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PROTEIN ENHANCEMENT OF YAM (*DIOSCOREA ROTUNDATA*) PEELS WITH SINGLE- OR CO-INOCULATION OF *ASPERGILLUS NIGER* VAN TIEGHEM AND *TRICHODERMA VIRIDE* PERS EX FR. UNDER SOLID-STATE FERMENTATION

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ABSTRACT

This study assessed the protein enrichment of sterilized and non-sterilised yam peels substrates fermented for 21 days at 25°C with mono- and co-cultures of *Aspergillus niger* and *Trichoderma viride*. Yam substrates were harvested at 0, 7, 14, and 21 days intervals for protein content and other chemical composition analyses. Results showed an overall percentage increase in protein contents of sterilised yam peels by 71.80% for *A. niger*, 58.03% for *T. viride*, and 80.60% for co-culture of *A. niger* and *T. viride*. Protein contents in non-sterilised yam peels increased by 113.30%, 95.00%, and 96.45% for *A. niger*, *T. viride* and co-culture of the test fungi, respectively. The significantly ($p \leq 0.05$) higher protein contents of the fermented, non-sterilised yam peels suggest possible successional microbial colonization of the substrate, and their combined, cumulative contributions to protein enhancement, unlike the sterilised yam peels. Ash content significantly ($p \leq 0.05$) increased in both sterilised and non-sterilised yam peels. These findings underscore the fact that, through fungal bioprocessing, protein contents of yam peels can be significantly enriched for value-addition. The practical implications of the findings are discussed.

Keywords: *Aspergillus niger*, *Dioscorea rotundata* peels, Protein content, Solid-state fermentation, *Trichoderma viride*

Introduction

Yam is cultivated for the tubers (storage organs), which may be subterranean (e.g. *Dioscorea rotundata*), or aerial (e.g. *Dioscorea bulbifera*), serving dual agricultural functions as source of food and planting material (Hahn, 1995; Ile *et al*., 2006). The most important species of yams in West Africa are *D. rotundata* and *D. alata* (Ile *et al*., 2006). It is one of the most important tuber crops cultivated in the world, particularly in Africa, Asia, the Americas, and the South Pacific (Aruna *et al*.,

2017). As a crop of cultural importance and prestige in West Africa, yam is consumed on a large scale by millions of people as a major source of nourishment and livelihood (Martin & Sadik, 1977). Nigeria remains the largest producer of yams in the world; between 2010 and 2018, it produced an annual average of 41.5 million metric tonnes, followed by Cote d'Ivoire and Ghana with 7.0 and 6.2 million metric tonnes, respectively (FAO STAT, 2018). Yams produced in these West African countries and other parts of Africa are processed into different food products. For example, in Ghana, yams are fried, roasted, boiled or pounded and consumed with accompaniments like fried fish, sauce, peanut, or soup. Before yam is processed into these and other processed food products, they are peeled, generating, in the process, large quantities of yam peels that are indiscriminately disposed of into the environment. The discarded yam peels are then left to rot, becoming environmental pollutants and nuisance with resultant health threats to animals and humans (Adu *et al*., 2018; Sadh *et al*., 2018; Yafetto, 2018; Yafetto *et al*., 2019).

Discarded yam peels, poor in protein contents, are typically carted away by some animal breeders and used as livestock feed, because they are cheap compared to imported conventional protein-enriched feed (Yafetto, 2018). The application of solid-state fermentation using fungi continues to be the most simple, but effective, easy-to-use biotechnological technique employed to convert agro-lignocellulosic residues of discarded agro-industrial wastes such as yam peels into value-added, protein-enriched products (Sadh *et al*., 2018; Yafetto, 2018). These protein-enriched products are potential solutions to inadequate animal nutritional needs of consumers around the world. Thus, the application of solid-state fermentation to convert agro-industrial wastes into protein-enriched feed is beneficial in two ways: (i) it curtails environmental pollution, and (ii) it improves the nutritional quality of animal feed, which consequently improves nutrition in humans (Aruna *et al*., 2017).

