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The biotechnological potential of using mono– and co–cultures of *Aspergillus niger* van Tieghem and *Trichoderma viride* Pers ex Fr. to enhance the protein content of cassava (*Manihot esculenta* Crantz) peels by solid substrate fermentation

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Abstract

The application of fungal biotechnology for nutrient value addition and improvement of agro-industrial wastes is critical in the development of protein-rich feed for livestock. This study evaluated conventional methods for protein enrichment of cassava peels by solid substrate fermentation using mono- and co-cultures of *Aspergillus niger* and *Trichoderma viride*. Sterile and non-sterile cassava peels inoculated with mono-cultures of *A. niger* or *T. viride* and co-cultures of *A. niger* and *T. viride* were spontaneously fermented for 21 days at 24°C. Fermented substrates were harvested at 0, 7, 14, and 21 days intervals, then oven-dried at 60°C, milled in a blender and further assayed for ash, carbohydrate, crude fibre, fat, and protein contents. Results showed that percentage increase in protein contents after 21 days incubation of sterile cassava peels were 71.02% (for mono-culture of *A. niger*) and 71.64% (for mono-culture of *T. viride*); co-culture of *A. niger* and *T. viride* yielded a 129.00% increase in protein. Enhanced percentage increase in protein content of the non-sterile cassava peels was 126.80% (for mono-culture of *A. niger*) and 168.34% (for mono-culture of *T. viride*) in contrast with 63.47% obtained for the co-culture of *A. niger* and *T. viride* in non-sterile cassava peels, which was attributable to possible antibiosis due to microbial competition. Ash content significantly ($p \leq 0.05$) increased for 21 days while carbohydrate, crude fibre and fat contents in both sterile and non-sterile cassava peels decreased over the same period. These findings underscore the fact that protein content of cassava peels can be significantly ($p \leq 0.05$) enriched through fungal biotechnology to produce value-added feed supplement for livestock. The practical implications of these findings are discussed.

Key words – agro-industrial wastes – environmental mycoremediation – fungal biotechnology – nutrient-enriched feed

Introduction

Cassava (*Manihot esculenta* Crantz) is a starchy root crop consumed by many people in the

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developing countries, especially the tropics – Africa, Asia, and Latin America (Bayitse et al. 2015). The cassava plant was introduced in Africa from Latin America by the Portuguese in 1558 (Guira et al. 2016). According to the Food and Agriculture Organization of the United Nations, by 2018, Ghana was the fifth largest producer of fresh cassava root tubers in the world to the tune of over 19 million tons; the second highest producer in Africa, and in West Africa, after Nigeria, who was the world's largest producer of fresh cassava root tubers accounting for 56 million tons of the world production (FAO 2018).

Like the root tubers, cassava leaves are edible, and they both serve as good sources of nutrition for more than 500 million people in the world. The cassava root tubers, for example, are considered highly enriched with carbohydrates (Bayitse et al. 2017). However, the root tubers have low shelf-life, low protein content and high content of the toxic cyanogenic glucosides (CNGs) – linamarin and lotaustralin (Cooke & Coursey 1981, Bayitse et al. 2017). The leaves and peels of cassava, which constitute 25% of the entire cassava plant, are mostly the by-products of harvesting and processing. These by-products of the cassava plants are reportedly used as feed for poultry, pigs and ruminants (Longe & Adetola 1983, Iyayi 1986, Iyayi & Tewe 1988, Sarwat et al. 1988). According to Bayitse et al. (2017), there are six key steps involved in traditional cassava processing, namely, peeling, chipping, grating, fermentations, sieving, and frying, drying or roasting. These six steps of processing remarkably reduce the high contents of CNGs before consumption. Peeling away the root tuber cortex generates huge amounts of cassava peels as waste. Cassava peels, which are known to contain high contents of toxic CNGs are simply disposed of to rot, polluting the environment in the process, or are carted away by interested livestock farmers to feed poultry, pigs, sheep and goats (Iyayi & Losel 2001, Adu et al. 2018). The use of cassava peels from cottage industries as feed is necessitated by the fact most farmers cannot afford to buy expensive, imported high-grade feeds; but the cassava peels generated by most of the local cottage industries are readily available at low, or at no cost, and can be easily used to produce value-added protein-enriched feed (Ikpesu et al. 2016). Moreover, cassava peels collected are rarely processed but are directly fed to livestock in its raw state with the high contents of the CNGs.

Although the removal of cassava peels is a critical step in the quality improvement of cassava for consumption, the peels generated as waste can be subjected to bioconversion to produce protein-enriched feed before using them to feed livestock. This bioconversion process is achievable biotechnologically through fermentation using mostly fungi and bacteria (Anupama & Ravindra 2000, Ikpesu et al. 2016). Fermentation, in addition to generally detoxifying agro-industrial wastes, also enriches their nutrient contents through biosynthesis of proteins, vitamins, and essential amino acids, and, in the process, improves nutrient quality and fibre digestibility (Pandey et al. 2000, Ikpesu et al. 2016, Aruna et al. 2017).

