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Protein Enrichment of Irish potato (*Solanium tuberosium*) peels through Solid Substrate Fermentation by Saccharomyces cerevisiae and Aspergillus Niger

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Abstract: *Flour prepared from dried potato peels were inoculated with pure strains of either Aspergillus niger or Saccharomyces cerevisiae and then left to ferment for seven days. Chemical analysis of the fermented peel mash revealed a significant (P< 0.05) increase in the crude protein content (13.62%) of the potato peel mash when compared with the unfermented potato peel (10.50%).The result also shows that fermentation increased significantly the crude protein, ash, lipid and fibre contents of the fermented potato peel mash, while a decrease in carbohydrate was observed. Moisture concentration of 125%, temperature of 30^{O}C and 25^{O}C and a pH of 3.5 and 5.5 were found to be optimum for the growth of A. niger and S. cerevisiae on the mash. Increase in all mass resulting from the growth of the two fungi contributed to the resultant increase in the crude protein content of the fermented mash. The fermented peels could therefore be a good source of cheap protein enriched feed.*
Keywords *- Potato peels; Fermentation; Crude Protein content; Fungi.*

I. Introduction

The white or Irish potato (*Solanum tuberosum*), also called the "earth apple", is grown in nearly all parts of the tropical and subtropical world and in warmer areas of the temperate regions. It has remained for centuries an important staple for many tropical communities [1]. *Solanum tuberosum* is the fourth largest yielding crop plant in the world, producing nearly 300 million metric tons of tubers per annum [2]. The potato peels are rich in phytonutrients [3], carbohydrates, high in starch (8-28%) but with only about 1-4% protein. Anon [4] reported that potato starch is a large-grained starch containing 25% amylose and 73% amylopectin and high phosphate content. A large amount of potato peels are discarded during processing for chips by many industries. These peels constitute a potential source of livestock feed ingredient. The major limitation in the use of potato peels for livestock feeding is its low protein content. Protein enrichment of potato peels through inexpensive means is therefore desirable.

Fermentation is one of the oldest and most widespread methods of preserving food. The fermentation of staples contributes significantly to food security by increasing the range of raw materials that can be used for the production of edible food products [5]. Fermentation enhances the nutrient content of foods through the biosynthesis of proteins, vitamins and essential amino acids. It also enhances micronutrient bioavailability and aids in degrading anti-nutritional factors [6].

Fungal fermentation has been identified as an inexpensive tool for increasing the protein level of substrates in a solid media fermentation technique. This study therefore sought to investigate how to increase the protein level of flour made from potato peels during solid state fermentation with *Aspergillus niger* and *Saccharomyces cerevisiae*.

II. Materials And Methods

2.1. Microorganisms

Local isolate of *A. niger* were obtained from decaying yam (*Dioscorea rotundata*) tuber and maintained on potato dextrose agar (PDA) slants at 28±2^{O}C. The subculturing was done once in a fortnight. Commercial dried baker's yeast (*Saccharomyces cerevisiae*) was purchased from Watt market in Calabar metropolis. The yeast was reactivated with 100 ml of warm (50^{O}C) sterile distilled water and maintained on slants of sterile Sabouraud dextrose agar (SDA) medium after subculturing. The inoculated slants were allowed to incubate at 30^{O}C for two days after which they were stored at 4^{O}C and sub-cultured once every fortnight. Spore suspensions were prepared in five ml sterile distilled water.

2.2. Substrate Preparation

The potato peels used in this study were obtained from fast food facilities in Calabar, Cross-River State. The peels were washed thoroughly with sterile distilled water, cut into tiny bits and dried in an oven at 60-^{O}C for 48 hours. The dried peels were milled in a hammer mill (Thomas Wiley Mill, Model ED-5, USA) and subsequently sieved with a 0.5mm screen mesh. The sieved flour was transferred into 250 ml Erlenmeyer flasks

and autoclaved at 121^{O}C for 15 minutes. They were then cooled to room temperature and subjected to chemical analysis to determine their proximate composition.

