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Nutritional Evaluation of the Protein-enrichment Potentials of Sweet Potato Peels Subjected to Submerge Fermentation with Bacteria Isolate from Pineapple Peels

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Abstract: As the global population rises, the demand for protein in food and animal feed also escalates, straining conventional sources and driving up the cost of animal feed. This highlights the need for alternative, cost-effective protein sources. Agricultural waste, particularly sweet potato peels, remains underutilized despite its potential as a valuable protein-enrichment resource. This study aimed to enhance the protein content of sweet potato peels through submerged fermentation using Bacillus isolates from pineapple peels. The fermentation process was conducted for three weeks with three groups: *Saccharomyces cerevisiae*, bacterial isolates from pineapple peels, and spontaneous fermentation as a positive control. The unfermented sample served as the negative control. Proximate analysis and amino acid profiling were performed on both fermented and unfermented samples. The fermentation process led to a significant increase in protein content, with 17 amino acids detected. This study demonstrates the potential of utilizing sweet potato peels, a common agricultural waste, as a protein-enriched resource, offering a sustainable alternative protein source for food and feed applications.

Keywords: Protein-enrichment, proximate analysis, amino acid profiling, gene expression analysis, RNA sequencing

Introduction

The increasing world demand for food and feed proteins has spurred the search for nonconventional protein sources that meet protein requirements [1]. The International Feed Industry Federation in 2019 predicted that there would be over 10 billion people on the planet by the year 2050 [2]. Moreover, it is anticipated that the growing population would eat nearly twice as much animal protein [3] and consumption of chicken meat and Pork is predicted to increase by 173 and 105%, respectively, between 2010 and 2050 [4]. This suggests that in the future, animal feed will play a crucial role in the integrated food chain. Therefore, there are needs to find alternative Protein sources for both Animal feed and Human consumption.

Protein-energy malnutrition is a serious public health issue in many developing countries and increasing daily protein intake, especially animal protein is necessary to solve this issue but the high cost, scarcity and competition makes difficult it. Low-cost sources of protein can be used to do meet up, such as agricultural waste and byproducts of food production [5]. Tuber crops, such as sweet potatoes, yams, and cassava, are high in carbs but low in protein, which poses a disposal problem for the environment.

In the food supply chain, almost one-third of all food produced for human consumption is lost or wasted globally [6]. This translates to an estimated 1.3 billion tons of food lost annually in addition to a significant loss of resources used in food production (such as land, water, and fossil fuels). Potatoes, cassava, sweet potatoes, yams and taro are the major roots and tubers produced globally. The use of waste materials for the production of protein is a promising approach towards sustainable and cost-effective protein production. Sweet potato peels are such waste materials which are generated in large quantities in many tropical countries and sweet potato peel contains high amounts of protein, fiber, and other nutrients [7]. However, due to its high lignin content, it is not readily digestible by animals or humans. It's noteworthy that the conventional protein sources are not enough to meet the protein demand, and converting food and agricultural wastes into nutritious products can be a great way to reduce pollution and provide valuable food and feed for humans and animals. Farms and food processing industries generate a considerable amount of waste every day, which makes up more than 30% of global agricultural productivity [8] and can be reutilized which conjugates the circular economy.

Consequently, a lot of plant science research focuses on developing new, high-yield crop, fruit, and vegetable types that are more suited to shifting climatic conditions in order to boost productivity [9]. However, there is still more space for enhancing food safety via reducing food loss and waste recycling, enhancing the nutritional value of byproducts, lengthening storage times, and boosting nutritional value. This is where fermentation enters the picture as an affordable, adaptable, and tried-and-true method that improves the nutritional value and shelf life of food items. Along with cooking, smoking, or air drying, fermentation has the potential to produce new food products from non-food biomass life and increase efficiency and product range [10].

Fermentation was fully industrialized millennia ago in ancient Mesopotamia and Egypt to produce bread and beer; it is also an elegant and simple technology as these microorganisms do most of the work without much human involvement [11]. The seminal discovery by Louis Pasteur regarding the role of microorganisms in the process of fermentation established a foundational framework for the advancement of technology, transitioning from conventional spontaneous fermentation methods to the application of precisely defined starter cultures [12].