Fungi, "*Generally Regarded As Safe"* (GRAS), because they do not usually produce metabolites of human health hazard, are important for application in solid-state fermentation, as a result of their traditional role in fermentation, and improvement of nutritional value of foods (Nasseri *et al*., 2011; Aruna *et al*., 2017). Studies have shown the potential for peels of some agro-industrial produce as suitable substrates for value-addition through solid-state fermentation using non-toxic fungi which fall into the category of GRAS (Iyayi & Losel, 2001; Akintomide & Antai, 2012; Dawish *et al*., 2012; Ezekiel & Aworh, 2013; Azam *et al*., 2014; Aruna *et al*., 2018; Maxwell *et al*., 2019). Few studies have focused on yam peels in the West African sub-region (Aruna *et al*., 2017; Aruna *et al*., 2018). Our group had previously studied protein enrichment of substrates such as cassava, watermelon, sweet potato, and pineapple wastes using *Aspergillus niger* and *Trichoderma viride* (Adu *et al*., 2018; Yafetto, 2018; Yafetto *et al*., 2019). There is paucity of information in Ghana on the potential use of other agricultural lignocellulose-like peels for enrichment of protein contents using fungal biotechnology. This study, therefore, sought to evaluate protein enrichment of yam peels by solid-state fermentation with mono- and co-cultures of *Aspergillus niger* and *Trichoderma viride* with the aim to developing a value-added product using fungal biotechnology. Findings from this study could confirm or approve the biotechnological potential of yam peels to improving protein contents by both *A. niger* and *T. viride*. Furthermore, the findings could serve as a springboard to pursue broader biotechnological studies involving the use of other agro-industrial wastes currently serving

as environmental pollutants, but which could be used beneficially to improve nutrient contents using either inoculation with single- or co-cultures of fungi placed in the category of GRAS.

Experimental

Study Design

This study followed the procedure of Yafetto *et al*. (2019), and it involved (i) collection and processing of yam peels, (ii) isolation and maintenance of pure cultures of *A*. *niger* and *T*. *viride* (ii) preparation of spore suspension of *A*. *niger* and *T*. *viride*, (iii) preparation, inoculation, and fermentation of yam peels, and (iv) proximate analyses of fermented yam peels.

Cultures of Aspergillus niger and Trichoderma viride

Yam (*Dioscorea rotundata*) tubers obtained from a local market in Cape Coast, Ghana, were peeled (peridium and cortex removed) and the fleshy tissue were cut into smaller cubes (1 cm x 1 cm). The cubes were exposed for 10 minutes in the sun and incubated in a bowl under ambient humid condition in the laboratory until they were colonized by fungi. *A*. *niger* colonizing the yam cubes was isolated and pure culture maintained on Potato Dextrose Agar (PDA) medium (200 g Irish potato; 20 g dextrose; 20 g agar; 1000 ml distilled water; autoclaved at a pressure of 1.1 kg/cm^2 at 121°C for 15 minutes) at 25°C – 30°C for 7 days. *Trichoderma viride* was obtained following the method of Yafetto *et al*. (2019). *A*. *niger* and *T*. *viride* isolated were identified based on their morphological features with the aid of identification manuals (Barnett & Hunter, 1995; Davet & Rouxel, 2000; Ellis *et al*., 2007; Pitt & Hocking, 2009; Watanabe, 2010; Campbell *et al*., 2013). Pure cultures of both *A*. *niger* and *T*. *viride* were maintained on PDA and *Trichoderma*-Selective Medium slants in McCartney tubes at 4°C, and sub-cultured fortnightly to preserve their viability until ready for use.

Preparation of conidial suspensions of Aspergillus niger and Trichoderma viride

Potato Dextrose Broth (PDB) amended with nutrient elements were prepared based on method described by Adu *et al*., (2018). The PDB-amended broth was used to prepare conidial suspensions of only *A*. *niger*, only *T*. *viride*, and a mixture of *A*. *niger* and *T*. *viride*, as previously described (Guarro *et al*., 1998; Yalemtesfa *et al*., 2010; Olorunnisola *et al*., 2017). Conidial suspensions of either *A*. *niger*, or *T*. *viride* and their mixture were used to inoculate yams peels for subsequent fermentation studies as described by Yafetto *et al*., (2019).