Fermentation studies have amply demonstrated that CNGs levels of cultured, fermented cassava peels with microorganisms such as the yeast, *Saccharomyces cerevisiae*, and, bacteria *Oenococcus oeni*, and *Lactobacillus plantarum* were significantly reduced (Westby & Choo 1994, Tweyongyere & Katongole 2002, Lambri et al. 2013). Fermentation studies with other microorganisms have proven that indeed protein contents of cassava peels can be significantly enriched (Ofuya & Nwanjuiba 1990, Iyayi & Losel 2001, Oboh 2006, Ezekiel et al. 2010, Ezekiel & Aworh 2013, Bayitse et al. 2015, Ikpesu et al. 2016). These studies provided useful evidence that the nutritional and economic values of cassava peels can be enhanced to serve as more appropriate feed devoid of CNGs for poultry, pigs, sheep, and goats. This present study aimed at protein enhancement of sterile and non-sterile cassava peels by fermentation with mono-cultures of *Aspergillus niger* or *Trichoderma viride*, and co-cultures of *A. niger* and *T. viride*. Findings from this study could fill the knowledge gap at the frontiers of fungal biotechnology and its application in (i) the production of protein-enriched feed from agro-industrial wastes, and (ii) environmental mycoremediation.

Materials & Methods

Cultures of *Aspergillus niger* and *Trichoderma viride*

Aspergillus niger used in this study was isolated following the procedures of Adu et al. (2018) and Yafetto (2018). *Trichoderma viride* was isolated from soil samples randomly collected from locations within the Botanical Gardens of the University of Cape Coast, Ghana. Three-fold serial dilutions of each soil sample were prepared in sterilized distilled water. One milliliter of the soil solution was added to approximately 20 ml of molten *Trichoderma*-selective medium (KH_2PO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; Peptone, 5 g; glucose, 10 g; Rose Bengal, 17 mg; Streptomycin sulphate, 30 mg; agar, 20 g; 1000 ml distilled water; autoclaved at a pressure of 1.1 kg/cm^2 at 121°C for 15 minutes) based on Davet & Rouxel (2000), after which the culture plates were incubated at 28 ± 2°C for 96 hours. Pure cultures of both *A. niger* and *T. viride* were maintained on Potato Dextrose Agar slants in McCartney tubes at 4°C, and sub-cultured fortnightly to preserve their viability until ready for use. *A. niger* and *T. viride* were identified based on 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).

Conidial suspensions of *Aspergillus niger* and *Trichoderma viride*

Amended Potato Dextrose Broth media (Irish potato, 200 g; dextrose, 20 g; distilled water, 1000 ml; autoclaved at a pressure of 1.1 kg/cm^2 at 121°C for 15 minutes and supplemented with 2 g Ammonium nitrate, 8 g Sodium chloride and 400 µg/L Thiamine solution with pH adjusted to 5) were prepared as described by Adu et al. (2018). The PDB-amended medium was used to prepare separate conidia suspensions of only *A. niger*, only *T. viride*, and a mixture of *A. niger* and *T. viride*, as described by Guarro et al. (1998), Yalemtesfa et al. (2010), Olorunnisola et al. (2017). Conidial suspensions of either *A. niger*, or *T. viride* and co-cultures of *A. niger* and *T. viride* were used to inoculate prepared cassava peel substrates for solid substrate fermentation.

Cassava peels

Cassava peels used in this study were obtained from a small-scale cassava-processing cottage industry in Cape Coast, Ghana. The periderms of cassava rind were removed and the cortex (peels) were divided into two portions of equal quantity. One portion was thoroughly washed under a running tap to remove all dirt and debris from the cassava peels; the other portion was not washed. The washed and unwashed peels were then sun-dried separately for 7 days, oven dried at 60°C for 24 hours, after which they were pulverized, by crushing and grinding, in a mortar with a pestle to obtain fine powders. One hundred and fifty grams of powdered, washed peels were then placed in a beaker, sealed with aluminum foil, sterilized by autoclaving at a pressure of 1.1 kg/cm^2 at 121°C for 15 minutes, and allowed to cool; 150 g of powdered, unwashed peels were not sterilized.