Currently, fermentation is extensively employed in the production of a diverse array of products, including alcoholic beverages, bread and pastries, dairy products, pickled vegetables, soy sauce, among others.
Recent advancements in genomics and synthetic biology facilitate precision fermentation for targeted compound production in the food, chemical, and medicinal sectors [13]. Moreover, the integration of genomics with fermentation enhances the potential for innovative food and product development, and fermentation is recognized for augmenting the protein content in high-sugar foods [14]. Since microbes are involved in fermentation and pineapple peels are often disposed as an agricultural waste despite its availability, it is on this note that we decided to use the bacteria isolate of the peels because Pineapple peel powder in probiotic yogurt enhances probiotic viability and maintains anti-mutagenic and antioxidant properties during refrigerated storage [15] and also Fruit residues, such as pineapple and papaya peels, contain excellent lactic acid bacteria species and abundant feed nutrients, making them potential food resources for livestock [16].

Materials and Methods

Sample collection and preparation

Sweet potato peels were collected from Lapai Market, Niger State, Nigeria, and processed into flour. Pineapple peels were also collected and stored for bacterial isolation.

Fermentation process

Bacterial isolates from pineapple peels were used to ferment sweet potato peels under controlled conditions. Three fermentation groups were established: (i) bacterial isolate fermentation (SPPB), (ii) yeast fermentation using *Saccharomyces cerevisiae* (SPPY), and (iii) spontaneous fermentation (SPPS). A control group (SPPC) was maintained without fermentation.

Proximate analysis

The proximate analysis methods, including protein, carbohydrate, moisture, ash, lipid, and crude fiber determination, were conducted according to standard procedures described in AOAC (2010).

Determination of protein content

A 0.2 gram of the sample were weighed into a filter paper and transferred into a clean, dried Kjeldahl flask. 1 mL of concentrated sulfuric acid was added to the flask in the presence of 0.5 grams of selenium catalyst, and subsequently, 4 mL of concentrated sulfuric acid was added to prevent loss of sample particles due to foaming. The flask was gently heated in a fume cupboard using a heating mantle in an inclined position and allowed to digest (digestion is complete when the liquid is clear and free from black or brown color). The flask was allowed to cool and then diluted with 50 mL of distilled water. 10 mL of the sample and 10 mL of NaOH were measured into a 250 mL glass tube.
Approximately 50 mL of boric acid solution was measured into a 500 mL Erlenmeyer titration flask, and a few drops of screened methyl red/bromocresol green indicator were added. The Erlenmeyer flask was placed on the receiving end of the delivery tube, dipping just below the level of the boric acid solution. An automatic Kjeldahl distillation system was used for distillation, taking 5 minutes for each sample. The distillate was titrated with 0.1 mL HCl until the first trace of pink color appeared.

$$\% \text{ Crude Protein} = (A - B) \times 0.05 \times 1.04007 \times 6.25 / 0.2$$

Where: - A = Titre value of the sample; - B = Titre value of the blank;
- 0.05 = Normality or molarity of acid;- 1.04007 = Nitrogen factor; - 6.25 = Conversion protein factor

Determination of moisture

The powdered sample was weighed at 5 g and put into a petri dish using a spatula. After that the sample was dried at 105^{o}C for about 2 h in the oven. The sample was weighed after it was cooled in the desiccator.
To determine the moisture content, the following formula was used
Calculation: % moisture = w2 - w3 /w2-w1 x 100
Where: W1 = initial weight if the empty crucible; W2 = weight of crucible + sample before drying
W3 = final weight of crucible + sample after drying; Total solid = 100% moisture

Determination of crude ash

The sample was weighed at 2 g using analytical weigh balance was put in the crucible and heated in the muffle furnace at 550°C for 5 hours. The sample of the ash was then removed and allowed to cool in a desiccator and weighed to determine its weight.
The following formula was used to analyze the preparation of ash.
Calculation: % Ash = W2– W1/W X 100
Where: W = weight of sample analyzed; W1 = weight of empty crucible; W2 = weight of crucible and ash