Preparation of yam peels for fermentation

Yam peels obtained after peeling the yam tubers were thoroughly washed under running tap water to rid them of dirt and debris, spread on aluminum trays and sun-dried in the open for 4 days. The yam peels were then oven-dried at 60°C for 24 hours. The dried yam peels obtained were pulverized with pestle and mortar and sieved to obtain a fine powder using 3.35–4.00 mm size sieve mesh. The powder was divided into two equal portions of 200 g each. One portion divided into 50 g each were placed into four 250 ml Erlenmeyer flasks and sterilized by autoclaving at a pressure of 1.1 Kg/cm^2 at 121°C for 15 minutes. The remaining portion, also divided into 50 g each and dispensed in four 250 ml Erlenmeyer flasks, were not sterilized. The sterilised and non-sterilised yam peel substrates were then prepared for fermentation.

Fermentation of yam peels powder and protein content estimation

Yam substrates adjusted to moisture content of 50% (w/v) were inoculated with spore suspension of *A. niger* only and incubated for 21 days under conditions as described by Yafetto (2018), Yafetto *et al*., (2019) and Correia *et al*. (2007) for solid substrate fermentation. The fermentation processes were repeated for mono-culture of *T. viride* and co-cultures of *A. niger* and *T. viride.* The conventional Kjeldahl method (Kjeldahl, 1883) was used to determine the initial nitrogen (N_2) content of the substrate (unfermented) and subsequently after 7, 14, and 21 days of fermentation. Preparation of yam peels and the subsequent fermentations were repeated for mono-culture of *T. viride* and co-cultures of *A. niger* and *T. viride*. The nitrogen contents were analysed in triplicates. The nitrogen contents, thus, determined after 0, 7, 14, and 21 days of fermentation of the yam substrate were used to estimate the percentage protein contents of the substrates by using % N_2 x 6.25 (where 6.25 is the protein conversion factor). Percentage increase in protein contents of the fermented yam peels was subsequently calculated as follows:

$$\textbf{Percentage increase in Protein Content (\%)} = \frac{\textbf{Final Protein Content} - \textbf{Initial Protein Content}}{\textbf{Initial Protein Content}} \times 100\%$$

Proximate composition analyses of sterilised and non-sterilised yam peels

Following the method of Yafetto *et al*., (2019), 20 g fermented yam samples were dispensed into sterile porcelain crucibles and heated at 105°C in a Sanyo Gallenkamp OMT benchtop oven for 48 hours, then placed in a desiccator to cool before weighing. The percentage moisture content of the yam samples was estimated as the difference in weight. The proximate analyses for dry matter, ash, carbohydrate, crude fibre, and fat contents of the yam peels substrates were performed in triplicates according to AOAC (2005) as detailed by Yafetto *et al*., (2019).

Statistical analysis

One-Way Analysis of Variance (ANOVA) was performed on the data using the Statistical Package for Social Sciences (SPSS) Version 25.0. Means were separated using Tukey post-hoc test at 95% confidence level ($p \leq 0.05$; $p \leq 0.001$). Final results were expressed as means ± standard deviation (SD).

Results

Protein enrichment of sterilised, fermented yam peels substrate

The initial protein content (%) of sterilised, unfermented yam peels was 7.72±0.29 at day 0 (Table 3). Surprisingly, the protein content of the substrate when inoculated with *T. viride* decreased ($p \leq 0.05$) to 6.60±0.03 after 7 days of fermentation (Table 3). It then increased significantly ($p \leq 0.05$) to 12.20±0.07 after 21 days. Similarly, after 21 days of fermentation, there was asignificant increase in the mean protein contents of sterilised substrates inoculated with *A. niger* (13.26±0.10) and co-culture of *A. niger* and *T. viride* (13.94±0.09) (Table 3) and the corresponding increase in protein contents were 71.80, 58.03 and 80.60%, respectively (Table 1).