2.3. Fermentation of Substrates

Twenty grams of the dried sieved sample was weighed out into 250 ml Erlenmeyer flasks in triplicates, and moistened by adding 25 ml of sterile distilled water. The flasks were plugged with sterile cotton wool wrapped in aluminum foil and the mash was left to ferment for seven days. The mash was then dried to a constant weight. Some quantity was chemically analysed while the remainder was stored for other experiments.

2.4 Preparation of Inoculum

Aspergillus niger inoculum was prepared from fresh mature (three to five days old) cultures grown on PDA slants. The spores from PDA slants were covered with 5 ml of sterile distilled water and 0.2% Tween 80 was added to facilitate the preparation of the fungal inoculum. The colonies were carefully rubbed with a sterile loop; the isolates were then shaken vigorously for 15 seconds and then transferred to a sterile tube. Saccharomyces cerevisiae inoculum was prepared from stock vials by subculturing a loopful onto SDA plates. Five colonies were picked from 48 hours-old cultures and suspended in 5ml of sterile 0.85% saline. The resulting suspension was shaken vigorously for 15 seconds and then transferred to a sterile tube. All suspensions were quantified by plating on PDA plate for the fungi and on SDA plate for the yeast. Five serial 10-fold dilutions of each inoculum suspension were performed in sterile distilled water, and a 10µl of each portion of each dilution was spread over the surface of the agar plates with a sterile glass rod. The plates were incubated at 30^{O}C and were observed daily for the presence of growth. The colonies were counted with an electric colony counter as soon as possible after the observation of visible growth.

2.5. Determination of Optimum Conditions For Fermentation of Mash

Procedures were as described above but the mineral salt solution used was adjusted to different pH levels of 3.5, 4.0, 5.5 and 6.0. The mash samples were incubated at varying temperatures of 25, 30, 35 and 40^{O}C respectively. The quantity of the mineral salt solutions added were adjusted to moisture levels of 50, 70, 100 and 125% moisture (v/w) to determine the optimum moisture level needed for fermentation of the mash.

2.6. Fermentation of Potato Peel Mash Inoculated with Organisms:

The potato peel (flour) was weighed out in 20g quantities into 250 ml Erlenmeyer flasks and 25 ml of sterile mineral solution was added to each container to moisten the mash. The flasks were then autoclaved for 15 min at 121^{O}C. After sterilization, the flasks were then aseptically inoculated with two (2ml) of each active inoculum ($3x10^{9}$ cfu.ml^{-1}), properly labeled and plugged with sterile cotton wool. The flasks were left to ferment at 28±2^{O}C for 7 days. At the end of the fermentation period, the mash was dried and subjected to chemical analysis.

2.7. Compositional Analysis

The nutritional composition (ash, crude lipid and crude fiber) of the fungi/yeast fermented potato product was evaluated using the standard AOAC method [7]. The protein content was determined using the microkjeldhal method (N x 6.25) while the carbohydrate content was estimated by the difference method.

2.8. Statistical Analysis

The results are presented as the mean standard values of three replicates each. A one-way analysis of variance (ANOVA) and the Least Significance Difference were carried out. Significance was accepted at $P < 0.05$.

III. Results

The chemical analysis of the unfermented and fermented mash prepared from potato peels (*Solanum tuberosum*) revealed a significant increase ($P < 0.05$) in the protein content from 10.50% (unfermented) to 13.62% (fermented) after seven days fermentation (**Table 1**).

Fig 1 shows the crude protein produced by *A. niger* or *S. cerevisiae* growing on potato peel mash which had been adjusted to contain different moisture levels. The highest amount of crude protein was obtained when 20g of mash was supplemented with 25 ml of water representing 125% moisture level.

The effects of pH on the protein yield of potato peel mash fermented by *A. niger* or *S. cerevisiae* are presented in Fig 2. While *A. niger* generated maximum crude protein yield of 15.01% at pH 3.5, *S. cerevisiae* yielded its maximum crude protein level of 15.27% at pH 5.5.

