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Protein enrichment of yam peels by fermentation with *Saccharomyces cerevisiae* (BY4743)

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ABSTRACT

Conventional sources of protein are becoming inadequate for protein-based food supply. Non-conventional sources are of much concern, but literature is sparse on the protein quality of yam peel biomass. Solid state fermentation (with and without ammonium sulphate) of yam peels was carried out for 96 h, using *Saccharomyces cerevisiae* (BY4743). Samples from fermented biomass were collected at 24 h interval for 96 h, oven dried at 60 °C, cooled, milled and assayed for chemical and amino acid composition. The crude protein, true protein, fat and ash contents increased significantly from 6.60%, 4.38%, 1.12% and 4.45% to 15.54%, 13.37%, 2.09% and 8.02% respectively when fermentation reached 96 h in the presence of ammonium sulphate. High protein content with good amino acids profile (leucine, valine, lysine and threonine as essential amino acids) was recorded in ammonium sulphate supplemented peels fermented for 96 h. Remarkable increase in protein and amino acids suggested that ammonium sulphate supplement influenced the biomass protein yield and quality.
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Introduction

Proteinaceous food supply from conventional protein sources are inadequate and pollution occurring from food and agricultural wastes have encouraged concern to convert them into nutritious, safe and valuable products thereby turning waste to either food or feed for human and animal use. Farms and food processing industries generate different kinds of wastes (solid, liquid or gaseous) on a daily basis. These make up a significant proportion (over 30%) of global agricultural productivity (Ugwuanyi et al., 2009). Adoption of mechanized farming techniques and application of inorganic fertilizers, herbicides and pesticides have not only increased yield and positively altered the quality and composition of agricultural outputs, but have also increased the quantity of agricultural wastes produced annually. Proper identification and management of these wastes are very important in order to ensure desirable environmental sanitation. One way by which this can be achieved is by biotransformation of wastes into commercially valuable products like protein-enriched products, single cell protein, mushroom production, etc. (Gomez et al., 2005). Therefore, special efforts have been made to ensure that agricultural and industrial by-products, that are at times detrimental to man and animal health, are positively utilized by converting them into value-added products (Iyayi and Losel, 2001). Some of these include wastes from root tubers such as yam and cassava peels, fruit and vegetable processing industries such as pineapple, citrus, banana, plantain and mango peels, coconut husks and shells, corn cobs and husks.

Yam (*Dioscorea* spp.) is an important food crop in Africa, Americas, Carribean, South Pacific and Asia. Nigeria is the leading producer of yams in the world accounting for over 65% (with 38 million metric tonnes) of the world production in 2012 and it is more widely distributed and abundantly available than cereals (Akpan et al., 2005). Ezeibekwe et al. (2009) reported that white yam (*Dioscorea rotundata*) to be the most important cultivated yam. It is processed into a wide range of products in different parts of Africa and other regions of the world. In tropical Africa, yam flour (poundo-yam), yam gruel (*amala*), pounded yam (*iyan*), fried yam (*dundu*), fried grated-spiced yam (*ojojo*)and other products are made from yam resulting in large amounts of yam peels which is very low in protein, consequently, it is either discarded or burnt resulting in pollution of air, land and water. Usually, the price of

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conventional protein feed is high, thus increasing cost of livestock production.

Fermentation is an essential technology that is required for converting lignocellulosic materials such as yam peels into value-added products which have potential to provide solutions to problems of animal nutrition such as inadequate intake of protein and calories. This will also reduce waste, pollution as well increase quality of animal by-products. According to Anupama and Ravindra (2000), fermented products contain proteins that can feed both humans and animals thereby replacing expensive conventional sources of protein like fishmeal and soymeal. However, it is important to use microorganism that are "Generally Regarded as Safe (GRAS)" for fermentation in order to promote its use. *Saccharomyces cerevisiae* is widely accepted due to long history of its use in traditional fermentation and its nutritional quality (Nasseri et al., 2011). Therefore, this study ought to develop protein-enriched and value-added products from yam peels by solid state fermentation and to evaluate the protein quality of the produced biomass.

Material and methods

Yam peels from white yam tubers were collected from a canteen in University of Ibadan, Oyo State, Nigeria during the raining season. *S.cerevisiae* (BY4743) was obtained from Department of Microbiology, University of Ibadan, Oyo State, Nigeria and maintained on yeast extract agar slants at 4 °C.

