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Solid State Fermentation of Cassava Peel with *Trichoderma viride* (ATCC 36316) for Protein Enrichment

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***Abstract*—**Solid state fermentation of cassava peel with emphasis on protein enrichment using *Trichoderma viride* was evaluated. The effect of five variables: moisture content, pH, particle size (p), nitrogen source and incubation temperature; on the true protein and total sugars of cassava peel was investigated.

The optimum fermentation period was established to be 8 days. Total sugars were 5-fold higher at pH 6 relative to pH 4 and 7-fold higher when cassava peels were fermented at 30^{o}C relative to 25^{o}C as well as using ammonium sulfate as the nitrogen source relative to urea or a combination of both. Total sugars ranged between 123.21mg/g at 50% initial moisture content to 374mg/g at 60% and from 190.59mg/g with particle size range of 2.00>p>1.41mm to 310.10mg/g with 4.00>p>3.35mm.True protein ranged from 229.70 mg/g at pH 4 to 284.05 mg/g at pH 6; from 200.87 mg/g with urea as nitrogen source and to 254.50mg/g with ammonium sulfate; from 213.82mg/g at 50% initial moisture content to 254.50mg/g at 60% moisture content, from 205.75mg/g in cassava peel with 5.6>p> 4.75mm to 268.30 in cassava peel with particle size 4.00>p>3.35mm, from 207.57mg/g at 25^{o}C to 254.50mg/g at 30^{o}C

Cassava peel with particle size 4.00>p>3.35 mm and initial moisture content of 60% at pH 6.0, 30^{o}C incubation temperature with ammonium sulfate (10g N / kg substrate) was most suitable for protein enrichment with *Trichoderma viride*. Crude protein increased from 4.21 % in unfermented cassava peel samples to 10.43 % in fermented samples.

***Keywords*—**Cassava peel, Solid state fermentation, *Trichoderma viride,* Total sugars, True protein.

I. Introduction

IN the developing countries, the pattern of food consumption does not show that enough protein is being consumed resulting in malnutrition [1]. Therefore, it is necessary to increase daily intake of protein in sufficient proportion using cheap and non-conventional sources. A lot of food materials are discarded as wastes and some as by-products of food processing

However, value could be added to such food and agricultural wastes and by-products to further utilize them. This may result from inadequate knowledge of appropriate technology to optimally utilize these materials. Any safe means, therefore, to make available food nutrients of high value from cheap sources such as wastes and by-products is of high interest.

Major sources of wastes as far as food is concerned include food processing wastes, agricultural wastes and industrial wastes or by products of some manufacturing processes which are not been utilized optimally. Examples of food wastes and by products are cassava peels, cheese and whey permeates, banana peels, citrus peels, peanut shells, and sugarcane baggase. Also, large amount of lignocellulosic waste that pose environmental pollution problem are generated through forestry and agricultural practices of many agro-based industries. Interests in recovery of waste or by-products have been increasing for both economic and ecological reasons as well as for nutritional reasons [2]. In the last two decades in Nigeria, there have been concerted efforts at finding ways of complete utilization of agro-industrial by–products, which sometimes constitute environmental hazards [3].

Cassava (*Manihot* spp.), a staple food of the majority of people in Tropical Africa, Central and South America [4], [5] has been subjected to series of fermentations in the different countries to produce similar or different products [6]. The various production processes are usually accompanied with some waste products that act as environmental pollutants [7].

Cassava peels; leaves and starch residues constitute 25% of the cassava plant [3]. These are usually discarded as wastes after harvesting and processing, with limited utilization due to low protein, high crude fibre and cyanide contents [8]. Chief among the waste obtained from cassava plant is cassava peel, which form high mounds of heap in production areas with the attendant smelly odors [9]. Technology exists for recovery of food materials in waste by chemical and microbiological means either directly for human food or indirectly by conversion to animal feed. One of the ways to appropriately utilize this waste is protein enrichment using solid-state fermentation (SSF) technique to add value to it [10]. The economic interest in biotechnological production of protein-enriched products can be enhanced if the carbon source (glucose) needed could be obtained from low-cost lignocellulosic waste, using SSF process, an inexpensive and efficient food processing technique.