The percentage crude protein yields of potato peel mash inoculated with *A. niger* or *S. cerevisiae* and fermented at varying temperatures are presented in Fig 3. A temperature of 30 ^{O}C and 25^{O}C gave the highest crude protein levels of 13.35% and 14.17% respectively.

Fermentation of the substrates with moisture levels of 125%, at incubation temperatures of 30^{O}C and 25^{O}C and with pH of each substrate adjusted to 3.5 and 5.5 respectively for *A. niger* and *S. cerevisiae* resulted in high growth rate of the organisms. Considerable cell mass was generated at these fermentation conditions as depicted by the further increase in the crude protein content with *A. niger* yielding 15.68% and *S. cerevisiae* yielding 18.62% respectively (**Table 2**).

IV. Discussion

The results obtained from this study revealed that fermentation can bring about desirable changes in the nutrient composition of potato peels. From this study, both fungi showed potential to increase the protein content of the potato peel mash. The yeast S. cerevisiae demonstrated the best ability to enrich the peel mashes in seven days. The results obtained for *A. niger* are comparable to those of [8] who reported similar findings using sweet potato peels in solid state fermentation.

There was an increase in the protein content compared to the unfermented peel from 10.50% to 15.68% when fermented with *A. niger*. Also the peels when fermented with *S. cerevisiae* had an improvement to 18.62%. This implied that the fungi and yeast had significant ($P< 0.05$) effect on the protein content. The increase in the crude protein observed could be attributed to the additional crude protein (extracellular enzymes) such as amylases produced by the fungal mycelia [9][10][11][12] and thus secreted into the fermenting mash in an attempt to make use of the starches as a carbon source [13].

Furthermore, increase in the growth and proliferation of the microorganisms in the fermenting potato peel mash may possibly account for the apparent increase in the protein content of the fermented peel mash [14] [15]. Similar results have been reported using sweet potato in solid state fermentation [8][16].

An optimum temperature and pH range of 25^{O}C and 5.5 respectively supported the highest crude protein formation when *S. cerevisiae* was grown on the potato peel mashes. This finding is in agreement with that of [17] who reported a temperature range between 25^{O}C and 30^{O}C to be favorable for the growth of most yeast. Similar findings were also reported by [14]. This observation further confirms that the increase in crude proteins observed is as a result of an increase in cell mass generated by the organism. A temperature range of 30^{O}C was observed to support the highest crude protein formation in the case of *A. niger,* when the potato peel mash was fermented. This finding agrees with that of [18] who reported an optimum temperature of 30^{O}C for *A. niger.* This temperature has also been reported to support extracellular enzyme production during the organism's growth [19].

The levels of moisture concentration were found to affect crude protein yields by both microorganisms. The lowest moisture concentration (50%) supported the production of the least crude protein, whereas the highest moisture concentration (125%) investigated supported the formation of the highest amount of crude protein. These results agrees with the reports of [20] who stated that maximal production of microbial protein required certain amount of moisture in order to render the contents of the substrate soluble for the fungi to assimilate and grow.

V. Conclusion

The results obtained in this study have shown that growing of fungi and yeast on potato peel mash can greatly enrich its protein content. In view of this significant protein yield increase, this by-product could be a good supplement in compounding animal feed provided that it is acceptable and highly digestible.

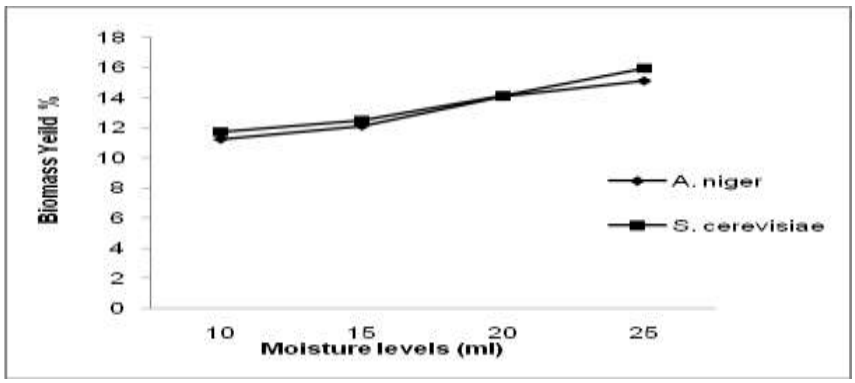

Figure 1. Effect of moisture levels on the crude protein yield of *A. niger* and *S. cerevisiae* on potato peel mash after fermentation