Sample preparation

Yam peels (1.0 kg) were washed to remove dirt, thereafter drained and dried at 60 °C for 12 h, cooled and milled. It was then sieved using 3.35–4.00 mm sieve mesh sizes, packaged and stored prior to further analysis.

Solid State Fermentation (SSF) of yam peels (without nitrogen supplementation) using S. cerevisiae (BY4743)

Yam peels (50 g) were weighed into conical flask, moisture content was adjusted to 60% by adding calculated amount of distilled water, mixed, sterilized at 121 °C for 15 min and allowed to cool to room temperature (27 ± 2 °C). 15 ml (2.6×10^8 CFU/ml) of fermented yeast broth was added to the sterilized yam peels in the conical flasks, mixed gently, loosely covered and incubated at 27 °C for 96 h with intermittent manual shaking (Fig. 1). During incubation, samples were collected at interval of 24 h, spread on metallic trays and oven-dried at 60 °C for 5 h, cooled to 27 ± 2 °C, milled and packaged in air tight containers.

SSF of yam peels (with addition of inorganic nitrogen) using S. cerevisiae (BY4743)

About 50 g each of the substrate (yam peels) was weighed separately into 250 ml conical flask, moisture contents were adjusted appropriately. Inorganic nitrogen was added, in the form of ammonium sulphate (10 g of N/kg substrate) to the substrate, sterilized at 121 °C for 15 min, cooled to ambient temperature, inoculated with fermented yeast broth and incubated at 27 °C for 96 h. The control sample was properly labelled and not inoculated. Samples were taken 24 hourly during incubation and fermentation was terminated by oven drying at 60 °C for 24 h (Ezekiel et al., 2010).

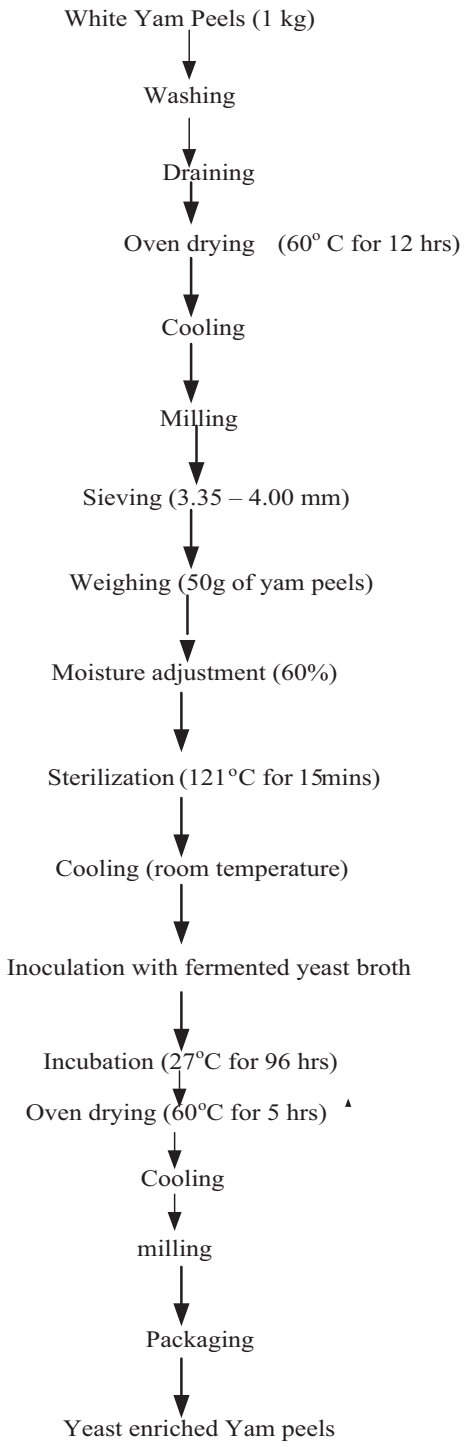


Fig. 1. Flow sheet of production of yeast enriched yam peels.

Chemical composition

Ash, crude fiber, fat, protein, carbohydrate and moisture contents were determined as described by AOAC (2005). Ash was done by incinerating the sample (1 g) at 550 °C, crude fiber content was determined by treating the sample with H_2SO4 and NaOH which was followed by rinsing with water and acetone, drying in the oven at 105 °C for 3 h and incineration at 550 °C for 1 h. Fat was determined by Soxhlet petroleum ether extraction, crude protein content was calculated using % N × 6.25, carbohydrate was

estimated by difference and moisture content was determined by oven - drying the sample (5 g) at 105 °C until constant weight was obtained.

