

Protein Enrichment of Potato Peels Using *Saccharomyces cerevisiae* via Solid-State Fermentation Process

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Abstract

In order to add value to potato peels and also curb their environmental pollution problems, this study investigated the protein enrichment of potato peels with *Saccharomyces cerevisiae* via Solid-State Fermentation (SSF). SSF is a fermentation process which involves solid matrix and is carried out in absence or near absence of free water. SSF of potato peel mashed was carried out with *S. cerevisiae* at 30°C, pH of 5.5, moisture adjustment between 40 and 90%, addition of ammonium sulphate and urea salts as nitrogen supplements for the microorganisms for 3 days. The results showed that the percentage crude protein content of all the fermented samples increased significantly when compared with the unfermented sample. 40% moisture content adjustment and ammonium sulphate as nitrogen source gave the best result. The crude protein increased from 12.5% to 21.86%, which is 74.88% increment for ammonium sulphate supplementation, and 12.5% to 18.42%, which is 47% increment for urea supplementation. Therefore, the fermented peels could serve as good source of cheap protein enriched feed for livestock.

Keywords

Potato Peel, *Saccharomyces cerevisiae*, Urea, Ammonium Sulphate, Crude Protein, Solid-State Fermentation, Protein Enrichment, AOAC 1990

1. Introduction

Protein-energy malnutrition remains a major public health problem in many developing countries and there is need to increase the daily intake of protein, especially animal protein, using cheap and non-conventional sources such as agricultural wastes and by-products of food processing [1]. Nigeria has now an

alarming rate of population growth of both human and livestock, which include goats, sheep, poultry, etc. [2]. Food security for these teeming millions of people and their domestic animals is of immense concern to the authorities as most staple foods for humans also serve as livestock feeds, thereby exalting serious competition between human and livestock for these scarce food items [3]. Among the nutritional requirements, the need for energy is most paramount, and an animal fuel consists largely of carbohydrate, protein and fat [3] [4]. The potato peels are rich in phytonutrients [5], carbohydrates, high in starch (8% - 28%) but with only about 1% - 4% protein [6]. 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 [6].

Micro-organisms have the ability to upgrade low protein plant material to high protein feed via fermentation [7]. 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 [8]. Fungal fermentation has been identified as an inexpensive tool for increasing the protein level of substrates in a solid-state fermentation technique [6].

Solid-state fermentation (SSF) is defined as the fermentation process in which micro-organisms grow on solid materials without the presence of free water [9]. The selection of a proper substrate is essential in SSF, where the substrate will act as a physical support and nutrient source for the micro-organism [10]. In Solid state fermentation, different agro-industrial wastes are used extensively and the ability of the microorganisms to utilize this substrate depends on various parameters like moisture level, particle size and nutrient compositions of the substrates. Fungi have been exploited to convert carbohydrates, lignocelluloses and other industrial waste into feedstuffs rich in protein due to the following characteristics: ability for a very fast growth rate which can be easily modified genetically for growth on a particular substrate under particular culture conditions, high protein content varying from 35 to 60 percent, ability to grow on solids and their nutritional values are as good as other conventional foods rich in protein [6].

The objective of this study was to evaluate the performance of *S. Cerevisiae* in the protein enrichment of potato peels by solid-state fermentation.

2. Materials and Method

2.1. Micro-Organism

Saccharomyces cerevisiae was obtained from the culture collection of the department of Microbiology Enugu State University of Science and Technology (ESUT) Nigeria and maintained on slants of sterile yeast agar medium after sub-culturing. The inoculated slants were allowed to incubate at 30°C for two days after which they were stored at 4°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 through hand peeling of purchased Irish potatoes from Ogbete main market Enugu. Before the peeling process the potatoes were washed with water and rinsed with distilled water. The peels obtained were also washed with sterile distilled water and dried in an oven at 105°C for 4 hours. The dried peels were milled inside the Laboratory, sieved to obtain 0.5 mm mesh sized flour and kept ready to be used for SSF process while some quantity was chemically analysed.