SSF processing is a relevant, initial approach to lignocellulose bioconversion appropriate for developing countries. It offers the possibility of using by-products and wastes from food and agricultural industries [11] for food, feed, chemicals and fuel. The greatest socio-economical potential of SSF is the raising of living standards through the production of protein rich foods for human consumption [12].

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This can be achieved by exploring two alternatives: production of protein-enriched fermented foods for direct human consumption and production of fermented materials for animal feeding. Starch substrate protein-enriched by SSF could be fed to monogastric animals or poultry.

Bioconversion of lignocellulosic materials to useful high value added products normally require multi-step processes [13], which include: pre-treatment (mechanical, chemical or biological) [14], [15]; hydrolysis of the polymers to produce readily metabolizable molecules (e.g. hexose or pentose sugars); bio-utilization of these molecules to support microbial growth or to produce chemical products; the separation and purification [16].

Many physical, chemical and microbial pre-treatment methods for enhancing bioconversion of cellulosic materials have been reported [17]- [19]. Pretreatment of cellulose opens up the structure and removes interaction between glucose chains [20], [21]. Reference [22] produced cellulase from sawdust, bagasse and corncob from *Aspergillus flavus* and also reported production of cellulase of 0.056425IU/ml from the growth of *Aspergillus flavus* on bagasse pre-treated using ballmill and caustic soda. Environmental factors such as temperature, pH, water activity, oxygen levels and concentrations of nutrients and products significantly affect microbial growth and product formation.

Trichoderma species are highly efficient producers of many extracellular enzymes. They are used commercially for production of cellulases and other enzymes that degrade complex polysaccharides. They are frequently used in the food and textile industries for these purposes [23]. The occurrence of pathogenic *Trichoderma* strains may be restricted to species of section Longibrachiatum; the species in this section are *Trichoderma longibrachiatum* and *Trichoderma citrinoviride* [24].

The objective of this work is to establish suitable condition for the solid state fermentation of cassava peels with *Trichoderma viride* for protein enrichment by evaluating the effect of different levels of fermentation pre-treatments and variables which include initial pH, initial moisture content, particle size, incubation temperature and nutrient supplementation.

II. Materials and Method

The peel from fresh cassava tubers, variety TME I, obtained from a farm at Ajibade village in Akinyele local government area, Ibadan was used for this study.

A. Micro-organism

The filamentous fungi *T. viride* ATCC 36316 was obtained from American Type Culture Collection.

B. Maintenance Medium

T. viride ATCC 36316 was maintained on malt extract agar slants and stored at 4^{o}C. The organism was sub-cultured once every 3 months.

C. Inoculum Preparation

The fungus was subcultured on malt extract agar in petri dishes for 5 days. Spore suspension containing approximately $3x10^{6}$ spores per ml was prepared in Ringer solution.

D. Substrate Preparation for Solid State Fermentation

The cassava tubers were washed, peeled and dried immediately in the oven at 60^{o}C to constant moisture content. The sample was evaluated chemically.

The sample was milled into the following ranges of particle size (p), — 5.6>p>4.75 mm, 4.75>p>4.00 mm, 4.00>p>3.35 mm, 3.35>p>2.00 mm, 2.00>p>1.41 mm, 0.60>p>0.30 mm standard mesh sizes and were stored in the desiccators until needed.

The sample (30 g) was weighed into several 250 ml conical flasks and moisture content and pH were adjusted appropriately. Moisture content of 50%, 60%, 70% and 80%; pH of 4, 5 and 6 were used. The pH values were adjusted using 0.1M HCl and 0.1M NaOH respectively until desired pH was achieved. The mouth of the flasks were clogged with cotton wool and then covered with aluminum foil. The flasks containing the substrates were autoclaved at 121^{o}C for 15 minutes. The autoclaved samples were allowed to cool to ambient temperature before inoculation. All experiments were performed in triplicates.

E. Nutrient Supplementation

Samples prepared as described were supplemented with nitrogen sources in the form of urea and ammonium sulfate as follows: 10g of N as Ammonium Sulfate per kg substrate; 10g of N as Urea per kg substrate; 5g of N from Ammonium Sulfate with 5g of N from Urea per kg substrate, in 30g of pre-treated cassava peels for each addition ratio.

