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Protein Enrichment of Wastes from Three Nigerian Yam Varieties using Mono and Co-cultures of Amylolytic Fungi

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Protein enrichment of wastes from *Dioscorea rotundata, Dioscorea cayenensis* and *Dioscoreaalata* was investigated by solid state fermentation using mono and co-cultures of four amylolytic fungi. The wastes provided the sole carbon source while mineral elements were from $(NH_4)SO_4$, urea and $KHPO_4$. Fermentation was allowed to progress for 5days. pH, moisture and protein contents were assessed on the first day and on the 5th day for the various yam varieties. Protein contents increased from the value of 3.82±0.29% (w/w) to values that ranged from 8.46±0.30% (w/w) from *S. cerevisiae* on *D.rotundata* to 10.10±0.50% (w/w) from *Mucor* sp on *D.alata*. Addition of booster doses of nitrogenous supplements resulted in further increases in protein yield. The use of co-cultures of the fungi showed a further increase in protein although not up to double for the values obtained from those where mono cultures were inoculated. There were decreases in pH values with increasing moisture contents. Results indicate the possibility of the use of the bioconverted wastes as feeds for poultry and other animals after appropriate toxicological studies.

Key words: Protein, Enrichment, Mono, Co-cultures, Amylolytic fungi.

Nigerian commonly cultivated yams belong to the family Dioscoreceae and genus Dioscorea. They serve as staple food in many tropical countries of the world (Coursey, 1967). Nigeria is known to account for about 50% of global output, about 22million tonnes (FAO, 1989). The commonly cultivated varieties are *Dioscorea rotundata* (white yam), *Dioscorea cayenesis* (yellow yam) and *Dioscorea alata* (water yam). Apart from these there are other wild types that are also good for consumption Okigbo and Nmeka, 2005).

Yams as good source of carbohydrate are peeled and processed in various ways before eating. The processing methods depend on the taste of the consumer at a particular time. It may be by frying (yam chips), boiling for eating, pounding (pounded yam) or as yam pudding. As a result many end products are obtained (Okigbo and Ikediugwu, 2000). These wastes which are mainly yam peels are usually dumped indiscriminately. They are low in protein and in some cases used to feed ruminant animals just as cassava wastes, but they still can be found littering the environment thereby constituting aesthetic problems.

The fact that wastes can be converted to other useful products through the action of microoganisms has been documented (Oyewole and Odunfa, 1988; Akpan and Ikenebomeh, 1995; Nwafor and Ejukonemu, 2004). The increasing desire to rare animals and poultry for domestic and commercial purposes has brought about a high cost of animal feeds in Nigeria today. There is therefore the need to explore other sources of cheaper and easily available protein rich animal feeds. Microbial proteins being cheaper because

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fast growth rate of microorganisms and smaller space required will be able to fill this gap. This can be achieved through bioconversion of yam wastes using appropriate microorganisms.

Bioconversion refers to the use live organisms often microorganisms to carry out chemical reaction that is more costly or not feasible biologically (Adrie *et al*., 2004). The organisms convert the substrates to chemically modified forms. Yam wastes can serve as good substrates for the production of protein rich feed stuffs using solid state cultivation. Similar wastes have been investigated (Yang *et al*., 1990).

The conventional use of yam wastes as feed stuffs is not a proper way of waste management as it does not solve the aesthetic environmental problems. It is therefore necessary that present day waste management procedures should apply product oriented bioconversion processes that yield products of desired quality that can be sold in high priced markets.

This research is therefore designed to study the bioconversion potentials of some amylolytic fungi of wastes from three varieties of yams for protein enrichment.

MATERIALS AND METHODS

Saccharomyces cerevisiae, Rhodotorula graminis, Mucor sp *and Rhizopus donosus* all of which show amylolytic activity were isolated from decaying yam wastes from three Nigerian yam varieties. These were *Dioscorea rotuntata* (white yam), *Dioscorea cayenensis* (yellow yam) and *Dioscorea alata* (water yam). They were identified using cultural and morphological characteristics (Harrigan and Mcance, 1976; Samson and Reenen-Hoekstra, 1988; Bounds *et al*., 1993).