2.3. Determination of Optimum Day(s) for Fermentation of the Substrate

Twenty grams of the dried sieved sample was weighed out into 250 ml Erlenmeyer flasks in triplicates and moistened by adding 26.83 ml of sterile distilled water, which corresponded to 60% moisture content adjustment [11]. Also 1.32 g of Ammonium sulphate was added as a nitrogen source supplement, 0.1 g of Streptomycin was added to avoid cross contamination and sterilization was carried out with an autoclave for 15 minutes at 121°C. The flasks were plugged with sterile cotton wool wrapped in aluminium foil and the mash was left to ferment for five days. The mash was dried to a constant weight, and then followed by the determination of the Crude Protein content and reducing sugar of the fermented sample for ascertaining the optimum day(s).

For the calculation of the volume of sterile distilled water used for moisture content adjustment of the sample, the formula below was used [11];

$$A = \frac{B \times (C - D)}{100 - C} \quad (1)$$

A = Amount of water to be added in ml; B = measured weight of the sample; C = % of water to be added; D = Moisture content of the sample obtained by proximate or chemical analysis.

2.4. Fermentation of Potato Peel Mash Inoculated with Organisms

Twenty grams of the dried sieved samples were weighed out into 250 ml Erlenmeyer (Conical) flasks in duplicates and moistened with sterile distilled water according to the calculated volume of the moisture content adjustments of 30%, 40%, 50%, 60%, 70%, 80% and 90% respectively. In studying the effects of nitrogen supplements and their input on the protein enrichment, the mixture were supplemented with each of the mineral salts: 10 g of N_2 as $(NH_4)_2SO_4$ /kg of the weighed sample used and 10 g of N_2 as $CO(NH_2)_2$ (Urea)/kg of the weighed sample used respectively. Temperature of 30°C and pH of 5.5 were found to be the optimum for the growth of *S. cerevisiae* on the mash [12]. In all the media, initial pH was adjusted to 5.5 using 1 N NaOH and 1 N HCl. Furthermore 0.1 g of Streptomycin was added to the mixture to avoid cross contamination and autoclaved for 15 minutes at 121°C. After sterilization, the flasks were then aseptically inoculated with two (2 ml) of active inoculums, properly labelled and

plugged with sterile cotton wool. The flasks were left to ferment at 30°C for 3 days. At the end of the fermentation period, the mash was dried and subjected to chemical (Proximate) analysis.

2.5. Compositional Analysis

The samples were analysed for proximate composition (moisture content, crude fat, crude fibre, crude protein, ash and carbohydrate) before and after the yeast fermentation process using the standard AOAC 1990 method [13].

3. Results and Discussion

3.1. Estimation of the Fermentation Period

Twenty grams of the dried sieved samples were weighed into 250 ml Erlenmeyer flasks in triplicates and moistened by adding 26.83 ml of sterile distilled water which corresponded to 60% moisture adjustment. 1.32 g of Ammonium sulphate was added to serve as a nitrogen source, the mixture was sterilized using an autoclave. Solid state fermentation was carried out for 5 days in order to establish a time profile for the SSF where effects of other parameters will be evaluated. **Figure 1** shows the time profile of reducing sugar and protein enrichment during the fermentation period.

It was observed from **Figure 1** that after 3 days of fermentation, the reducing sugars dropped drastically from 38.43% to 3.07% and the crude protein increased from 12.50% to 20.66%. Crude Protein content of the fermented samples increased almost linearly during the first 3 days, and after which a significant decrease was observed probably due to proteolysis after 3 days [14]. Therefore 3 days was obtained to be the optimum fermentation period. The relatively high protein content improvement obtained after 3 days showed the ability of *S. cerevisiae* to grow in this substrate. Also it showed that reducing sugars were promptly