F. Procedure

A basis for fermentation was chosen for all the fermentation conditions - substrate particle size (0.6 mm > p> 0.3 mm), initial pH (5.00 $\pm$ 0.1), nitrogen source (Ammonium sulfate) and incubation temperature (30^{o}C). Each conical flask containing prepared cassava peel was inoculated with 5ml of prepared inoculums.

To establish a time profile for the fermentation process, samples were withdrawn daily and analysed for true protein and soluble solids, the optimum fermentation period corresponding to the day with the highest protein and soluble solid content was noted.

Subsequently, the fermentation parameters were varied as indicated in the substrate preparation procedure and samples withdrawn on the noted day corresponding to the day with the highest true protein and soluble solid content for subsequent analysis to evaluate the effect of the variables on protein enrichment and total sugars. This procedure is expected to help facilitate the establishment of suitable fermentation conditions for cassava peels using *Trichoderma viride* under solid state fermentation process.

Solid State Fermentation was carried out in 250 ml conical flasks containing the autoclaved substrates. The set of flasks were aseptically inoculated with 5 ml of the spore suspension of each of the organisms and properly labelled while control sample was not inoculated. Incubation was carried out at two temperatures 25 ± 0.1^{o}C and 30± 0.1^{o}C. Fig. 1 showed the flow chart for protein enrichment of cassava peel using solid state fermentation.

Subsequent fermentation was carried out using the most suitable fermentation conditions obtained and the resulting protein enriched cassava peel from solid state fermentation using *Trichoderma viride* was subjected to analysis.

G. Compositional Analysis of Cassava Peel

Moisture content of the cassava peel was determined by drying at 105^{o}C to constant weight [25]. The crude protein was by Kjeldahl method (total nitrogen x 6.25), crude fibre, fat, ash, carbohydrates (estimated by difference); total dietary fibre, nitrogen free extract and gross metabolizable energy were quantified as described by AOAC methods [26]. True protein content was determined by the method of Lowry [27]. Total sugars were estimated using phenol sulfuric acid method [28]. Total cyanide of dried samples was determined by phosphoric acid extraction, hydrolysis of cyanogenic glucosides with linamarase from cassava, followed by colorimetric determination of cyanide [29].

III. Results and Discussion

A. Establishment of Time Profile for Protein Enrichment of Cassava Peels during Solid State Fermentation Process Using Trichoderma Viride

The time profile of true protein production by *Trichoderma viride* is shown in Fig. 1. Protein production with incubation time closely followed the pattern of fungal growth. The production started after a lag phase of one day and increased till the 8th day and began to decline thereafter. The optimum time of incubation for maximal true protein production observed from the result was 8 days; further increase in incubation period did not result in increase in protein. This may be due to the age of the fungi and depletion of the sugar content in the fermentation substrate.

B. Effect of Initial pH on Fermentation of Cassava Peel

True Protein increased from 230 mg/g at pH 4 to 260 mg/g at pH 5 and 270 mg/g at pH 6 in cassava peels of particle size 0.30-0.60 mm and moisture content of 60 % when fermented with *Trichoderma viride* for 8 days (Fig. 2a). Total sugars were 5-fold higher at pH 5 and 6 relative to pH 4 (Fig. 2b). At pH less, than 5 mycelia growths would have been inhibited by the high acidity resulting in reduced bioconversion of sugars to protein.

C. Effect of Incubation Temperature

True protein was greater at 30^{o}C relative to 25^{o}C (Fig. 3a). Total Sugars were 7-fold higher when cassava peels were fermented at 30^{o}C relative to 25^{o}C presumably because the induction of amylase and cellulose enzymes required for starch and cellulose hydrolysis by *Trichoderma viride* occurred readily at 30^{o}C than at 25^{o}C (Fig. 3b).

D. Effect of Nitrogen Source

Addition of exogenous nitrogen sources considerably increased total sugars and true protein production during fermentation of cassava peels with *Trichoderma viride* (Fig.s 4a and 4b). This is consistent with the observations of [30] on the effect of nitrogen supplementation on fungal species. Better utilization of urea as a nitrogen source by *Aspergillus oryzae* when compared with other sources has been reported [31]. However, in this study urea was utilized inefficiently relative to ammonium sulfate as a nitrogen source in biomass and protein production. The combination of urea and ammonium sulfate was less efficient than ammonium sulfate alone.