Determination of lipid

This was determined using the continuous solvent extraction gravimetric method. Accordingly, a measured weight of the sample (2 g) was wrapped tightly in a previously weighed 15 cm porous paper (Whatman No 1 paper). The wrapped sample was put in a porous thimble and placed in a Soxhlet reflux flask. The reflux flask was mounted

onto an oil extraction flask containing about 200 ml of n-hexane. The upper end of the reflux flask was coupled to a condenser. The solvent in the flask was heated to vaporize and condense into the reflux flask enveloping the wrapped sample (extracting the oil in it). When the reflux flask is filled up, Here's the corrected version of the text:
The oil extracted is siphoned into the extraction flask, and the cycle continues. The processes of vaporization, condensation, extraction, and reflux were allowed to go on repeatedly for up to 8 refluxes. The extracted wrapped samples were removed with the aid of long forceps and dried, while the solvent was measured.
The fat content was calculated as the percentage weight of the oil extracted from the sample using the formula below
Calculation: % crude fat content = W2-W3/W2-W1 x 100
Where: W1 = weight of the empty filter paper; W2 = weight of the paper and sample before extraction
W3 = weight of the paper and sample after fat extraction and drying

Determination of crude fibre
Two grams of the sample were weighed and boiled in 12.5 mL of 1.25% H_2SO_4 solution under reflux for 30 minutes. It was washed repeatedly with hot distilled water using a two-fold Muslin cloth to retain the particles. The washed sample was carefully transferred quantitatively back into the flask and boiled for another 30 minutes in 12.5 mL of 1.25% NaOH solution. Thereafter, it was thoroughly washed again with hot distilled water, flushed with n-hexane to remove any traces of fat, and dried in the oven for 30 minutes at 100°C, cooled in a desiccator, and weighed using analytical weighing balance (Presica Switzerland).
The crude fiber contained in the sample was burnt out in the muffle furnace at 550°C for 2 hours, leaving the ash in the crucible, which was cooled in the desiccator and weighed.
The formula below was used to calculate the crude fiber content:
% crude fiber = (W2 - W3) / W × 100
Where: - W = Weight of the sample analyzed; - W2 = Weight of the sample and crucible after boiling, washing, and drying; - W3 = Weight of the crucible and sample after burning the washed, dried sample in the furnace

Determination of carbohydrate
The nitrogen-free extract (NFE) method was used. Carbohydrate content was calculated as the weight difference between 100 and the summation of other proximate components as Nitrogen Free Extract (NFE).
Calculation: % NFE = 100 - (A + B + C + D + E)
Where: - A = Protein content; - B = Lipid content; - C = Ash content; - D = Fiber content; - E = Moisture content

Amino acid analysis
Amino acid composition was determined following the method described by AOAC (2016) using reverse-phase high-performance liquid chromatography (HPLC). The defatted samples were utilized at the end of the 3rd wee to estimate amino acids. The sample (30 mg) was hydrolyzed with 6N HCI at 110°C for 24h. Amino acid analysis was performed on reverse phase-high pressure liquid chromatography (HPLC) (Buck scientific BLC 10/11 USA) equipped with UV 338 nm detector. A C18, 2.5 x 200 mm, 5 um column and a mobile phase of 1:2:2 (100 mM sodium phosphate, pH 7.2: Acetonitrile: methanol) was used at a flow rate of 0.45 mL/minute and an operating temperature of 40°c. Mixed standards were analyzed in a similar manner for identification. Peak identification was conducted by comparing the retention times of authentic standards and those obtained from the samples, these data were integrated using peak Simple chromatography data system processor; (Buck SCi. chromatopac data processor).

Results and Discussion

Proximate composition
After three weeks of fermentation, Table 1 shows the proximate analysis (Moisture, Ash, Fibre, Lipid, Protein and Carbohydrate) of the unfermented sweet potato peels, fermented via spontaneous process of fermentation, sweet potato peels fermented using *S. cerevisiae*, and the sweet potatoes peels fermented using the Bacterial isolates.
The proximate analysis revealed significant changes in the nutritional composition of sweet potato peels following fermentation. Notably, protein content increased substantially in the fermented samples compared to the unfermented control (SPPC). The highest protein enrichment was observed in the spontaneously fermented sweet potato peels (SPPS) at 28.87%, followed by SPPB (fermentation using bacterial isolates) at 25.28%, and SPPY (fermentation using *S. cerevisiae*) at 23.0%. This increase in protein content aligns with previous studies that demonstrate the ability of microbial fermentation to enhance the nutritional value of agro-waste products through microbial metabolism and enzymatic activity [17].
The carbohydrate content decreased significantly in the fermented samples, with the lowest observed in SPPS (53.19%). This reduction is indicative of microbial carbohydrate utilization as an energy source during fermentation [18]. Additionally, an increase in ash content was recorded, particularly in SPPY and SPPB, suggesting a