Moulds were cultivated on potato dextrose agar slants while yeasts were cultivated on yeast/malt agar slants and both were incubated at 29±2°C. Before use, the moulds were inoculated into potato dextrose broth while yeasts were inoculated into yeast/ malt extract broth and incubated at 29± 2°C for 48h. Portions of each broth culture were adjusted to between 10^3 and 10^5 spores or cells/ml sterile deionised water

Yam wastes were obtained from three major restaurants in Obiaruku, Delta state, Nigeria, where pounded yam and boiled yam are sold. They were separately sun dried, ground into powder and further oven dried to constant mass at 104°C in the Department of Microbiology, Delta State University, Abraka. They formed the substrates for growth of the fungi. The substrates were prepared separately with 80g portion (powder) each of wastes from the various yam varieties in 250ml conical flasks to which $(NH_4)_2SO_4$(1.25g), urea(1.25g), and $KHPO_4$ (10g) were added. There solid substrates were autoclaved at 121°C for 15mins. and allowed cool before mixing with spores or cells of the microorganisms both as mono (single) and co-cultures (combinations). Sterile deionized water was added to the flasks to suitable moisture levels (63%) and incubated on a water-bath shaker (70rpm) for 5days at 29±20°C. To another set of similar experiments were added a second dose of the nitrogenous supplements after 48h incubation.

pH Determination

The pH was determined for each fermenting medium by mixing aliquots with deionized water (1:2 ratio) and dipping the electrode of Model 291MK2 (PyeUnicam, UK) pH meter into it after standardizing with phosphate buffer of pH 4.00. Readings were taken in triplicates (AOAC 2000) on the first day and after 5days.

Moisture Content Determination

Moisture content was determined by oven- drying 20g of sample portions at 104°C to constant mass(AOAC 2000). Readings were taken in triplicates.

Protein content Determination

Protein contents were determined using Micro-Kjeldal method as described by Melon and Pomeranz (1980), AOAC (2000).

Statistical Analyses

The data obtained were subjected to statistical analyses of mean, standard deviation and analysis of variance (ANOVA). The significant values were determined by t- distribution test using appropriate computer software (Ogbeibu, 2005).

RESULTS

Changes in pH, moisture and protein contents of wastes from *D. rotundata* (white yam), *D. cayenensis* (yellow yam) and *D. alata* (water yam) fermented with amylolytic fungi are shown in Table 1 - 4. Generally, there were decreases in pH

values while water and protein contents increased in all the experiments at 29±20°C as recorded on the 5th day.

The results obtained when *S. cerevisiae, Rhodotorula graminis, Mucor* sp and *Rhizopus donosus* were inoculated as mono cultures are shown in tables 1-3.*Rhizopus donosus* produced the highest amount of protein, 8.60±04 % (w/w) with *D. rotundata*(white yam) while the lowest, 8.03±0.20% (w/w) was obtained with *Rhodotorula graminis* (Table 1). The trend was the same with *Rhizopus donosus* on *D. cayenensis* (yellow yam) where the highest protein content of 9.080.4% (w/w) was obtained while *Rhodotorula graminis* produced the lowest, 8.00±0.30% (w/w) as in Table 2. *Mucor* sp produced the highest protein content of 10.00±0.30% (w/w) when the organisms were grown on *D. alata* (water yam) waste substrate with *S. cerevisiae* producing the lowest quantity of protein, 8.53±0.20% (w/w).