E. Effect of Substrate Particle Size

Similarly, particle size had a profound effect on biomass and protein production. Considerable increase in total sugars and true protein were obtained from cassava peels with particle size 3.35 – 4.00 mm (Fig.s 5a and 5b). Lower particle sizes provided a greater saturated surface area but a lower interparticular porosity than larger particles [32] which pose difficulty in aeration/respiration. Larger particles provide better aeration/respiration opportunities but provide lesser surface area [33] which does not encourage the growth of the filamentous organism. An optimal particle size of 5mm for biomass production in *Saccharomyces cerevisiae* on sugar cane baggase has been reported [32]

Depending upon the nature of the substrate, the amount of water absorbed could be one or several times more than its dry weight, which leads to relatively high water activity on the solid/gas interface in order to allow higher rate of biochemical process. Low diffusion of nutrients and metabolites takes place in lower water activity conditions whereas compaction of substrate occurs at higher water activity. Hence, maintenance of adequate moisture level in the solid matrix along with suitable water activity is essential elements for SSF processes [33].

F. Effect of Initial Moisture Content

Fig.s 6a and 6b showed that the total sugars and true protein production was reduced at 50, 70, and 80% when compared with the production observed at 60% initial moisture content. The reduction in protein production at high initial moisture content of 70 and 80% may be due to the steric hindrance of the growth of *Trichoderma viride* which can be caused by compaction of the substrate, reduction in porosity of the solid matrix and consequent interference with oxygen transfer. On the other hand, if the quantity of the water becomes insufficient and does not allow a good diffusion of solutes and gas, the cell metabolism slows, or can stop, because of a lack of substrates or through too high concentration of inhibitive metabolites in or near the cell [34]. Lower moisture content

can cause reduction in solubility of nutrients of the substrate, low degree of swelling and high water

G.Chemical Composition of Fermented Cassava Peel during Solid State Fermentation

The changes in the chemical composition of the fermented cassava peel are presented in Table I. The result showed the effect of solid state fermentation with *Trichoderma viride* on chemical composition of cassava peel samples. Dry biomass increased in crude protein, true protein and ash while crude fat, crude fibre, carbohydrate, starch, cyanide and total dietary fibre reduced.

The crude protein increased from 4.21 % in unfermented samples to 10.43 % in fermented samples. Carbohydrate content reduced significantly ($p \leq 0.05$) while starch content of about 17.34 % was lost in solid state fermentation. The crude protein obtained was comparable to similar published systems. Reference [3] reported an increase in protein content of cassava peel from 5.6-14.4 % when fermented with *Aspergillus niger* for 20 days and a corresponding increase to 16.74 % when fermented with *Saccharomyces cerivisiea* during solid state fermentation. Using *Trichoderma* species, maximum reduction in starch in cassava wastes was observed during the first 12 days [35]. This period also coincided with optimum sugar and protein levels. The increased protein and decreased carbohydrate levels obtained in this study are an indication of the ability of *Trichoderma viride* to secrete enzymes such as amylolytic and cellulolytic enzymes which breaks the starch and non- starch polysaccharides to monomer sugars which are easily metabolized [35]. The bioconversion of starch or sugars to protein does occur, leading to the protein enrichment of the cassava peel. Similar studies on protein enrichment of solid waste have previously been carried out on barley, wheat and dehydrated beet pulp [36] as well as cassava [37]. This implied that *Trichoderma viride* had significant ($p \leq 0.05$) effect on the protein content. The increase in the crude protein was probably due to the additional crude protein produced in the fungal mycelia [38] or the mycelia protein and this is influenced by carbon to nitrogen ratio. Similar results had been reported [39] using sweet potato in solid state fermentation. Also this result is in line with [3], reportedly enriched protein of cassava peel and pulp with different fungi type. The fiber component decreased over the period of degradation which may be due to the hydrolytic nature of the fungi used for the biodegradation. The result here agreed with the earlier claim that disruption of cell walls and their degradation by microbial enzyme could be beneficial to host [40]. It was reported that available cell wall carbohydrate not attacked by digestive enzymes now seem wildly optimistic after biodegradation. The author then stressed that total breakdown requires the action not only of the enzymes responsible for the primary attack on the cell wall polysaccharide and glucan hydrolases but also of a second set of glycosidases able to reduce oligosaccharides to their monomeric components. During biodegradation, the enzymes from fungi breakdown polysaccharides into less complex structures. The ease of degrading any fiber component is a function of the enzyme composition of fungi and the physicochemical properties of the substrate.