True protein determination

True protein content was determined by Folin-Ciocalteau method as described by Lowry et al. (1951).

Amino acid determination

Amino acid composition was determined according to the method described by Holmes et al. (2013).

Statistical analysis

All analyses were performed in triplicates. Data was subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) V. 17.0. Means were separated using Duncan Multiple Range Test (DMRT) at 95% confidence level ($P \leqslant 0.05$).

Results and discussion

Chemical composition

Table 1 presents the chemical composition of unfermented and fermented yam peels without the addition of inorganic nitrogen. Crude protein, true protein, fat and carbohydrate contents ranged between 6.60–11.08%, 4.38–9.56%, 1.12–1.52%, 78.81–73.03% respectively when fermentation period reached 96 h. As fermentation period progresses, the values of all chemical composition increased except for carbohydrate content which declined. The increase in protein contents (crude and true) of yam peels after fermentation was presumably due to extracellular protein secretion by *S. cerevisiae* (BY4743) in metabolizing yam peels constituents or its multiplication in the form of single cell protein (Correia et al., 2007). This corroborates with the findings of Oboh and Akindahunsi (2003) who observed increase in protein level in cassava products due to possible secretion of some extracellular enzymes (proteins) such as amylase and cellulose into the substrates which break the starch and other polysaccharides into simpler sugars that are easily metabolized by yeast as a source of carbon. Low fat content of yam peels agrees with the findings of Ekeyem et al. (2006) and Akinmutimi and Onen (2008) who reported similar results for yam peels meal. Decrease in the carbohydrate content could be as a result of significant increase in the protein content in the fermented peels (Oboh et al., 2002). It could also due to the ability of *S.cerevisiae* to hydrolyze complex carbohydrate into sugars that serve as carbon source in synthesizing microbial biomass that are high in protein during the fermentation process (Akintomide and Antai, 2012).

Table 2 showed the effects of ammonium sulphate supplementation on the chemical composition of yam peels fermented with *S. cerevisiae* (BY4743). Addition of ammonium sulphate to yam peels prior to fermentation significantly increased the crude protein, true protein as well as fat contents of the yam peel biomass throughout the 96 h fermentation. Crude protein, true protein and fat increased to 15.54%, 13.37% and 2.09% respectively. The significant increase in crude and true protein contents obtained during the study could probably be due to the fact that nitrogen is a nutritive compound and it has positive influence on the pH of the growth medium (Oboh and Akindahunsi, 2003). This result followed the same trend as reported by Yang (1988) in which the protein content of sweet potato residue supplemented with $(NH4)_2SO_4$/urea (1:1, w/w), increased from 6.01% to 14.04% after 72 h of fermentation using *S. cerevisiae*. Increase in fat content may be as a result of possible bio-conversion carbohydrate to fat (Oboh et al., 2002), and also certain fungal species are capable of building up fat during the fermentation process (Akindumila and Glatz, 1998). The ash content of the ammonium sulphate supplemented yam peels biomass was higher than the ash level in the non supplemented peels. Oboh and Akindahunsi (2003) reported increase in ash content in *Saccharomyces cerevisiae* fermented cassava products. Ezekiel et al. (2010) also reported an increase in ash content of the fermented cassava peels during submerged fermentation of cassava peels with *Trichoderma viride* and this could be due to addition of ammonium sulphate to the fermenting medium. Also, at the end of the fermentation period, there was a significant decrease ($P < 0.05$) in carbohydrate level obtained in the ammonium sulphate supplemented fermented peels when compared with the non-supplemented fermented peels which could likewise be attributed to the depletion of sugars during fermentation. Table 3 showed the comparism between unfermented, non-supplemented and supplemented *S.cerevisiae* (BY4743) fermented yam peels. Supplemented *S.cerevisiae* (BY4743) fermented yam peels had the highest composition for all the proximate parameters followed by non-supplemented biomass while unfermented yam peel had the lowest. Increase in protein, crude fat and fiber contents observed in this study after fermentation was similar to the findings of Pandey et al. (2000) who reported that fermented products had better and improved nutrient compositions as well as digestibility. Higher protein yield obtained in the study also corroborates with the findings of Zadrazil et al. (1996) who reported bioconversion of lignocelluloses into protein enriched feed for ruminants using white-rot fungi. On the other hand, the value for carbohydrate is the reverse with unfermented yam peel having the highest and supplemented biomass having the least. Also, low carbohydrate values recorded after fermentation with or without the addition of inorganic nitrogen may be due to adequate nitrogen and carbon sources utilization by the fermenting organism (Ezekiel et al., 2010).