Protein enrichment of non-sterilised, fermented yam peels substrate

The initial protein content (%) was 6.77±0.32 at day 0 (Table 4). After 21 days of fermentation, the protein contents of the substrate inoculated with *A. niger*, *T. viride* and co-cultures of *A.*

niger and *T. viride* increased significantly ($p \leq 0.05$) to 14.44±0.21, 13.19±0.12, and 13.29±0.03, respectively (Table 4), with corresponding increase in protein contents of 113.30, 95.00 and 96.45%, respectively (Table 1).

Comparative Analyses of Protein Enrichment of Sterilised and Non-sterilised Yam Peels Substrate

Protein contents of the yam substrates were greatly enriched by the fermentation treatments of the test fungi. However, comparative analyses among the fungal treatments of sterilised substrates showed that fermentation with co-culture of *A. niger* and *T. viride* significantly ($p \leq 0.001$) enriched the protein content higher than fermentation by mono-cultures of *A. niger* and *T. viride* (Table 2). For non-sterilised substrates, fermentation with mono-culture of *A. niger* significantly ($p \leq 0.001$) enriched the protein content better than the mono-culture of *T. viride* and co-culture of *A. niger* and *T. viride* (Table 2).

Proximate analyses of sterilised yam peels substrate

After 21 days, changes in the chemical composition of sterilised, fermented yam peels treated with *A. niger* showed significant ($p \leq 0.05$) decrease in fat content, marginal increase in crude fibre and carbohydrate, and significant ($p \leq 0.05$) increase in ash content (Table 3). For *T. viride*, there was a significant increase in both fat and ash contents after fermentation (Table 3). In yam peels fermented with co-culture of *A. niger* and *T. viride*, there was no significant ($p \leq 0.05$) increase in crude fibre, but significant ($p \leq 0.05$) decrease in carbohydrate; there were, however, significant ($p \leq 0.05$) increases in both fat and ash contents (Table 3).

Proximate analyses of non-sterilised yam peels substrate

Changes in the chemical composition of non-sterilised, fermented yam peels treated with *A. niger* for 21 days showed that there was a significant ($p \leq 0.05$) decrease in fat content (Table 4). Crude fibre content recorded no significant change after the fermentation process with *A. niger*, although there was a significant ($p \leq 0.05$) decrease in carbohydrate and increase in ash contents (Table 4). For *T. viride*, there was a significant ($p \leq 0.05$) decrease in crude fibre, carbohydrate, and fat contents, except ash content that recorded a significant increase after fermentation (Table 4). In yam peels fermented with co-culture of *A. niger* and *T. viride*, there was a significant ($p \leq 0.05$) decrease in crude fibre, carbohydrate, and fat contents; there was a significant ($p \leq 0.05$) increase in ash content after fermentation (Table 4).

TABLE 1

Percentage (%) increase in protein content of yam peels after 7, 14, and 21 days of fermentation

	Percentage increase in protein content								
	A. niger			***T. viride***			***A. niger* and *T. viride***		
Nature of substrate	**7 Days**	**14 Days**	**21 Days**	**7 Days**	**14 Days**	**21 Days**	**7 Days**	**14 Days**	**21 Days**
Sterilised yam peels	15.93	18.52	71.80	-14.51	28.37	58.03	60.75	31.50	80.60
Non-sterilised yam peels	40.03	62.78	113.30	21.71	34.86	95.00	40.62	48.15	96.45

TABLE 2

Comparative protein content of sterilised and non-sterilised yam peels after 21 days of fermentation with mono-cultures of A. niger, T. viride, and co-culture of A. niger and T. viride. [a-c]*Means within a column with different superscripts are significantly different (p≤0.001).*

Results expressed as mean (n=3) ± SD (Standard deviation).

	Protein content of yam peels	
Fungal culture	**Sterilised**	**Non-sterilised**
A. niger	13.26±0.10[a]	14.44±0.21[a]
T. viride	12.20±0.07[b]	13.19±0.11[b]
A. niger and *T. viride*	13.94±0.09[c]	13.28±0.03[b]

TABLE 3

Changes in the chemical composition of sterilised, fermented yam peels with mono-cultures of A. niger or T. viride, and a co-culture of A. niger and T. viride (data presented are based on the dry matter). [a-d]*Means within a column with different superscripts are significantly different (p ≤ 0.05).*

Results expressed as mean (n=3) ± SD (Standard deviation).