Solid substrate fermentation and estimation of the protein content of cassava peels

Sterile and non-sterile cassava peels were treated and prepared for solid substrate fermentation using the method as described by Adu et al. (2018), using mono- and co-cultures of *A. niger* and *T. viride*. The initial percentage nitrogen (% N_2) of the sterile and non-sterile cassava peels were determined at day 0 using the conventional Kjeldahl method (Kjeldahl 1883), after which the % N_2 of inoculated and incubated cassava peels were determined after 7, 14, and 21 days of fermentation. The percentage protein contents of the substrates were subsequently determined as described by Adu et al. (2018) and Yafetto et al. (2018).

Proximate analyses of sterile and non-sterile cassava peels

Twenty grams of fresh cassava samples were dispensed into already cleaned and oven-dried porcelain crucibles. The crucibles containing the cassava samples were evenly spaced in a thermostatically controlled oven and heated at 105°C for 48 hours, after which they were removed, placed in a desiccator to cool, and weighed. The percentage moisture content of the cassava samples was then calculated by difference in weight. All procedures for proximate analysis were performed in triplicates.

Determination of dry matter of cassava substrates

The dried samples obtained after the determination of the percentage moisture were weighed and expressed as the percentage dry matter of the cassava samples.

Determination of ash content of cassava substrates

Dried cassava peel samples in a porcelain dish were heated in an oven at 105°C for 1 hour, after which they were transferred to a furnace and further heated at a temperature of 550°C for 12 hours; the samples were heated till all the carbon particles were completely burnt into ash. The dishes containing the ash were removed from the furnace, cooled in a desiccator and weighed. The ash content was then calculated as a percentage of the original sample as follows:

$$\text{Ash (\%)} = \frac{\text{Weight of Ash (g)}}{\text{Weight of Sample (g)}} \text{ x 100}$$

Determination of fat content of cassava substrates

Ten grams of dried cassava peel samples were weighed and dispensed into a 50 × 10 mm Soxhlet extraction thimble. The fat content of the cassava samples was determined by Soxhlet petroleum ether extraction as described by AOAC (2005). Percentage of crude fat was calculated as follows:

$$\text{Fat (\%)} = \frac{\text{Weight of Oil (g)}}{\text{Weight of Sample (g)}} \text{ x 100}$$

Determination of carbohydrate content of cassava substrates

Carbohydrate was determined using the method described by Dubois et al. (1956) and AOAC (2005).

Determination of crude fibre content of cassava substrates

Approximately 1 g of dried cassava peels was weighed and dispensed into a boiling flask, after which 100 ml of 1.25% sulphuric acid solution was added and the mixture boiled for 30 minutes; filtration was carried out in a sintered glass crucible. The resultant residue was transferred back into the boiling flask, 100 ml of 1.25% NaOH solution was added and the mixture was boiled for 30 minutes. Filtration was continued after boiling and the residue washed with boiling water and methanol. The sintered glass crucible with the residue was dried in an oven at 105°C for 12 hours and weighed. The crucible and the residue were placed in a furnace at 500°C for 3 hours, after which they were slowly cooled to room temperature in a desiccator and weighed.

The percentage crude fibre was calculated as follows:

$$\text{Crude Fibre (\%)} = \frac{\text{Weight loss through Ashing (g)}}{\text{Weight of Sample (g)}} \text{x100}$$

Study design

This study was a laboratory-based research. The procedure for preparing spore suspension media, cassava substrates for fermentation, and proximate analysis of fermented cassava peels are summarized in Fig. 1 following a modified method of Aruna et al (2017).

Statistical analysis

All data were collected and analyzed in triplicates by subjecting them to One-Way Analysis of Variance (ANOVA) with Statistical Package for Social Sciences (SPSS) Version 25.0. The means were separated using Tukey post-hoc test at 95% confidence level ($P \leq 0.05$).

Results

Protein enrichment of fermented, sterile cassava peels

The mean initial protein content (%) of the sterile, dried, and pulverized cassava peel was 8.04±0.61 (Fig. 2). The protein contents of the sterile cassava peel treated with mono-cultures of *A. niger*, and *T. viride* only increased to commensurate with the incubation periods of 7, 14, and 21 days of fermentation (Fig. 2), The samples of sterile cassava peel treated with co-cultures of *A. niger* and *T. viride* recorded a greater percentage increase in protein of 129.00% as compared to 71.02% and 71.64% for sample inoculated with mono-cultures of *A. niger* and *T. viride* only (Table 1).

Protein enrichment of fermented, non-sterile cassava peels

The mean initial protein contents of non-sterile, dried, and pulverized cassava peels was 6.57±0.23. Akin to the sterile cassava peels, the protein contents of the non-sterile cassava peels treated with mono-culture of *A. niger* and *T. viride* only also showed a commensurate increase after 7, 14, and 21 days of fermentation (Fig. 3). Curiously, the non-sterile cassava peels inoculated with mono-cultures of *A. niger* and *T. viride* recorded an enhanced percentage increase in protein by 126.8% and 168.34%, respectively, in contrast with the non-sterile cassava peels, which recorded an increase of 63.47% (Table 1).