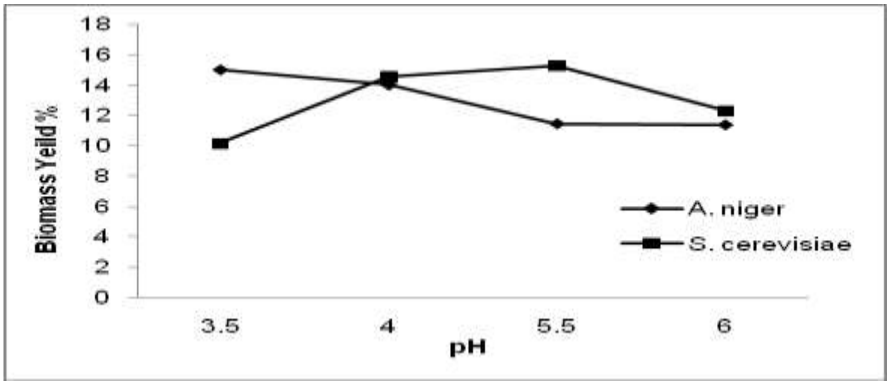

Figure 2. Crude protein yields resulting from the growth of *A. niger* and *S. cerevisiae* on potato peel mash adjusted to different pH levels

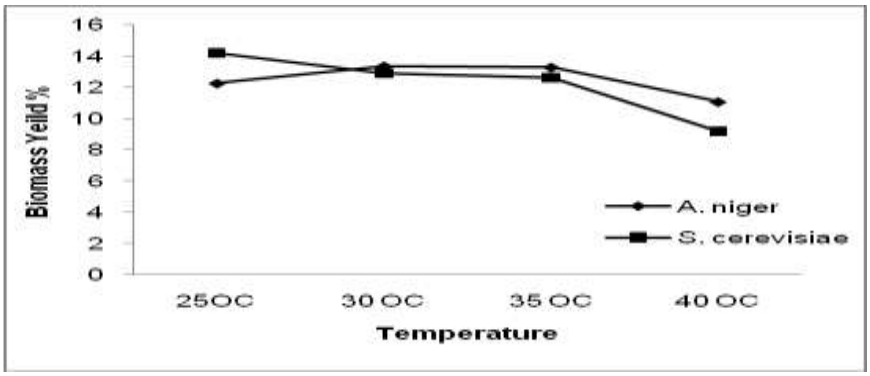

Figure 3. Crude protein yields resulting from the growth of *A. niger* and *S. cerevisiae* on mash prepared from potato peels and incubated at different temperatures

Table 1: Proximate compositions of potato peel mash samples after processing

Nutrients (%)	Unfermented	Fermented
Crude Protein	10.50 ± 0.1	13.62 ± 1.3
Crude Lipid	1.32 ± 0.2	0.48 ± 0.5
Crude Fiber	6.20 ± 0.2	8.75 ± 0.3
Carbohydrate	76.65 ± 0.2	69.78 ± 0.1
Ash	5.33 ± 0.6	7.37 ± 0.4

Values are mean ± standard deviation, based on three replicate values

Table 2: Chemical compositions of potato peel mash inoculated with *A. niger* and *S. cerevisiae* and fermented for seven days.

	Mash inoculated with	
Nutrients (%)	*A. niger*	*S. cerevisiae*
Crude Protein	15.68 ± 0.2	18.62 ± 1.3
Crude Lipid	0.96 ± 0.4	1.42 ± 0.2
Crude Fiber	10.25 ± 1.3	8.50 ± 1.1
Carbohydrate	64.85 ± 2.5	63.48 ± 0.1
Ash	8.26 ± 0.5	7.98 0.5

Values are mean ± standard deviation, based on three replicate values.

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