The increase in the ash content may be suggesting that microbial fermentation increased the ash content of cassava peel which could be useful in animal feeds. This agreed with [41] with observed increase in ash content in cassava products subjected to fermentation with *Saccharomyces cerevisae*. Similar trend was reported by [42].

Cyanide reduced significantly ($p \leq 0.05$) in the fermented sample from 0.72 in the unfermented to 0.21mg/100g in the fermented samples. It is evident that *Trichoderma viride* was very efficient in cyanide detoxification.

IV. Conclusion

The optimal processing conditions for protein enrichment of cassava peel using solid state fermentation with *Trichoderma viride* are particle size 3.35 – 4.00 mm, initial moisture content of 60% at pH 6.0, and 30^{o}C incubation temperature with ammonium sulfate (10g N / kg substrate) as additional nitrogen source.

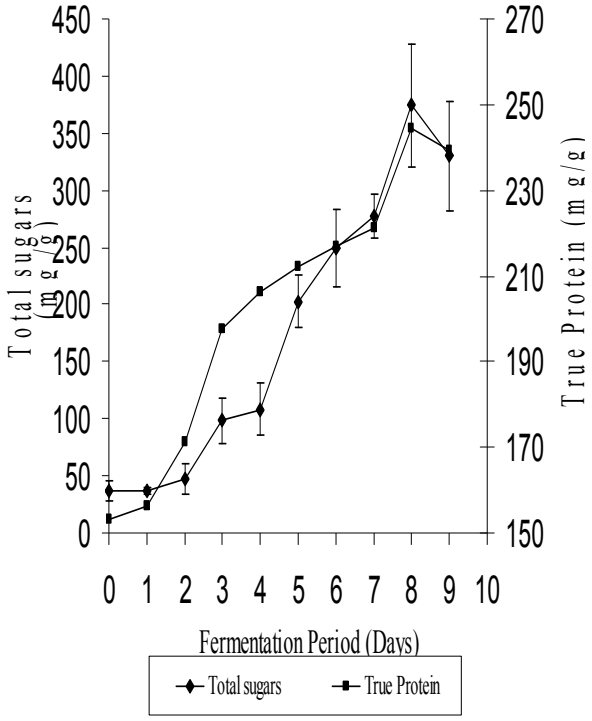


Fig. 1 Time Profile for soluble solid and true protein production in cassava peels during solid state fermentation using *Trichoderma viride.* The error bars indicate the standard deviation among three parallel replicates

FERMENTATION CONDITIONS
Cassava Peels: Unsoaked
Particle size: 0.60mm>p> 0.30mm
Initial Moisture Content: 60%
Initial pH: 5.00 +0.1

Nitrogen source: Ammonium Sulfate (10g N/kg substrate)

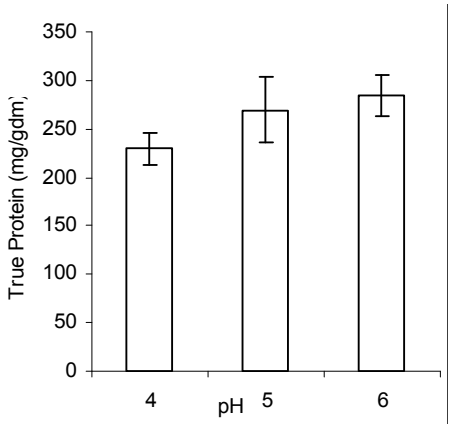


Fig. 2(a)