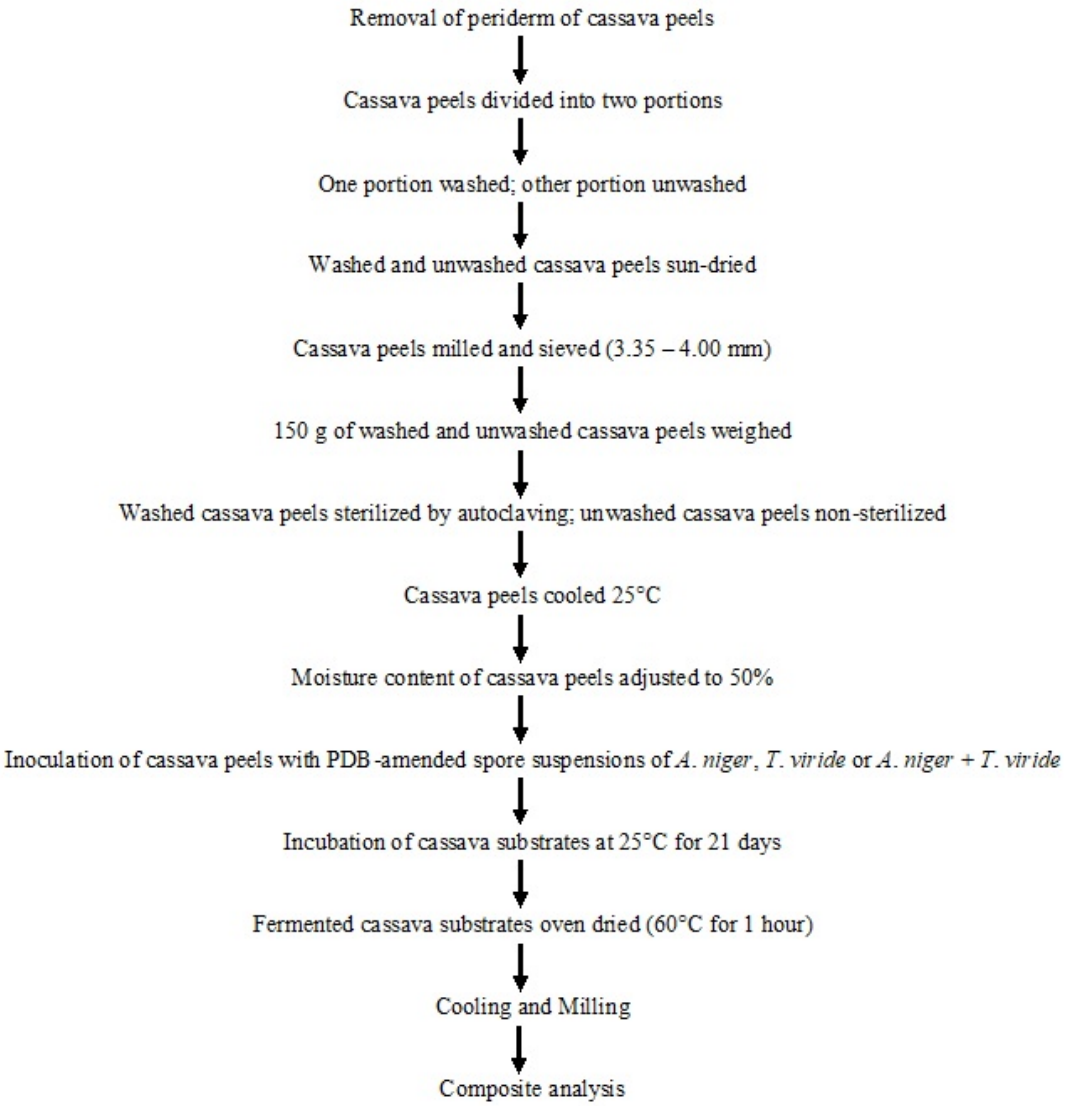

Fig. 1 – Flow diagram of the steps for production of protein-enriched cassava peels and composite analysis. Adopted with modifications from Aruna et al (2017).

Table 1 Percentage (%) increase in protein content of sterile and non-sterile cassava peels after 7, 14 and 21 days of fermentation at 24°C.

