutical Research*. 4(2): 35-41.
- Akinyele, B.J., Olaniyi, O.O. and Arotupin, D.J. (2011). Bioconversion of selected agricultural wastes and associated enzymes by *Volvariella volvacea*: An edible mushroom. *Research Journal of Microbiology*. 6(1): 63-70.
- Aletor, V. A. and Adeogun, O.A. (1995). Nutrient and anti-nutrient components of some tropical leafy vegetables. *Food Chemistry*. 53: 375-379.
- Amadou, I., Kamara, M.T., Tidjani, A. and Foh, M.B.K. (2010). Physicochemical and nutritional analysis of fermented soybean protein meal by *Lactobacillus plantarum* Lp6. *World Journal of Dairy and Food Science*. 5 (2): 114-118.
- AOAC (2005). Official Methods of Analysis of the Association of Official Analytical chemists, Washington, USA. 2000; 2: p. 1234.
- Aro, S.O., Aletor, V.A., Tewe, O.O., Fajemisin, A.N., Usifo, B. and Adesida, J.A. (2008). Studies on the nutritional potentials of cassava tuber wastes (CTW) collected from a factory. Proc. 4th Annual Conf. SAAT, Federal University of Technology, Akure, Nigeria. 21st May, 2008: 86-92.
- Day, R.A. and Underwood, A.L. (1986). Quantitative analysis, 5th edition, preistice hall publication USA. pg: 701.
- De boland, A.R., Garner, G.B.O., Dell, B.L. (1975). Identification and properties of phytate in cereal grains and oil seed products. *Journal of Agriculture and Food Chemistry*. 23: 1186-1189.
- Egounlety, M. and Aworh, O.C. (2003). Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Harms). *Journal of Food Engineering*. 56: 249-254.
- El-Naggar, M.Y., El-Aassar, S.A., Youssef, A.S., El-Sersy, N.A. and Beltagy, E.A. (2006). Extracellular β -mannanase production by the immobilization of the locally isolated *Aspergillus niger*. *International Journal of Agriculture and Biology*. 8: 57-62.
- Fardiaz, D. and Markakis, P. (1981). Degradation of phytic acid in oncom. *Journal of Food Science*. 46: 523-525.
- Frias, J., Song, Y.S. and Martinez-Villaluenga, C. (2008). Immunoreactivity and amino acid contents of fermented soybean products. *Journal of Agriculture and Food Chemistry*. 56: 99-105.
- Gowthaman, M.K., Krishna, C.H. and Moo-Young, M. (2001). Fungal solid state fermentation- an overview. *Applied Mycology and Biotechnology*. 1: 305-352.
- Hanson, J. R. (2008). The Chemistry of Fungi. 1st ed. RSC Publishing. 221pp.
- Hong, K.J., Lee, C.H. and Kim, S.W. (2004). *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and soybean meals. *Journal of Medicinal Food*. 7 (4): 430-435.
- Ibrahim, D., Puspitaloka, H., Abdul Rahim, R. and Sheh Hong, L. (2012). Characterization of Solid State Fermentation Culture Conditions for

- Growth and Mannanase Production by *Aspergillus niger* USM F4 on Rice Husk in Tray System. *British Biotechnology Journal*. 2(3): 133-145.
- Igbabul, B.D., Amove, J., and Twadue, I. (2014). Effect of fermentation on the proximate composition, anti-nutritional factors and functional properties of cocoyam (*Colocasia esculenta*) flour. *African Journal of Food Science and Technology*. 5(3): 67-74.
- Iyayi, E.A. (2004). Changes in the cellulose, sugar and crude protein contents of agro-industrial by-products fermented with *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* sp. *African Journal of Biotechnology*. 3 (3): 186-188.
- Iyayi, E.A. and Aderolu, Z.A. (2004). Enhancement of the feeding value of some agro industrial by products for laying hens after their solid state fermentation with *Trichoderma viride*. *African Journal of Biotechnology*. 3: 182-185.
- Karunanandaa, K., Varga, G.A., Akin, D.E., Rigsby, L.L. and Royse, D.J. (1995). Botanical fractions of rice straw colonized by white-rot fungi: changes in chemical composition and structure. *Animal Feed, Science and Technology*. 55(3-4): 179-199.
- Kayode, R.M.O. and Sani, A. (2008). Physicochemical and proximate composition of mango (*Mangifera indica*) kernel cake fermented with mono-culture of fungal isolates obtained from naturally decomposed mango kernel. *Life Science Journal*. 5 (4): 55-63.
- Khalil, A. (2006). Nutritional improvement of an Egyptian breed of mung bean by probiotic lactobacilli. *African Journal of Biotechnology*. 5 (2): 206-212.