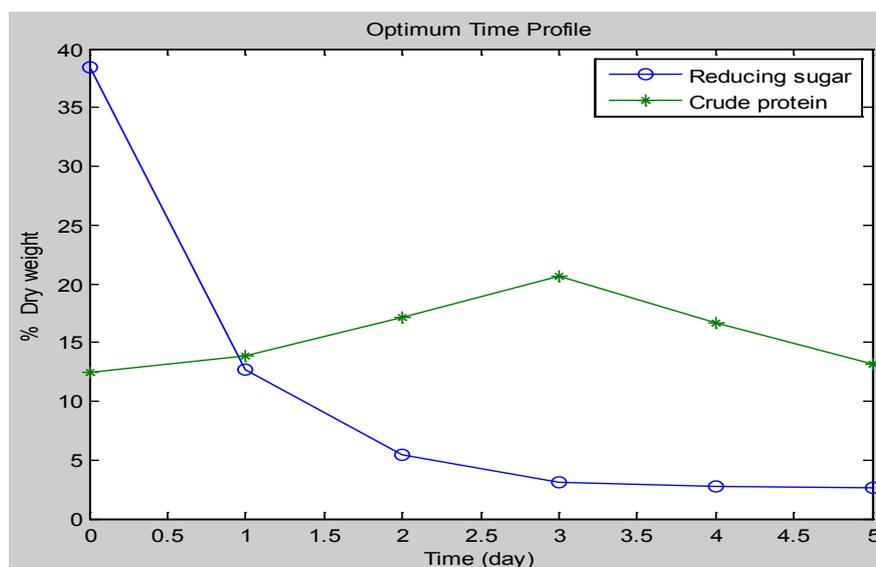


Figure 1. Determination of the optimum fermentation time.

utilized by *Saccharomyces*, since they were nearly exhausted after 3 days. This behaviour shows that *S. cerevisiae* is able to efficiently metabolise available sugars as its carbon source for protein enrichment of the residue [14].

3.2. Effect of Nitrogen Source Supplementation

Next to carbon, nitrogen has a pronounced influence on microbial growth and activity. In general inorganic nitrogen sources in their most reduced forms are more efficiently utilized than other forms of nitrogen sources by fungi. In micro-organism, nitrogen (both organic and inorganic forms) is metabolized to produce amino acids, nucleic acids, proteins and cell wall components. It is clear that organisms show slight differences in the growth pattern in the presence of different nitrogen sources due to their relative solubility. In the present study, inorganic compounds such as ammonium sulphate and urea at different moistures were used. It was observed that in the presence of these compounds there was significant increase in the microbial growth and activity, which was buttressed by the increase of the crude protein after fermentation.

From **Table 2**, it was observed clearly that there was protein enrichment via solid state fermentation using *S. cerevisiae* at different moisture levels and nitrogen source supplementation after 3 days. This implies that fungi and yeast have significant effect on the protein content. The crude protein concentration for the unfermented potato peel sample from **Table 1** is 12.5% with a standard deviation 0.15, while the least crude protein concentration for the fermented potato peel sample is 12.60% with a standard deviation of 0.14 and maximum value of 21.86% with a standard deviation of 0.03. 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 [15] [16] [17] secreted into the fermenting mash in an attempt to make use of the starches as carbon source [18]. So it is evident that availability of nitrogen is a major controlling factor in the final biomass and consequently crude protein yields.

Among the two nitrogen sources investigated, the supplementation of potato peel mash with ammonium sulphate resulted in the highest crude protein yield of 21.86% compared to the best value of 18.42% obtained using urea as a nitrogen supplement. This could be attributed to high solubility of ammonium sulphate in water, and also to the fact that apart from providing nitrogen, it also provides sulphur, an element required by *S. cerevisiae* for growth in the medium.