	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**
Sterile peels	31.20	38.0	71.02	25.00	56.10	71.64	51.74	86.82	129.00
Non-sterile peels	48.10	52.21	126.80	61.34	103.50	168.34	36.53	60.12	63.47

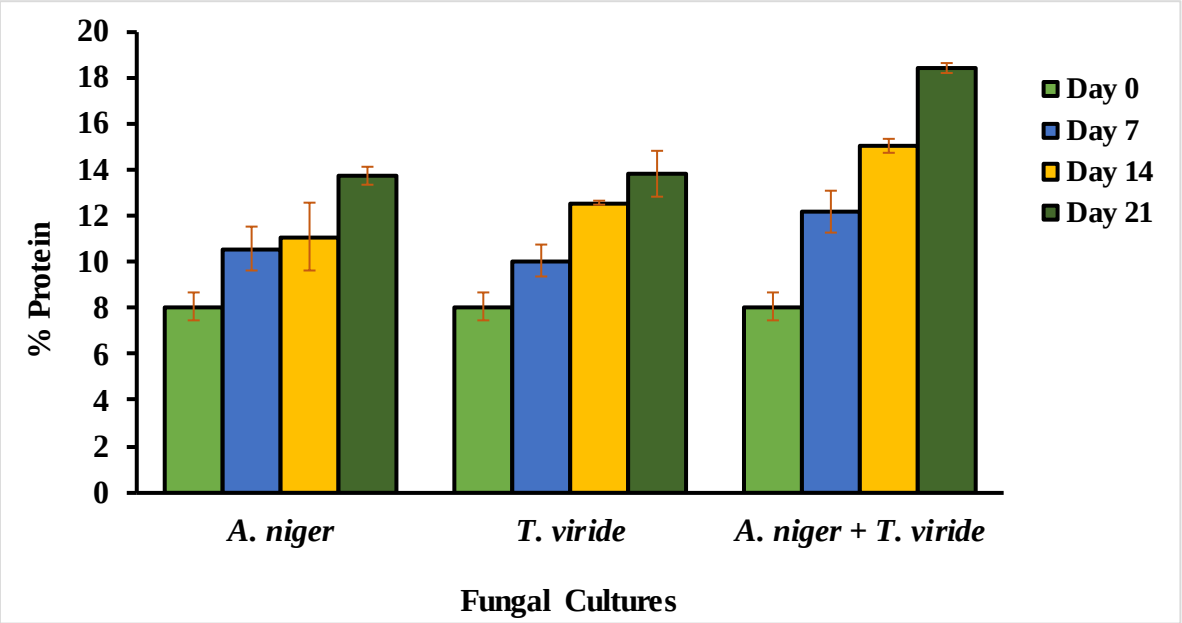

Fig. 2 – Percentage protein contents of sterile cassava peels after solid-state fermentation with mono-cultures of *A. niger*, *T. viride*, and co-culture of *A. niger* and *T. viride* incubated at 24°C.

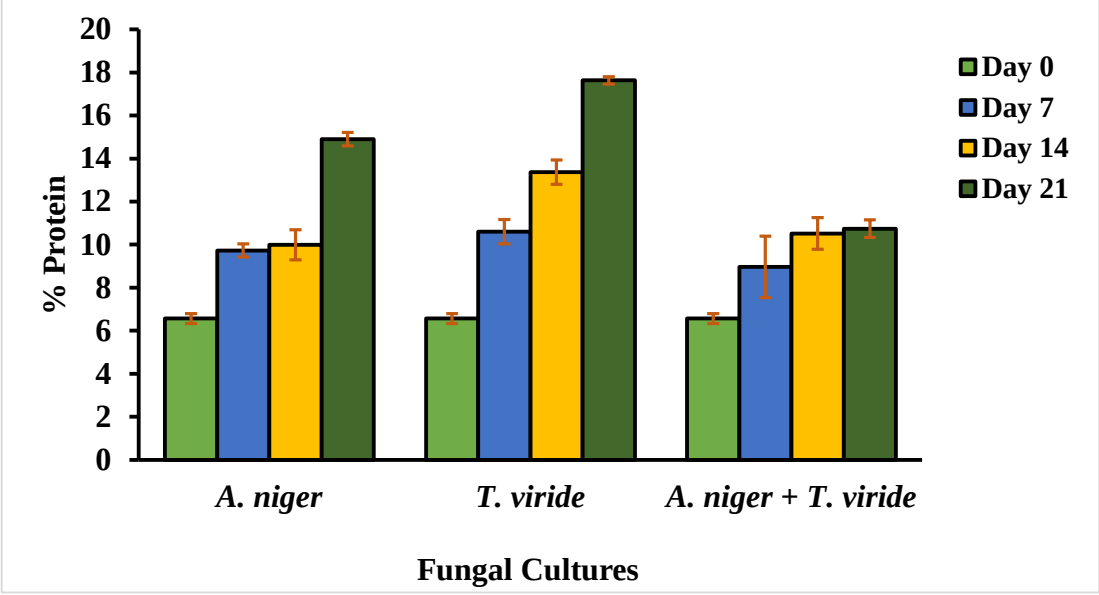

Fig. 3 – Percentage protein contents of non-sterile cassava peels after solid-state fermentation with mono-cultures of *A. niger*, *T. viride*, and co-culture of *A. niger* and *T. viride* incubated at 24°C.