Addition of nitrogenous supplements after fermentation for 48hs resulted in higher protein yield on the 5th day in all the experiments (Table 1-3). *Rhizopus donosus* grown on *D. rotundata* waste produced the highest amount of protein, 9.05±0.03% (w/w) while the lowest of 8.03± 0.20 %(w/w) was obtained with *Rhodotorula graminis* (Table 1). Similarly, the

Table 1. pH, Moisture Protein contents of wastes from *D. rotundata* (white yam) after growth of four fungi species for 5days at 29±20° C

Organisms	Fermentation Time in Days					
	pH moisture content %(w/w)		Protein content (%w/w)			
	1	5	1	5	1	5
S.cerevisiae	5.50±0.10	3.30±0.20	63.00±0.50	65.00±0.40	3.82±0.20	8.46±0.30
	#	3.60±0.20		67.00±0.30		8.06±0.1
R.graminis	5.50±0.10	3.35±0.10	63.00±0.50	65.50±0.20	3.82±0.20	8.03±0.2
	#	3.62±0.20		69.10±0.30		9.10±0.2
*Mucor*sp	5.50±0.10	3.41±0.30	63.00±0.50	64.80±0.30	3.82±0.20	8.83±0.4
	#	3.28±0.10		66.30±3.00		8.69±0.10
R.donosus	5.50±0.10	3.29±0.30	63.00±0.50	65.60±0.50	3.82±0.20	8.60±0.04
	#	3.10±0.02		66.30±0.30		9.60±0.03

Note: Each value is mean ± standard deviation of triplicate determinations.
=Addition of nitrogenous supplement after 48hs.

Table 2. pH, Moisture and Protein contents of wastes from yellow yam (*Dioscoreacayenesis*) after growth of four fungi species for 5days at 29±20°C

Organisms	Fermentation Time in Days					
	pH moisture content %(w/w)			Protein content (%w/w)		
	1	5	1	5	1	5
S.cerevisiae	5 .50±0.10	3.32±0.06	63.0±0.50	64.80±0.50	3.82±0.20	8.53±0.05
#		3.42±0.05		67.70±0.04		9.01±0.03
R. graminis	5.50±0.10	3.36±0.02	63.0±0.50	64.40±0.03	3.82±0.20	8.00±0.03
#		3.63±0.03		65.30±0.02		10.10±0.05
Mucorsp	5.50±0.10	3.41±0.02	63.0±0.05	65.60±0.05	3.82±0.20	9.30±0.40
#		3.30±0.10		66.50±0.01		10.20±0.30
R. donosus	5.50±0.10	3.29±0.30	63.0±0.4	65.60±0.30		10.30±0.20
#		3.26±0.20		65.30±0.30		10.30±0.20

Note: Each value is mean ± standard deviation of triplicate determinations.
=Addition of nitrogenous supplement after 48hs.

highest value of 10.30±0.12% (w/w) was obtained from *Rhizopus donosus* on *D. cayenensis* (yellow yam) with the lowest of 9.01±0.03 % (w/w) obtained from *S. cerevisiae* (Table 2). *Mucor* sp on the hand produced the highest amount of protein,10.40 ±0.02 (w/w) as in table 3.

The pH, moisture and protein contents of wastes from the three yam varieties fermented withco-cultures (combinations) of the fungi species are shown in Table 4-6. There were appreciable increases in the protein contents compared with when the organisms were inoculated individually. Generally, higher protein values were obtained where two moulds were inoculated together. For instance, a combination of *Rhizopus donosus* and *Mucor* sp generated a protein content of 11.50±0.20 % (w/w) on *D. rotundata* (Table 4),11.45±0.50% (w/w) *D. cayenensis* (Table 5) and11.65±0.50% (w/w) on *D.alata* (Table 6) compared with when two yeast species are inoculated together.

In all cases, there were gradual decreases in pH values with concomitant increases in moisture and protein contents after 5 days of fermentation. The results revealed that pH decreased as moisture and protein contents increased.

