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 10^5spores/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. 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

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(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 sitophile*, *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 posses 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; $MgSO_4.7H_2O$, 0.5; KCl, 0.5 and $FeSO_4.7H_2O$ 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 (%)	Control (unfermented)	*T. oedocephalis* (Spores/ml) 10^3	10^5
Moisture	$7.76^{c}\pm0.34$	$5.43^{a}\pm0.10$	$6.29^{b}\pm0.35$
Fat	$12.73^{b}\pm0.41$	$7.32^{a}\pm0.16$	$6.86^{a}\pm0.07$
Ash	$5.64^{a}\pm0.08$	$7.91^{b}\pm0.00$	$9.03^{c}\pm0.12$
Protein	$11.55^{a}\pm0.17$	$13.25^{b}\pm0.78$	$11.94^{a}\pm0.80$

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 (%)	Control (unfermented)	*P. italicum* (Spores/ml) 10^3	10^5
Moisture	$7.76^{b}\pm0.34$	$5.59^{a}\pm0.25$	$5.99^{a}\pm0.13$
Fat	$12.7^{3}c\pm0.41$	$8.69^{b}\pm0.01$	$7.41^{a}\pm0.03$
Ash	$5.64^{a}\pm0.08$	$10.01^{c}\pm0.19$	$9.33^{b}\pm0.12$
Protein	$11.55^{a}\pm0.17$	$13.57^{b}\pm0.01$	$13.37^{b}\pm0.26$

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 (%)	Control (unfermented)	*A. flavus* (Spores/ml) 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	Control (unfermented)	*T. oedocephalis* (Spores/ml) 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^{c}±0.00	3.84^{a}±0.48	5.76^{b}±0.00
Tannin (mg/g)	0.06^{a}±0.00	1.96^{b}±0.00	2.13^{b}±0.31
Saponin (%)	1.27^{c}±0.03	1.18^{b}±0.02	0.96^{a}±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)

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

		A. flavus (Spores/ml)	
Parameters (%)	Control (unfermented)	10^3	10^5
Alkaloid (%)	$1.73^{b}\pm0.18$	$0.87^{a}\pm0.04$	$0.75^{a}\pm0.02$
Oxalate (mg/g)	$1.57^{b}\pm0.05$	$1.36^{a}\pm0.06$	$1.39^{ab}\pm0.15$
Phytate (mg/g)	$12.36^{c}\pm0.00$	$6.57^{b}\pm0.04$	$3.66^{a}\pm0.42$
Tannin (mg/g)	$0.06^{b}\pm0.01$	$0.02^{a}\pm0.00$	$0.02^{a}\pm0.00$
Saponin (%)	$1.27^{b}\pm0.01$	$0.74^{a}\pm0.12$	$0.76^{a}\pm0.22$

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 bioavailablity 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 neutraceutical 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 sitophile* 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.

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