Selected proximate analysis of sterile cassava peels for ash, carbohydrate, crude fibre and fat contents

Results obtained are summarized in Table 2. There was general consistent decrease in carbohydrate, crude fibre and fat contents in sterile cassava peels after 21 days of fermentation for samples inoculated with mono-cultures of *A. niger* and *T. viride* only as well as co-cultures of *A. niger* and *T. viride*. The only exception was recorded after 7 days when there was a momentary increase in fat content in the samples, which thereafter declined (Table 2). Ash contents, on the other hand, increased with period of incubation for sterile cassava peels (Table 2).

Table 2 Changes in the chemical composition of sterile, fermented cassava 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 dry matter). Each value is a mean of triplicates of proximate analyses of cassava samples. Means with different subscripts in the same column are significantly different ($p \leq 0.05$).

Fungal Culture	Day	Chemical composition (%)			
		Ash	Carbohydrate	Crude fibre	Fat
A. niger	0	$2.20 \pm 0.01_a$	$80.00 \pm 0.55_a$	$2.84 \pm 0.07_a$	$0.76 \pm 0.03_a$
	7	$3.55 \pm 0.13_b$	$76.00 \pm 0.74_b$	$2.30 \pm 0.02_b$	$0.88 \pm 0.03_b$
	14	$3.50 \pm 0.24_b$	$75.00 \pm 1.42_b$	$2.21 \pm 0.10_b$	$0.60 \pm 0.00_c$
	21	$3.51 \pm 0.24_b$	$72.20 \pm 0.70_c$	$2.21 \pm 0.10_b$	$0.64 \pm 0.00_d$
T. viride	0	$2.20 \pm 0.01_a$	$80.00 \pm 0.55_a$	$2.84 \pm 0.07_a$	$0.76 \pm 0.03_a$
	7	$3.32 \pm 0.22_b$	$76.84 \pm 0.81_b$	$2.32 \pm 0.10_b$	$1.02 \pm 0.01_b$
	14	$3.28 \pm 0.14_b$	$73.80 \pm 0.61_c$	$2.33 \pm 0.05_b$	$0.63 \pm 0.00_c$
	21	$3.71 \pm 0.12_c$	$72.54 \pm 1.00_c$	$2.26 \pm 0.04_b$	$0.63 \pm 0.00_c$
A. niger* and *T. viride	0	$2.20 \pm 0.01_a$	$80.00 \pm 0.55_a$	$2.84 \pm 0.07_a$	$0.76 \pm 0.03_a$
	7	$3.60 \pm 0.04_b$	$73.10 \pm 0.88_b$	$2.64 \pm 0.11_{ab}$	$0.71 \pm 0.01_b$
	14	$3.50 \pm 0.24_b$	$71.52 \pm 0.10_c$	$2.43 \pm 0.10_b$	$0.67 \pm 0.01_b$
	21	$3.53 \pm 0.13_b$	$67.50 \pm 0.44_d$	$2.44 \pm 0.07_b$	$0.69 \pm 0.00_b$

Selected proximate analysis of non-sterile cassava peels for ash, carbohydrate, crude fibre and fat contents

Results of the listed analysis of non-sterile cassava peels fermented for 21 days followed similar trends of decrease in carbohydrates, crude fibre and fat (Table 3), irrespective of whether the samples were treated with mono-cultures of *A. niger* and *T. viride* or with co-cultures of the test fungi. Ash content, on the other hand, increased with period of incubation of both fermented sterile and non-sterile cassava peels (Tables 2, 3), irrespective of whether samples were inoculated with mono-cultures of *A. niger* and *T. viride* or co-cultures of the two fungi.

Discussion

The increasing demand for livestock feeding products in developing countries has necessitated the exploitation of large quantities of agro-industrial wastes such as cassava (*Manihot esculenta*), coffee (*Coffea arabica*), maize (*Zea mays*), oil palm (*Elaeis guineensis*), pineapple (*Ananas sativa*, *A. comosus*), etc., to produce protein-enriched substrates using fungi (Ezekiel & Aworh 2013, Yafetto 2018). In this present study, mono-culture of *A. niger* used to inoculate sterile peels and non-sterile peels of cassava yielded 71.02 and 126.80% increase in protein in 21 days as compared to 71.64 and 168.34% when inoculated with the mono-cultures of *T. viride* for the same period (Figs 2, 3, Table 1). The combined treatment with *A. niger* and *T. viride* also increased protein content by 129.00 (for sterile) and 63.47% (for non-sterile) cassava peels. Clearly, findings from this study show that the protein contents of both sterile and non-sterile wastes of cassava peels