Table 3. pH, Moisture and Protein contents of wastes from water yam *(Dioscorea alata)* after growth of four fungi species for 5days at 29±20°C

Organisms	Fermentation Time in Days					
	pH moisture content %($^{w}/_{w}$)			Protein content (%$^{w}/_{w}$)		
	1	5	1	5	1	5
S.cerevisiae	5 .50±0.10	3.33±0.02	63.00±0.50	65.20±0.04	3.82±0.20	8.52±0.30
#		3.30±0.10		66.30±0.30		9.60±0.30
R. graminis	5.50±0.10	3.36±0.50	63.0±0.50	65.0±0.10	3.82±0.20	8.62±0.3
#		3.60±0.4		64.0±0.30		9.30±0.4
Mucorsp	5.50±0.10	3.70±0.20	63.0±0.50	65.10±0.5	3.82±0.20	10.10±0.5
#		3.50±0.11		66.12±0.13		10.40±0.20
R. donosus	5.50±0.10	3.50±0.20	63.0±0.50	68.14±0.20	3.82±0.20	8.95±0.4
#		3.32±0.02		68.50±0.10		9.60±0.20

Note: Each value is mean ± standard deviation of triplicate determinations.
=Addition of nitrogenous supplement after 48hs.

Table 4. pH, Moisture and Protein contents of wastes from white yam *(Dioscorea rotundata)* after growth of four fungi species for 5days at 29±20°C

Organisms	Fermentation Time in Days					
	pH moisture content %($^{w}/_{w}$)			Protein content (%$^{w}/_{w}$)		
	1	5	1	5	1	5
S.cerevisiae&	5 .50±0.10	3.0±0.10	63.0±0.50	66.30±0.4	3.82±0.20	10.30±0.10
R.donosus #		2.90±0.20		65.0±0.40		10.60±0.20
R. cerevisieae&	5.50±0.10	2.60±0.30	63.0±0.50	67.0±0.30	3.82±0.20	9.90±0.2
R. graminis#		2.50±0.40		68.0±0.20		10.10±0.30
R. donosus&	5.50±0.10	2.40±0.2	63.0±0.50	68.00±0.2	3.82±0.2	10.20±0.30
Mucorsp #		2.30±0.10		69.30±0.30		10.50±0.20
R. graminis&	5.50±0.10	2.30±0.03	63.0±0.50	68.30±0.30	3.82±0.20	10.10±0.20
Mucorsp #		2.20±0.02		69.20±0.30		10.40±0.20

Note: Each value is mean ± standard deviation of triplicate determinations.
=Addition of nitrogenous supplement after 48hs.

Table 5. pH, Moisture and Protein contents of wastes from yellow yam (*Dioscorea caryenesis*) after growth of combination of different fungi species for 5days at 29±20°C

Organisms	Fermentation Time in Days					
	pH moisture content %(w/w)			Protein content (%w/w)		
	1	5	1	5	1	5
S.cerevisiae&	5 .50±0.10	3.20±0.2	63.0±0.50	66.30±0.4	3.82±0.20	10.31±0.10
R.donosus #		3.10±0.3		67.10±0.4		9.90±0.10
R. cerevisiae&	5.50±0.10	2.50±0.01	63.0±0.50	66.50±0.30	3.82±0.2	10.31±0.20
R. graminis#		2.40±0.02		67.50±0.4		11.0±0.20
R. donosus&	5.50±0.10	2.45±0.3	63.0±0.50	68.30±0.10	3.82±0.2	10.10±0.10
Mucorsp #		2.30±0.10		64.10±0.40		11.40±0.50
R. graminis&	5.50±0.10	2.20±0.02	63.0±0.50	67.10±0.2	3.82±0.2	10.60±0.3
Mucorsp #		2.10±0.03		68.10±0.2		11.00±0.2

Note: Each value is mean ± standard deviation of triplicate determinations.
=Addition of nitrogenous supplement after 48hs.