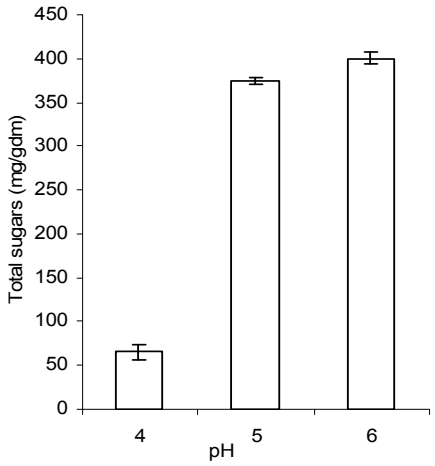
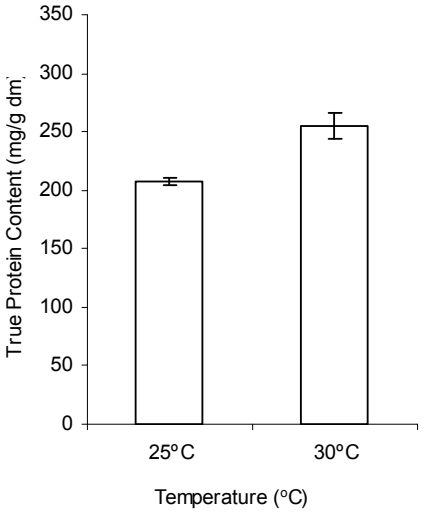


Fig. 2(b)

Fig. 2(a) and 2(b) Effect of Initial pH on protein enrichment and soluble sugars of cassava peels by *Trichoderma viride* during solid state fermentation for 8 days.The error bars indicate the standard deviation among three parallel replicates.



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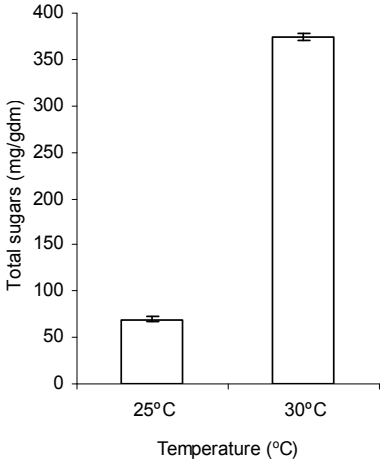

Fig. 3 (b)

Fig. 3(a) and 3(b) Effect of Incubation temperature on protein enrichment and soluble sugars of cassava peels by *Trichoderma viride* during solid state fermentation for 8 days. The error bars indicate the standard deviation among three parallel replicates

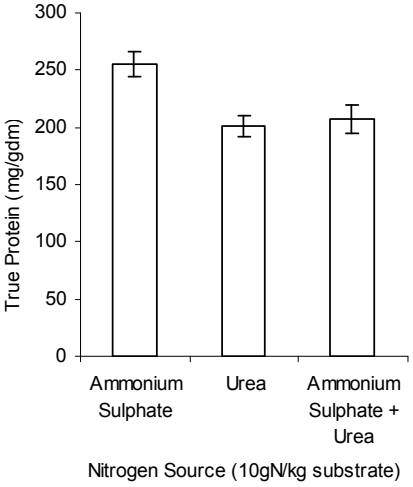

Fig. 4(a)

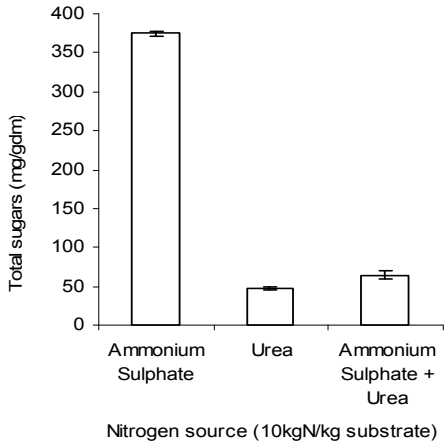

Fig. 4(b)

Fig. 4(a) and 4(b) Effect of nitrogen source on protein enrichment and soluble sugars of cassava peels by *Trichoderma viride* during solid state fermentation for 8 days. The error bars indicate the standard deviation among three parallel replicates.