have biotechnological potential to be enriched by single cultures of *A. niger* and *T. viride*. But the combined co-culture of *A. niger* and *T. viride* was more effective (129.00%) in the sterile cassava peel substrates than in the non-sterile cassava peels (63.47%) after 21 days of fermentation (Figs 2, 3, Table 1). This could be attributable to antibiosis effect being more pronounced after 21 days on the non-sterile cassava peels with co-cultures of *A. niger* and *T. viride*. The absence of competitors in the sterile cassava peels accentuated by low cyanide level due to sterilization potentiated the activity of *A. niger* and *T. viride* (Table 1). The increase in protein content could presumably be attributed to the utilization of complex carbohydrates and subsequent protein formation (Correia et al. 2007).

Table 3 Changes in the chemical composition of non-sterile, fermented cassava 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 dry matter). Each value is a mean of triplicates of proximate analyses of cassava samples. Means with different subscripts in the same column are significantly different ($p \leq 0.05$).

Fungal Culture	Day	Chemical Composition (%)			
		Ash	Carbohydrate	Crude fibre	Fat
A. niger	0	$2.20 \pm 0.01_a$	$80.60 \pm 0.41_a$	$3.05 \pm 0.10_a$	$0.74 \pm 0.02_a$
	7	$3.44 \pm 0.30_b$	$78.00 \pm 0.43_b$	$2.45 \pm 0.10_b$	$1.02 \pm 0.01_b$
	14	$3.40 \pm 0.11_b$	$76.30 \pm 0.50_c$	$2.46 \pm 0.10_b$	$0.60 \pm 0.00_c$
	21	$3.33 \pm 0.12_b$	$71.00 \pm 0.54_d$	$2.41 \pm 0.10_b$	$0.70 \pm 0.00_d$
T. viride	0	$2.20 \pm 0.01_a$	$80.60 \pm 0.41_a$	$3.05 \pm 0.10_a$	$0.74 \pm 0.02_a$
	7	$3.50 \pm 0.23_b$	$76.50 \pm 1.00_b$	$3.00 \pm 0.10_b$	$1.00 \pm 0.04_b$
	14	$3.60 \pm 0.13_b$	$73.00 \pm 0.40_c$	$2.63 \pm 0.03_b$	$0.70 \pm 0.01_{ac}$
	21	$3.60 \pm 0.30_b$	$69.00 \pm 0.20_d$	$2.50 \pm 0.07_c$	$0.70 \pm 0.00_c$
A. niger* and *T. viride	0	$2.20 \pm 0.01_a$	$80.60 \pm 0.55_a$	$3.05 \pm 0.10_a$	$0.74 \pm 0.02_a$
	7	$3.60 \pm 0.10_b$	$75.30 \pm 1.10_b$	$3.04 \pm 0.10_a$	$1.05 \pm 0.10_b$
	14	$3.10 \pm 0.03_c$	$75.40 \pm 1.00_b$	$3.00 \pm 0.10_a$	$0.70 \pm 0.01_a$
	21	$4.20 \pm 0.12_d$	$74.00 \pm 0.20_b$	$2.71 \pm 0.04_b$	$0.71 \pm 0.01_a$

Recent studies by Adu et al. (2018) and Yafetto (2018) have shown that protein contents of both sterile and non-sterile pulp wastes of pineapple, cassava (pulp) and watermelon have the biotechnological potential to be enriched with *A. niger* activity using solid substrate fermentation. Other workers (Iyayi & Losel 2001) reported a significant increase in percentage protein content of cassava peels by 192.85% with *Saccharomyces cerevisiae* (yeast) after 21 days of fermentation, and that *A. niger* yielded a higher percentage in cassava peels, than the pulp of same plant tuber. On the other hand, Ofuya & Nwanjuiba (1990) found an 185.00% increase in protein content using a single culture of *Rhizopus*. Varying degrees of protein enrichment of peels of different agro-industrial wastes have been reported using *Trichoderma viride* and *T. pseudokoningii* single cultures or combination of *A. niger* and *S. cerevisiae* (Ezekiel et al. 2010, Ezekiel & Aworh 2013, Omwango et al. 2013, Ahmadi et al. 2015, Bayitse et al. 2015, Aruna et al. 2018). Findings in this paper extend the list of agriculture wastes, like cassava peels, whose protein contents can be enhanced by biotechnological technique of inoculating the peels with single and co-cultures of *A. niger* and *T. viride*, and allowed to undergo spontaneous fermentation for 21 days. Application of co-cultures of *A. niger* and *T. viride* to ferment cassava peel substrate for 21 days has shown good promise for enhancing protein content, at least synergistically, on the sterile cassava peels. This gives a new insight into the potential of this technique in the preparation of nutrient-enhanced animal feed. The application of co-cultures of *A. niger* and *T. viride* to synergistically enrich protein of especially sterile cassava peels provides also a novel approach to the use of fungi to