Table 6. pH, Moisture and Protein contents of wastes from water yam *(Dioscorea alata*) after growth of combination of different fungi species for 5days at 29±20°C

Organisms	Fermentation Time in Days					
	pH moisture content %(w/w)			Protein content (%w/w)		
	1	5	1	5	1	5
S.cerevisiae&	5 .50±0.10	3.30±0.3	63.0±0.50	66.50±0.30	3.82±0.20	10.10±0.10
R.donosus #		3.10±0.3		67.00±0.20		11.20±0.40
R. cerevisiae&	5.50±0.10	2.60±0.02	63.0±0.50	65.80±0.3	3.82±0.20	9.90±0.30
R. graminis#		2.50±0.2		66.40±0.20		10.40±0.40
R. donosus&	5.50±0.10	2.60±0.2	63.0±0.50	68.12±0.20	3.82±0.2	9.93±0.30
Mucorsp #		2.52±0.10		64.10±0.40		11.65±0.5
R. graminis&	5.50±0.10	2.30±0.02	63.0±0.50	68.80±0.20	3.82±0.2	9.93±0.3
Mucorsp #		2.10±0.40		69.10±0.50		

Note: Each value is mean ± standard deviation of triplicate determinations.
=Addition of nitrogenous supplement after 48hs.

DISCUSSION

One major benefit derivable from bioconversion of wastes is that it get rid of wastes that would otherwise constitute nuisance in the environment. Apart from this, high protein rich feedstuffs can be obtained at a cheaper rate from them. This fact has already been documented some other wastes (Anupama and Ravinda, 2001, Esenwah and Ikenebomeh, 2008).

This study has revealed the capacity of *Saccharomyces cerevisiSae, Rhodotorula graminis, Rhizopus donosus* and *Mucor* sp to convert wastes from three popular Nigerian yam varieties namely *Dioscorea rotundata* (white yam), *Dioscorea cayenensis* (yellow yam) and *Dioscorea alata* (water yam) to protein rich feed stuff during solid state fermentation at 29±2°C in 5days. These organisms are known to be able to elaborate the enzyme amylase which aids in the breakdown of the wastes leading their growth and production of crude protein in the growth medium. Similar results have been earlier obtained (Yang and Yaun, 1990, Nwafor and Ejukonemu 2004). The ability of moulds to produce high amount of crude protein maybe due to their filamentous nature. They are able to

penetrate the medium and spread through the solid substrate leading good growth and protein formation. This in line with earlier report by Yang *et al.,* (1993) on the bioconversion of cassava wastes. On the other hand, that yeasts could cause the formation of appreciable amount of protein within the period may be due to their short generation time (fast multiplication). Yeasts have been shown to produce protein from starchy materials although not as high as with moulds (Yang and Yaun, 1990) as now established in the present report.

The observed increase in protein contents when co-cultures of the organisms were inoculated is an indication of synergism among them since the values were not double those obtained with singular treatments.

Increase in moisture content could be an indication of production of metabolic water or release of water as a result of carbohydrate oxidation. The increase was however, within the limit that would not allow for anaerobic growth to occur. These agree with previous reports by Wang (1981);Yang (1988).

Progressive decreases in pH values may be attributed to the release metabolic products such as organic acids. This corroborates the reports (Ogiehor*et al*., 2004, Ogiehor and Nwafor, 2004).

In conclusion, this study has shown that protein content of *D. rotundata* (white yam), *D. cayenensis* (yellow yam) and *D. alata* (water yam) wastes can increased using some amylolytic fungi. The products so obtained would be suitable as feeds for poultry and other animals after proper toxicological studies