- Khanongnuch, C., Sa-Nguansook, C. and Lumyong, S. (2006). Nutritive Quality of β -Mannanase Treated Copra Meal in Broiler Diets and Effectiveness on Some Fecal Bacteria. *International Journal of Poultry Science*. 5(11): 1087-1091.
- Kiers, J.L., Meijer, J.C., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A. and Meulen, J. (2003). Effect of fermented soya beans on diarrhoea and feed efficiency in weaned piglets. *Journal of Applied Microbiology*. 95: 545-552.
- Kim, S.W., Heugten, E., Ji, F., Lee, C.H. and Mateu, R.D. (2010). Fermented soybean meal as a vegetable protein source for nursery pigs: I. Effects on growth performance of nursery pigs. *Journal of Animal Science*. 88: 214-224.
- Krishna, C.H. (2005). Solid-state fermentation systems-an overview. *Critical Review on Biotechnology*. 25: 1-30.
- Mukherjee, R. and Nandi, B. (2004). Improvement of *in vitro* digestibility through biological treatment of water hyacinth biomass by two *Pleurotus* species. *International Biodeterioration and Biodegradation*. 53(1): 7-12.
- Obadeni, B.O. and Ochuko, P.O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some Hemostat plants in Edo and Delta states of Nigeria. *Global Journal of Pure and Applied Sciences*. 22: 283-285.
- Ojokoh, A.O., Adetuyi, F.C. and Akinyosoye, F.A. (2005). Nutritional evaluation of fermented roselle (*Hibiscus sabdariffa*) calyx. *Journal of Food Technology*. 3(3): 423-426.
- Olaniyi, O.O. (2014). Effect of Beta-Mannanase Treatment on Nutritive Quality of Palm kernel Meal. *African Journal of Microbiology Research*. 8(25): 2405-2410.
- Omid, S., Mehrdad, F., Chris, C., Bagher, Y. and Masoomeh, M.S. (2012). Study on the effect of solid state fermentation with *Aspergillus niger* on antinutritional factors of canola protein concentrate with aim of

- using in the diet of rainbow trout (*Oncorhynchus mykiss*). The 1th International and the 4th National Congress on Recycling of Organic Waste in Agriculture 26–27 April 2012 in Isfahan, Iran. Pg 1-12.
- Price ML, Van Scoyoc S, Butler LG (1978). A critical evaluation of the vanillin reaction as an assay for tannins and sorghum grain. *Journal of Agriculture and Food Chemistry*. 26(5):1214-1218.
- Pal Vig, A. and Walia, A. (2001). Beneficial effects of *Rhizopus oligosporus* fermentation on reduction of glucosinolates, fiber and phytic acid in rapeseed (*Brassica napus*) meal. *Bioresource Technology*. 78: 309-312.
- Pitt, J.I. and Hocking, A.D. (1997). Fungi and food spoilage, 2th Edition, Blacki Academic, London, U.K. pp. 414.
- Price, M.L., Van Scoyoc, S. and Butler, L.G. (1978). A critical evaluation of the vanillin reaction as an assay for tannins and sorghum grain. *Journal of Agriculture and Food Chemistry*. 26(5): 1214-1218.
- Rakariyatham, N. and Sakorn, P. (2002). Biodegradation of glucosinolates in brown mustard meal (*Brasica juncea*) by *Aspergillus* sp. NR-4201 in liquid and solid culture. *Biodegradation*. 3: 395-409.
- Sallam, S.M.A., Bueno, I.C.S., Godoy, P.B., Nozella, E.F., Vitti, D.M.S.S. and Abdalla, A.L. (2008). Nutritive value in the assessment of the artichoke (*Cynara scolymus*) by products as an alternative feed resource for ruminant. *Tropical Subtropical Agroecosystem*. 8: 181-189.
- Singhanian, R.R., Patel, A.K., Soccol, C.R. and Pandey, A. (2009). Recent advances in solid-state fermentation. *Biochemistry and Engineering Journal*. 44: 13-18.
- Song, X.N., Fang, R.J., Wang, X.Z. and Wang, H.T. (2009). Research on utilization techniques of rape straw as resources. *Journal of Natural Bioresource*. 24(6): 984-990.
- Villas-Bôas, S.G., Esposito, E. and Mendonça, M.M. (2003). Bioconversion of apple pomace into a nutritionally enriched substrate by *Candida utilis* and *Pleurotus ostreatus*. *World Journal of Microbiology and Biotechnology*. 19(5): 461-467.
- Yamamoto, M., Saleh, F., Tahir, M., Ohtsuka, A. and Hayashi, K. (2007). The effect of koji-feed (fermented distillery by-product) on the growth performance and nutrient metabolizability in broiler. *Journal of Poultry Science*. 44: 291-296.
- Zhang, R., Li, X. and Fadel, J. (2002). Oyster mushroom cultivation with rice and wheat straw. *Bioresource Technology*. 82(3): 277-284.