3.3. Effect of Moisture Adjustment

The moisture content is one of the critical factors in SSF media that attributes to biosynthesis and secretion of the enzymes [19]. According to [19] the critical importance of moisture level in SSF media and its influence on the biosynthesis or secretion of enzymes can be attributed to the interference of moisture in the physical properties of the solid particles. Generally the moisture level of substrates

Table 1. Chemical compositions (Proximate analysis) of unfermented potato peel.

Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	Moisture Content (%)	Carbohydrate (%)	Ash (%)
12.5 ± 0.15	2.2 ± 0.10	8.71 ± 0.10	6.35 ± 0.05	66.74 ± 0.00	3.5 ± 0.26

Values are mean ± standard deviation, based on triplicate values.

in solid state fermentation processes vary between 30% - 85% and have marked effect on growth kinetics of fermenting organism [20] [21] [22].

Moisture optimization can be used to regulate and to modify the metabolic activity of the microorganisms [19]. According to [19] report, high moisture levels of substrate lead to decreased porosity, lower oxygen diffusion, increased risk of bacterial contaminations, enhanced aerial mycelial formation, reduction in gas volume, decreased gaseous exchange and change in the degradation of the lignin. Likewise low moisture levels might lead to reduction in the solubility of the nutrients of solid substrates, lower degree of swelling and higher water tension according to [19]. Therefore initial moisture content plays an important role in microbial growth and activity during SSF. Most of the microbial growth and product formation takes place at or near the surface of the solid substrate [19], thus it is very crucial to provide optimized water levels that control the water activity of the fermenting substrate for achieving maximum product [23]. Since the water acts as a vehicle for substrate transport and as a reactant it may be expected that activity of water affects enzymatic conversion during fermentation [24]. **Table 2** shows the moisture content variation and its effects on crude protein production via solid state fermentation. The results showed that at relatively lower moisture content adjustment, the higher the amount of crude protein produced by *S. cerevisiae*. This finding could be related to the fact that when water content was raised in the substrate, pores were filled and oxygen mass transfer was diminished [25].

This agrees with the report of [26] who stated that maximum production of microbial protein requires a certain amount of moisture in an optimum volume in order to render the contents of the substrate soluble for the fungi to assimilate and grow. Therefore, from the results tabulated above, 40% moisture gave the best value for the crude protein enrichment for both ammonium sulphate and urea salts (nitrogen salts). It was also observed that the amount of crude protein tends to drop as the volume of water added increases. It can be inferred also that much water makes the system less saturated and as such reduces the concentration of the nutrient availability for the micro-organism.

Table 3 shows the corresponding volume of sterile distilled water added in ml to the mixture to aid the moisture content adjustment. The formula for the moisture content adjustment calculation is stated above.

According to literatures high moisture results in low substrate porosity which in turn prevents oxygen penetration, whereas low moisture content may lead to poor accessibility of nutrients resulting in hampered microbial growth. Also a previous work done in this area that investigated moisture contents from 10% to

Table 2. Crude Protein content of the potato peel mash inoculated with *S. Cerevisiae* and fermented for 3 days with different nitrogen sources.

Moisture Content in %	Ammonium sulphate supplementation (%)	Urea supplementation (%)
30	12.80 ± 0.14	12.60 ± 0.14
40	21.86 ± 0.03	18.42 ± 0.08
50	21.51 ± 0.01	17.48 ± 0.03
60	20.66 ± 0.11	15.01 ± 0.27
70	17.21 ± 0.07	14.79 ± 0.01
80	16.61 ± 0.04	13.66 ± 0.04
90	14.73 ± 0.01	13.01 ± 0.04

Table 3. The moisture content adjustment with their corresponding volumes of water added to the system in ml.

Moisture content in %	Corresponding volume of sterile water added in ml
30	6.76
40	11.22
50	17.46
60	26.83
70	42.43
80	73.65
90	167.30

60% [11], showed no significant protein enrichment when compared with the crude protein value of the unfermented sample between 10% to 30% moisture. Therefore, it can be inferred conclusively that 40% moisture content produced the best results for crude protein enrichment using *S. cerevisiae* in a solid state fermentation process with both nitrogen sources (Table 4).