		Chemical composition (%)						
Fungal culture	**Day**	**Dry Matter**	**Moisture**	**Ash**	**Fat**	**Protein**	**Crude fibre**	**Carbohydrate**
T. viride	0	51.56±1.13[a]	48.44±1.13[a]	2.67±0.30[a]	0.70±0.01[a]	7.72±0.29[a]	5.81±0.30[a]	76.76±0.49[a]
	7	47.37±0.28[b]	52.63±0.28[b]	2.81±0.43[a]	0.85±0.05[b]	6.60±0.03[b]	5.82±0.30[a]	83.91±0.53[b]
	14	34.23±0.30[c]	65.77±0.33[c]	2.81±0.17[a]	0.79±0.02[b]	9.91±0.03[c]	6.02±0.01[a]	80.47±0.15[c]
	21	26.77±0.36[d]	73.23±0.36[d]	4.85±0.17[b]	0.79±0.03[b]	12.20±0.07[d]	5.97±0.04[a]	76.19±0.29[a]
A. niger	0	51.56±1.13[a]	48.44±1.13[a]	2.67±0.30[a]	0.70±0.01[a]	7.72±0.29[a]	5.81±0.30[a]	76.76±0.46[a]
	7	39.79±0.28[b]	60.21±0.28[b]	2.38±0.03[a]	0.49±0.02[b]	8.95±0.09[bc]	6.66±0.09[b]	81.52±0.19[b]
	14	30.29±0.15[c]	69.71±0.15[c]	3.38±0.14[b]	0.47±0.02[b]	9.15±0.06[c]	6.02±0.02[a]	80.97±0.14[b]
	21	25.40±0.28[d]	74.60±0.28[d]	3.11±0.14[b]	0.48±0.04[b]	13.26±0.10[d]	6.09±0.06[ac]	77.06±0.11[a]
A. niger and *T. Viride*	0	51.56±1.13[a]	48.44±1.13[a]	2.67±0.30[a]	0.70±0.01[a]	7.72±0.29[a]	5.81±0.30[a]	76.76±0.49[a]
	7	39.96±0.47[b]	60.04±0.47[b]	2.49±0.18[a]	0.91±0.02[b]	12.41±0.10[b]	5.64±0.07[a]	78.76±0.20[b]
	14	25.28±0.33[c]	74.72±0.33[c]	2.83±0.15[a]	0.80±0.01[c]	10.15±0.00[c]	5.66±0.09[a]	80.55±0.14[c]
	21	28.63±0.31[d]	71.37±0.31[d]	3.88±0.19[b]	0.75±0.02[d]	13.94±0.09[d]	5.95±0.05[a]	75.48±0.17[d]

TABLE 4

Changes in the chemical composition of non-sterilised, fermented yam peels with mono-cultures of A. niger or T. viride, and a co-culture of A. niger and T. viride (data presented are based on the dry matter). [a-d]Means within a column with different superscripts are significantly different ($p \leq 0.05$). Results expressed as mean (n=3) ± SD (Standard deviation).