REFERENCES

1. Adrie, V., Vinnie, W., Hans, W. and Hamelers, B., Advanced bioconversion of biowaste for production ofa peat substitute and renewable energy. *Bioresource Technology* 2004; **92**: 121-131.
2. Akpan, I. and Ikenebomeh, M.J., Glutamic acid fermentation. *Nigerian Journal of Microbiology* 1995; **10**: 60-65.
3. Anapuma, E. and Ravinda, P., Studies on single cell protein by *Aspergillusniger* in solid state fermentation of rice bran Braz-Arch. *Biol. Technol*. 2001; **44**:79-88.
4. AOAC., Official methods of analysis. Association of Official Analytical Chemists (17thedn.). Washington D.C. USA., 2000; 1298pp.
5. Bounds,H. C.,Boyd, F.M. and Norman, J. R. A., Laboratory Exercises in general Microbiology, Cambridge University Press 1993; pp20-66.
6. Coursey, D. G., Yam storage 1. A review of storage practices and information on storage losses. *Journal of Stored Product Research* 1987; **22**: 227-244.
7. Esenwah, C.N. and Ikenebomeh, M.J., Processing effects on the nutritional and anti-nutritional contents of African locust bean (*Parkiabiglobosa*Benth) seed.*Pakistan Journal of Nutrition* 2005; **7**(2): 214-217.
8. FAO., Roles of root tuber crops in food security in sub-saharaAfrica. *Food production year book* 1989; **43**:14.
9. Harrigan, W.F., and Mcance,M.E., Laboratory methods in food and dairy Microbiology. Academic Press London. 1976; 41pp.
10. Meloan, C. E. and Pomeranz, Y.., Food analysis Laboratory Experiments (2ndedn.).Westport,CT. Avi Publishing, 1980.
11. Nwafor, O.E. and Ejukonemu, F.E., Bioconversion of cassava wastes for protein enrichment using Amylolytic fungi-Apreliminary report. *Global Journal of Pure and Applied Sciences* 2004; **10**(4):505-507.
12. Ogbeibu, A. E., Biostatistics. A practical approach of research and data handling. Mindex Publishing Company ltd. Benin- city 2005; 264pp.
13. Ogiehor, I.S. and Nwafor, O. E., Associated microbiological biochemical and chemical changes in zobo beverage production from *Hibiscus sabdariffa*-Linn. *Nigerain Annals of Natural Sciences* 2004; **5**(2):1-10.
14. Ogiehor, I. S., Enaigbe, E. and Okwu, G. I, The effect of different packaging materials and seasonal variations on the quality changes of tapioca (kpokpogarri) produced from cassava (*Manihotesculanta*- crantz) during storage. *Nigerian Annals of Natural Sciences* 2004; **5**: 16-25.
15. Okigbo, R. N. and Ikediugwu, F.E. O., Studies on the biological control of tuber surface microflora of yams (*Dioscorearotundata*). *Tropical Science* 2000; **41**: 85-89.
16. Okigbo, R. N. and Nmeka, I. A., Control of yam tuber rot with leaf extracts of*Xylopiaaethiopica* and *Zingiberofficinale*. *African Journal of Biotechnology* 2005; **4**(8): 804-807.
17. Oshoma, C. E. and Ikenebomeh, M.J.,

Production of *Aspergillusniger*biomas from rice bran.*Pakistan Journal of Nutrition* 2005; **4**(1): 32-36.

18. Oyewole, O. B.andOdunfa, S.A., Microbiological studies on cassava fermentation for 'lafun' production. *Journal of Food Microbiology* 1988; **5**:125-133.
19. Samson, R. A. and Reenen-Hoekstra, E. S., Introduction to food-borne fungi (3rdedn.). Central bureau vourSchimel culture Baarn, The Netherlands, 1988.
20. Wang H. H., Water absorption characteristics ofcellulosic wastes and solid-state fermentation. In: Proceedings of thesecond world congress of Chemical Engineering, 297-300. Montreal Canada Society of Chemical Engineering, 1981.
21. Yang, S. S. and Yaun, S. S., Oxytetracycline production by *Streptomyces rimosus*in solid-state fermentation of sweet potato residue. World *Journal of Microbiology and Biotechnology* 1990; **6**: 236-244.
22. Yang,S. S., Jang, H. H., Liew, C. M., and Perez, J. C., Proteinenrichment of sweet potato residue by solid-state cultivation with mono and co-cultures of amylolytic fungi. *World Journal Microbiology and Biotechnology* 1993; **6**: 336-244.