Based on the observation that 40% moisture content produced the best results for crude protein enrichment, below are the tabulated results for the proximate analysis of the fermented potato peel sample.

Micro-fungi solid state fermentation of potato peel mash caused a significant decrease in the carbohydrate content [12]. This decrease could be attributed to the ability of the fungi to hydrolyze starch into glucose and ultimately the glucose will be used as carbon source to synthesize biomass rich in protein [12]. The decrease in the carbohydrate content could also be attributed to the significant increase in the protein content of the peels fermented with microorganisms [12]. There was a slight decrease in the fat concentration of the fermented when compared with the unfermented sample at 40% moisture content, but the crude fat concentration decreased as the percentage moisture content increased. It can be inferred that at 40% moisture the microorganisms had enough (optimum) carbon source to grow from the easily hydrolysed starch and did not use the fat. Table 5 shows the variation in the crude fibre concentration at increasing moisture contents.

Table 4. Composition of potato peel samples treated at 40% moisture content.

Nutrients (%)	Fermented sample with Ammonium sulphate	Fermented sample with Urea	Unfermented potato sample
Crude Protein	21.86 ± 0.03	18.42 ± 0.08	12.50 ± 0.15
Crude Fat	2.00 ± 0.01	2.10 ± 0.14	2.20 ± 0.10
Ash	5.00 ± 0.03	5.21 ± 0.01	3.50 ± 0.26
Crude Fibre	12.81 ± 0.06	13.50 ± 0.44	8.71 ± 0.10
Carbohydrate	58.33 ± 0.07	60.77 ± 0.37	66.74 ± 0.00

Values are mean values ± standard deviation, based on duplicate values.

Table 5. Crude fibre concentrations of the potato peel mash inoculated with *S. cerevisiae* and fermented for 3 days.

Moisture Content in (%)	Ammonium sulphate supplementation (%)	Urea supplementation (%)
50	1.92 ± 0.04	1.93 ± 0.04
60	1.80 ± 0.00	1.86 ± 0.03
70	1.50 ± 0.11	1.68 ± 0.01
80	1.43 ± 0.01	1.46 ± 0.01
90	1.20 ± 0.01	1.30 ± 0.14

Values are mean values ± standard deviation, based on duplicates.

The ash and fibre concentrations of the fermented potato samples increased relatively to the increase in moisture content. The increment in the ash and fibre concentration could be attributed to the inorganic content of the nutrient medium [12].

4. Conclusion

It was demonstrated in this research that the percentage crude protein content of potato peels can be improved (protein-enrichment) via solid state fermentation (SSF). This research is an immense addition in areas of biotransformation of tuber peels (abundant agrowastes) that are rich in carbohydrate to equally serve as protein source for livestock feed, as well as curbing their pollution menace. Solid-state fermentation of potato peel mash was carried out with *S. cerevisiae* at temperature of 30°C, pH of 5.5 for 3 days with moisture adjustment ranging from 40% to 90%, and nitrogen supplementation using ammonium sulphate and urea salts respectively. The results obtained showed significant protein enrichment with both nitrogen salts and at different ranges of the moisture adjustments. The best results for both nitrogen salts supplementation were obtained at 40% moisture adjustment, which was in agreement with the finding that excessive moisture content could cause the pores to be over saturated with water, thereby reduce oxygen mass transfer [25]; therefore, optimum moisture content of a system in SSF was imperative. The protein enrichment was better with ammonium sulphate than with urea as nitrogen supplements, which could be as a

result of the high solubility of the ammonium sulphate in water and also to the fact that apart from providing nitrogen, it also provided sulphur, an element required by *S. cerevisiae* for growth in the medium [12]. The protein-enrichment of cassava and yam (water yam and cocoyam) peels is recommended for further studies as they are also abundant agrowastes that contribute to environmental pollution.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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