		Chemical composition (%)						
Fungal culture	Day	Dry Matter	Moisture	Ash	Fat	Protein	Crude fibre	Carbohydrate
T. viride	0	52.40±0.85[a]	47.60±0.85[a]	2.63±0.24[a]	0.66±0.02[a]	6.77±0.32[a]	6.11±0.21[ac]	77.93±0.52[a]
	7	44.26±0.44[b]	55.74±0.44[b]	3.30±0.20[a]	0.14±0.00[b]	8.24±0.74[b]	6.69±0.09[b]	80.38±0.85[b]
	14	38.71±0.41[c]	61.29±0.41[c]	2.85±0.06[b]	0.12±0.00[bc]	9.13±0.22[b]	6.04±0.06[c]	80.81±0.10[b]
	21	36.12±0.76[d]	63.88±0.76[d]	3.76±0.05[c]	0.11±0.00[c]	13.19±0.12[c]	5.99±0.22[c]	76.00±0.13[c]
A. niger	0	52.40±0.85[a]	47.60±0.85[a]	2.63±0.24[a]	0.66±0.02[a]	6.77±0.32[a]	6.11±0.21[a]	77.93±0.52[a]
	7	43.62±0.34[b]	56.38±0.34[b]	3.28±0.17[b]	0.12±0.00[b]	9.48±0.13[b]	5.98±0.04[a]	80.09±0.18[b]
	14	31.29±0.25[c]	68.71±0.25[c]	3.11±0.09[bc]	0.11±0.00[b]	11.02±0.07[c]	6.06±0.06[a]	78.71±0.14[a]
	21	25.65±0.22[d]	74.35±0.22[d]	2.83±0.18[ac]	0.10±0.00[b]	14.44±0.21[d]	6.11±0.10[a]	75.59±0.20[c]
A. niger and T. viride	0	52.40±0.85[a]	47.60±0.85[a]	2.63±0.24[a]	0.66±0.02[a]	6.77±0.32[a]	6.11±0.21[a]	77.93±0.52[a]
	7	38.27±0.58[b]	61.73±0.58[b]	2.39±0.11[a]	0.12±0.01[b]	9.52±0.37[b]	5.49±0.03[b]	81.41±0.29[b]
	14	32.99±0.11[c]	67.01±0.11[c]	3.19±0.07[b]	0.11±0.01[b]	10.03±0.06[b]	5.96±0.06[ac]	79.71±0.09[c]
	21	27.61±0.43[d]	72.39±0.43[d]	3.19±0.08[b]	0.10±0.01[b]	13.29±0.03[c]	5.80±0.03[c]	76.68±0.03[d]

Discussion

The study of solid-state fermentation and its application for the production of a plethora of industrial products using, fungal technology, is now the order of the day. In the area of protein-rich animal feed for livestock rearing and soil bio-fertilizer application for increased vegetable cultivation, there is significant global efforts made to research into the use of solid-state fermentation; there is now industrial scale production of bioethanol, biodiesel, enzymes, organic acids, useful secondary fungal metabolite production as medicines, single-cell proteins and enzymes (Kavanagh, 2005). The increasing population in the West African region necessitates that microbes are exploited as a source of releasing proteins, fat, vitamin contents of lignocellulose for human and livestock consumption.

This present study has demonstrated that protein content of sterilised and non-sterilised yam peels can be enriched using mono- and co-cultures of *A. niger* and *T. viride* especially with the co-cultures of *A. niger* and *T. viride*. (Tables 1 – 4). *A niger* has long been of importance in the fermentation industry as principal source of citric acid and in the production of useful enzymes including lipases which breakdown fatty compounds (Carlile, *et al*., 2001). It is not surprising that in the fermentation process, the fat content decreased releasing metabolic products to be incorporated in the process of protein formation (Kavanagh, 2005). *T. viride* and *T.*

reesei produce a miscellany of enzymes, the cellulose complex (eg. cellobiohydrolase, 1, 4-*β*-glucans, endoglucananses, exoglucananses, 1,4-*β*-glucan hydrolases, cellubiose, etc.), which act in concert with the degradation of carbohydrates and starch into simple sugars (Carlile *et al*., 2001). *T. viride* is also lipolytic and might have played a role in the decline of carbohydrates and fat content of the substrate during fermentation (Table 4).