Amino acid profile of S. cerevisiae (BY4743) enriched and unfermented yam peels

The amino acid profile of *S. cerevisiae* (BY4743) fermented and unfermented yam peels proteins are presented in Table 4. Results

Table 1
Changes in chemical composition of yam peels fermented with *Saccharomyces cerevisiae* without addition of inorganic nitrogen.

Components (%)	0 h	24 h	48 h	72 h	96 h
True protein	$4.38 \pm 0.01_d$	$6.52 \pm 0.03_c$	$7.45 \pm 0.08_b$	$7.73 \pm 0.00_b$	$9.56 \pm 0.00_a$
Crude protein	$6.60 \pm 0.04_d$	$9.10 \pm 0.03_c$	$9.64 \pm 0.03_b$	$9.94 \pm 0.06_b$	$11.08 \pm 0.01_a$
Crude fibere	$9.02 \pm 0.09_a$	$9.08 \pm 0.03_a$	$9.14 \pm 0.00_a$	$9.33 \pm 0.04_a$	$9.45 \pm 0.03_a$
Carbohydrate	$78.81 \pm 0.05_a$	$76.14 \pm 0.01_b$	$75.27 \pm 0.04_c$	$74.41 \pm 0.3_d$	$73.03 \pm 0.11_e$
Fat	$1.12 \pm 0.01_b$	$1.16 \pm 0.01_b$	$1.28 \pm 0.00_b$	$1.44 \pm 0.1_a$	$1.52 \pm 0.03_a$
Ash	$4.45 \pm 0.03_a$	$4.52 \pm 0.06_a$	$4.67 \pm 0.03_a$	$4.88 \pm 0.02_a$	$4.92 \pm 0.14_a$

Data presented on dry matter basis. Each value is a mean of three independent experiments. Means with the same subscripts in the same row are not significantly different ($p > 0.05$).

Table 2
Chemical composition of *S. cerevisiae* (BY4743) enriched yam peels with addition of inorganic nitrogen.

Composition %	Fermentation period (hours)				
	0	24	48	72	96
Crude protein	$6.60 \pm 0.04_d$	$13.57 \pm 0.03_c$	$14.78 \pm 0.03_b$	$15.43 \pm 0.14_a$	$15.54 \pm 0.22_a$
True protein	$4.38 \pm 0.01_e$	$10.12 \pm 0.06_d$	$11.0 \pm 0.01_c$	$12.86 \pm 0.04_b$	$13.37 \pm 0.00_a$
Crude fat	$1.12 \pm 0.01_b$	$1.87 \pm 0.01_b$	$1.92 \pm 0.03_b$	$2.03 \pm 0.01_a$	$2.09 \pm 0.02_a$
Crude fibere	$9.02 \pm 0.09_a$	$9.39 \pm 0.06_a$	$9.52 \pm 0.04_a$	$9.60 \pm 0.03_a$	$9.81 \pm 0.01_a$
Ash	$4.45 \pm 0.03_b$	$8.22 \pm 0.00_a$	$8.17 \pm 0.06_a$	$8.05 \pm 0.03_a$	$8.02 \pm 0.01_a$
Carbohydrate	$78.81 \pm 0.05_a$	$66.95 \pm 0.07_b$	$65.61 \pm 0.08_c$	$64.89 \pm 0.11_d$	$64.54 \pm 0.07_d$

Data presented on dry matter basis. Each value is a mean of three independent experiments. Means followed by the same subscript in the same row are not significantly different ($p > 0.05$).

Table 3
Comparism between unfermented, non-supplemented and nitrogen supplemented *S. cerevisiae* (BY4743) fermented yam peels.

Composition (%)	Unfermented yam peels	Non-supplemented *S. cerevisiae* fermented yam peels	Supplemented *S. cerevisiae* fermented yam peels
Crude protein	$6.60 \pm 0.04_c$	$11.08 \pm 0.00_b$	$15.54 \pm 0.23_a$
True protein	$4.38 \pm 0.01_c$	$9.56 \pm 0.00_b$	$13.37 \pm 0.0_a$
Crude fat	$1.12 \pm 0.01_c$	$1.52 \pm 0.03_b$	$2.09 \pm 0.02_a$
Crude fibere	$9.02 \pm 0.09_a$	$9.45 \pm 0.03_a$	$9.81 \pm 0.01_a$
Ash	$4.45 \pm 0.03_b$	$4.92 \pm 0.14_b$	$8.02 \pm 0.01_a$
carbohydrate	$78.81 \pm 0.05_a$	$73.03 \pm 0.11_b$	$64.54 \pm 0.07_c$

Data presented on dry matter basis. Each value is a mean of three independent experiments. Means followed by the same subscript in the same row are not significantly different ($p > 0.05$). Alphabets 'a to c' indicates values in decreasing magnitude.