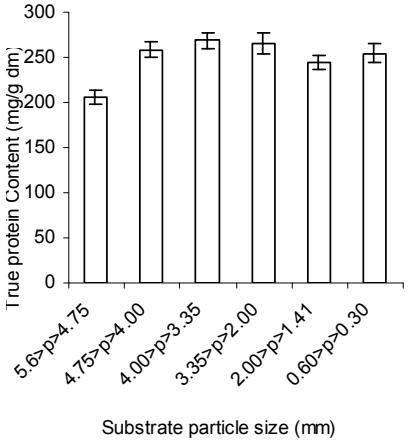

Fig. 5(a)

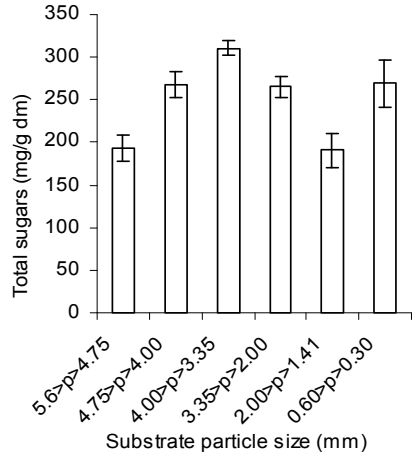

Fig. 5(b)

Fig. 5(a) and 5(b) Effect of particle size on protein enrichment and soluble sugars of cassava peels by *Trichoderma viride* during solid state fermentation for 8 days. The error bars indicate the standard deviation among three parallel replicates.

TABLE I
CHEMICAL COMPOSITION OF *TRICHODERMA VIRIDE* ENRICHED CASSAVA PEEL DURING SOLID STATE FERMENTATION

Composition (%)	Unfermented cassava peel	Fermented Cassava Peel
Crude Protein	4.21[b]	10.43[a]
True Protein	1.36[b]	7.90[a]
Moisture	8.73[a]	7.69[a]
Crude Fat	1.37[a]	1.20[b]
Crude Fibre	8.46[a]	6.37[b]
Ash	3.27[b]	7.82[a]
Carbohydrates by Difference	91.15[a]	80.55[b]
HCN(mg/100g)	0.72[a]	0.21[b]
Starch	51.93[a]	34.59[b]
Gross Calories (kcal/100g)	393.79[a]	374.72[b]
Total Dietary Fibre	24.96[a]	13.90[d]

Results are expressed on a dry matter basis. Each value is a mean of three independent experiments.
Means were separated using Duncan's Multiple Range Test
Means followed by the same superscript in the same row are not significantly different ($p \leq 0.05$)

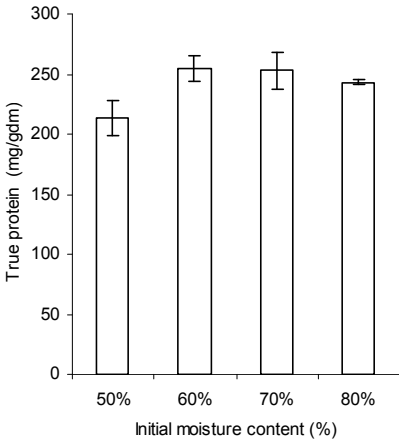

Fig. 6(a)

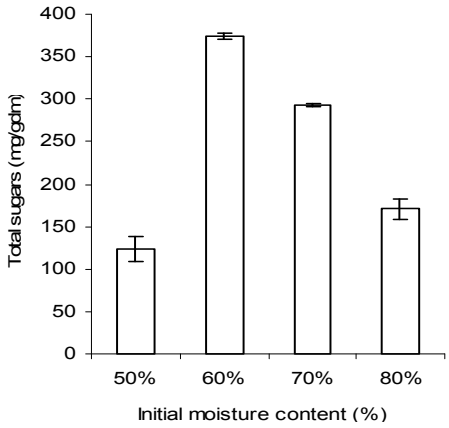

Fig. 6(b)

Fig. 6(a) and 6(b) Effect of Initial moisture content on protein enrichment and soluble sugars of cassava peels by *Trichoderma viride* during solid state fermentation for 8 days. The error bars indicate the standard deviation among three parallel replicates

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