Recent studies have demonstrated that co-cultures of *A. niger* and *T. viride* synergistically enriched protein content of fermented cassava (*Manihot esculenta* Crantz) peel supporting the findings of this study of protein enrichment with yam peels (Yafetto *et al*., 2019). The pertinent literature show that the co-culture of *Pleurotus ostraetus* and *Saccharomyces cerevisiae* upgraded the nutritional protein status of maize stalk (Darwish *et al*., 2012), while Olorunnisola *et al*., (2017) optimized protein enrichment of fruit peel using a mixed culture of *Phanerochaete chrysosporium* and *Schizophyllum commune*. Earlier studies also showed that co-cultures of *S. cerevisiae* and *Lactobacillus spp* synergistically increased protein content of cassava peel (Oboh, 2006). This simple biotechnological process is therefore feasible.
In this study, there was a remarkable increase in protein content of the non-sterilised yam peels after 21 days of fermentation. The protein contents increased by 113.3% for *A. niger* only, 95% for *T. viride* only, and 96.45% for the co-culture of *A. niger* + *T. viride* (Table 1). This contrasts with what was obtained with the sterilised yam peel substrate with protein content increase of 71.8% (*A. niger* only), 58.05% (*T. viride* only) and 80.6% (co-culture of *A. niger* + *T. viride*) (Table 1). The differences could be attributed to possible successional colonization of non-sterilised yam substrates by other competing microbes. Although fungal succession was not determined in this present studies, it is conjectured that the presence of the first resident colonizing fungal species in the non-sterilised yam peels could possibly enhance metabolism of substrate to produce intermediate compounds for the synthesis of proteins. In the sterilized yam peels, such early-colonizing decomposers would presumably be absent or depleted. In our future studies, fungal succession during solid substrate fermentation of yam peels by *A. niger*, *T. viride*, or both will be determined with the view to elucidating the difference in the level of protein formed in the differential treatments of sterilised and non-sterilized yam peel substrates.

Aruna *et al*., (2017) demonstrated that the protein content of yam peels could be increased from 6.6% to 11.05% in 4 days using *S. cerevisiae*. This paper shows that *A. niger* and *T. viride* inoculated singly or as mixture into yam peels substantially increased the protein content (58.03 – 113.3% depending on whether the substrate was sterilised or non-sterilised) (Table 1). The prolonged incubation period of 21 days might have increased and potentiated the protein content, i.e., more protein could be synthesized from the residual intermediate complex sugar content from solid-state fermentation being used in the enhancement of protein. Data from this present study therefore extends the list of low-cost domestic and industrial agro-lignocellulose wastes whose protein content can be increased using *A. niger* and *T. viride*, producing in the process, appropriate enzymes capable of converting complex carbohydrates and non-starch polysaccharides into simple mono-sugars, these simple mono-sugars can then be converted into value-added protein to increase nutrient value to agro-industrial wastes (Ezekiel and Aworh, 2013). The ash contents of both sterilised and non-sterilised fermented yam peels inoculated singly or with mixture of *A. niger* and *T. viride* increased significantly ($P \leq 0.05$) with prolonged period

of fermentation up to 21 days (Tables 3 and 4). this result agrees with other reports (Aruna *et al*., 2017; Yafetto *et al*., 2019; Oboh *et al*., 2002; Ezekiel *et al*., 2010). This significant increase in the ash content may be attributed to the probable attendant overall increase in fungal biomass during the fermentation period. However, variations in changes in carbohydrates, crude fibre, and fat during fermentation as summarized in Table 4 may be due to the different strains of organisms carrying out fermentation as well as the type and chemical nature of the substrates not excepting the period of fermentation.

This present study has demonstrated that protein content of sterilised and non-sterilised yam peels substrate could be enriched by fermetation with mono- and co-culture of *A. niger* and *T. viride*. It can be stated that there is a biotechnological potential for protein enrichment of yam peels using co-culture of both *A. niger* and *T. viride* to synergistically increase protein formation by 80.60% and 96.45%, respectively, in the sterilised and non-sterilised yam peels. Yam peels extend the list of agro-lignocellulose wastes whose protein content can be enriched to supplement animal feed nutrition in Ghana. Findings from our study suggest that prolonged period of fermentation could predispose the substrate to irregular changes in protein, fat, crude fibre, carbohydrates, etc., in the medium as reflected in previous studies reported by other workers.

Recommendations

We suggest that a future study using yam peels with *A. niger* and *T. viride* should assess protein contents and other chemical compositions between 7 and 14 days of incubation to avoid the resultant predisposition of substrates to irregular changes in nutritional components observed in prolonged fermentation periods. There is a broader spectrum of safe and harmless fungi in the Orders *Mucorales* and *Saccharomycetales*, and also Lactic Acid Bacteria (*Lactobacillus* spp) whose potential for bioconversion in co-culture fermentation studies of agro-lignocellulose wastes awaits our future studies.

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