Table 4
Amino acid profiles of unfermented and fermented yam peels.

Amino Acid	Unfermented Yam peels (mg/100 g)	Fermented yam peels (mg/100 g)
Glycine	280 ± 1.90	860 ± 17.22
Alanine	260 ± 1.30	1300 ± 25.31
Serine	180 ± 1.52	710 ± 4.90
Proline	300 ± 2.24	790 ± 6.23
Valine	210 ± 1.81	1100 ± 23.41
Threonine	160 ± 2.33	980 ± 16.24
Isoleucine	190 ± 1.25	870 ± 14.51
Leucine	260 ± 1.48	1200 ± 28.35
Aspartic acid	310 ± 2.66	1350 ± 26.42
Lysine	200 ± 1.20	1090 ± 22.80
Methionine	190 ± 1.52	400 ± 5.26
Glutamic acid	520 ± 3.83	1710 ± 24.63
Phenylalanine	380 ± 1.36	730 ± 3.93
Histidine	100 ± 1.62	510 ± 11.85
Arginine	210 ± 1.40	860 ± 5.83
Tyrosine	90 ± 1.21	390 ± 4.66
Cystine	120 ± 1.83	300 ± 2.31
Total A. acid Content (mg/100 g)	3852	14700

showed that fermented and unfermented yam peels contain essential and non-essential amino acids such as valine, phenylalanine, tyrosine, threonine, leucine, isoleucine, lysine, methionine and so on (McDougall, 2002), they are nevertheless abundant in aspartic and glutamic acid. Solid state fermentation greatly improved the amino acid profile of yam peels when *S. cerevisiae* (BY4743) was used. Total amino acid content of the fermented yam peels was approximately four times (14700 mg/100 g) the total amino acid content of the unfermented yam peels (3852 mg/100 g) and glutamic acid (1710 and 520 mg/100 g respectively) was the most abundant amino acid. Higher and better amino acid composition

Table 5
Chemical score of *S. cerevisiae* fermented yam peels.

Essential amino acids	FAO/WHO amino acid values (mg/g)	Essential amino acids in *S. cerevisae* fermented yam peel (%)
Threonine	40	35.00
Valine	50	26.19
Methionine + cystine*	35	18.18
Isoleucine**	40	20.71
Leucine	70	25.00
Phenylalanine + tyrosine	60	26.07
Lysine	55	25.95
Histidine	–	–
Tryptophan	10	–

* Limiting amino score – Methionine.
** Limiting amino score – Isoleucine.

of the *S. cerevisiae* (BY4743) fermented yam peels over the unfermented yam peel could be as a result of presence of high protein fungal biomass in the fermented yam peels. Similar results were observed by Ezekiel et al. (2010) when cassava peels were fermented with *Trichoderma viride*. Chemical score of *S. cerevisiae* (BY4743) enriched yam peels is presented in Table 5 using FAO/WHO amino acids reference pattern and it varied from 18.18 to 35.0% (Harper, 1981). The limiting amino acids are methionine and isoleucine and their chemical scores were 18.18% and 20.71% respectively. The two sulphur-containing amino acids are limiting as they are from plant source (McDougall, 2002). Better amino acid score showed by the fermented yam peels could be due to increase in protein content of the peels by *S. cerevisiae* (BY4743) that metabolised complex carbohydrate polymer into high protein biomass.

Conclusion

Solid state fermentation of white yam peels using *S. cerevisiae* (BY4743) with and without the addition of inorganic nitrogen (in form of ammonium sulphate) resulted in protein production. However, higher yield was obtained when ammonium sulphate was added thereby upgrading its low initial protein contents. Amino acid profile revealed that *S. cerevisiae* (BY4743) enriched and ammonium sulphate supplemented biomass had better amino acid composition than the unfermented sample. Considering these significant enhanced protein contents in the fermented yam peels, it is a potential protein supplement in animal diet.

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