maximize the bioconversion of agricultural lignocellulose wastes into useful value-added products for livestock and fish feed. The use of co-cultures for this purpose has been demonstrated by Oboh (2006) who used *S. cerevisiae* and *Lactobacillus* to increase protein content of cassava peels by 21.50% attended by a significant reduction in cyanide content. Darwish et al. (2012) also used co-cultures of *Pleurotus ostraetus* and *S. cerevisiae*, and succeeded in increasing protein content of maize stalk by only 11.80%.

There were other interesting observations. We found that ash content of the fermented cassava peels treated with single cultures of *A. niger* and *T. viride*, as well as co-cultures of *A. niger* and *T. viride*, significanlty ($P \leq 0.05$) increased with increasing period of incubation up to 21 days for both sterile and non-sterile cassava peels (Tables 2, 3). Correspondingly, there was a significant ($P \leq 0.05$) decrease in carbohydrates, crude fibre, and fat content in both fermented sterile and non-sterile cassava peels in 21 days (Tables 2, 3). Aruna et al. (2017) reported an increase in ash and fat contents, and a decrease in carbohydrate content of yam peels after 4 days of fermentation with *S. cerevisiae*. These constrasting results may be attributed to the differences in substrates and microorganisms employed. The increase in fat content of substrates in some instances may be presumably due to (i) bioconversion of carbohydrates into fat and (ii) accumulation of fat by certain fungi during the fermentation process (Akindumila & Glatz 1998, Oboh et al. 2002, Aruna at al. 2017). The significant decrease in carbohydrate content in our study may partly play a role in the corresponding increase in protein content of cassava peels fermented by *A. niger* and *T. viride*, and their ability to metabolize complex carbohydrates into single sugars required for microbial bioconversion into protein during fermentation (Akintomide & Antai 2012, Correia et al. 2007).

Ash content increased with the period of incubation of both fermented sterile and non-sterile cassava peels (Tables 2 and 3). This increase in ash content could be partly attributed to the overall fungal biomass accumulation during the fermentation period. This finding agrees with the report of Oboh et al. (2002) and Ezekiel et al. (2010). Findings from this present study not only demonstrate, for the first time in Ghana, that protein contents of both sterile and non-sterile cassava peels could be enriched by biotechnological fermentation process using *A. niger* and *T. viride* alone, but show also a near synergistic effect with co-inoculation with both *A. niger* and *T. viride* especially in the sterile cassava peels after 21 days of fermentation. The combined co-inoculation with *A. niger* and *T. viride* increased protein content by 129.00% (sterile cassava peels) and 63.47% (non-sterile cassava peels) after 21 days. The increased formation of protein recorded in this study for both sterile and non-sterile cassava peels gives credence to the ability of *A. niger* and *T. viride* to secrete appropriate enzymes that are capable of converting complex starch and non-starch polysaccharides into simple monomer sugars, which are then metabolized into proteins (Ezekiel & Aworh 2013). The depression in protein content of the non-sterile peels co-inoculated with *A. niger* and *T. viride* could be attributed to the presence of chemical antibiosis among the resident microbes which contributed to the lowering of protein content detected after 21 days. Ash content of the fermented sterile and non-sterile cassava peels inoculated singly with *A. niger* or *T. viride*, or co-inoculated with both fungi increased with increasing period of incubation, whereas carbohydrates, crude fibre, and fat contents generally decreased with time (Tables 2, 3).

We conclude that there is a biotechnological potential for protein enrichment of cassava peels using single cultures of either *A. niger* or *T. viride*, and a co-culture of both *A. niger* and *T. viride* to synergistically enhance protein formation by 129.00%, at least, in the sterile cassava peels. This finding extends the list of Ghanaian agro-lignocellulose substrates which can, together with fungi, obtain value addition under Ghanaian tropic conditions (Adu et al. 2018, Yafetto 2018). Our recommendation is that, in future studies, other early colonizing decomposers of cassava peel, especially members of Mucorales (*Rhizopus*, *Mucor*, *Actinomucor*, and *Syncephalastrum*), which are harmless, could be employed to enrich agro-industrial wastes using both mono- and co-cultures of these fungi. Findings from these studies should be tested *in vitro* for use as animal feedstuff. If this is done, this paper would serve as a springboard for using fungal biotechnology to enhance nutrient status of animal feed in Ghana, and, at the same time, assist in the bioremediation of environmental pollution by our unrestricted disposal of agro-industrial wastes in the tropical region.

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