concentration of mineral elements post-fermentation. The lipid content, on the other hand, showed a reduction in all fermented samples, which may be attributed to microbial lipid metabolism [19].

Table 1: Result for proximate analysis of unfermented and fermented samples

Samples	SPPC (%)	SPPS (%)	SPPB (%)	SPPY (%)
Moisture	5.59±0.20^{c}	3.93±0.60 ^{a}	4.42±0.30^{b}	4.17±0.21^{a}
Ash	4.07±0.20^{a}	3.85±0.10^{a}	5.70±0.20^{b}	6.12±0.20^{c}
Fibre	3.70±0.20^{a}	6.91±0.06^{c}	7.15±0.08^{c}	6.41±0.10^{b}
Lipid	4.79±0.04^{c}	3.77±0.20^{b}	3.04±0.11^{a}	3.22±0.10^{a}
Protein	11.87±0.30^{a}	28.87±0.90^{d}	25.28±0.84^{c}	23.0±0.17^{b}
Carbohydrate	69.93±0.20^{c}	53.19±1.50^{a}	54.43±0.92^{a}	57.10±0.74^{b}

SPPC=Unfermented Sweet potato peels, **SPPS**=Spontaneously fermented sweet potato peels**, SPPB**=Sweet potato peels fermented using Bacteria isolates**, SPPY**=Sweet potato peels fermented using *Saccharomyces cerevisiae*

Table 2: Results showing the amino acids profile of the unfermented and the fermented samples

Samples	SPPC%	SPPS%	SPPB%	SPPY%
Methionine	1.22±0.01^{b}	1.21±0.01^{b}	1.18±0.01^{b}	1.01±0.01^{a}
Lysine	1.88±0.01^{b}	1.64±0.01^{a}	2.01±0.01^{c}	1.73±0.02^{a}
Threonine	3.53±0.02^{a}	3.83±0.02^{c}	3.87±0.02^{c}	3.71±0.02^{b}
Leucine	1.75±0.02^{b}	1.85±0.02^{b}	1.25±0.01^{a}	2.82±0.01^{c}
Isoleucine	1.01±0.01^{a}	1.01±0.01^{a}	1.84±0.01^{b}	1.08±0.01^{a}
Tryptophand3	1.21±0.02^{a}	2.14±0.01^{c}	1.82±0.01^{b}	1.95±0.01^{b}
Phenylalanine	2.29±0.01^{c}	1.78±0.01^{b}	1.34±0.01^{a}	2.81±0.01^{d}
Histidine	1.07±0.02^{a}	1.34±0.01^{b}	1.13±0.01^{a}	1.56±0.01^{c}
Valine	1.83±0.03^{a}	1.87±0.01^{a}	3.34±0.01^{b}	1.84±0.04^{a}
Asparagine	1.22±0.01^{a}	1.32±0.01^{b}	1.22±0.01^{a}	1.53±0.40^{c}
Arginine	0.51±0.01^{a}	0.71±0.01^{b}	0.83±0.01^{b}	1.16±0.01^{c}
Alanine	2.87±0.01^{b}	5.13±0.01^{d}	2.01±0.01^{a}	4.32±0.02^{c}
Aspartate	2.84±0.02^{c}	2.85±0.01^{c}	2.62±0.01^{b}	2.47±0.02^{a}
Glutamate	0.75±0.01^{a}	1.11±0.01^{b}	0.72±0.01^{a}	1.52±0.01^{c}
Glycine	1.55±0.01^{b}	1.33±0.01^{a}	1.12±0.01^{a}	1.86±0.02^{c}
Tyrosine	2.13±0.01^{a}	2.12±0.01^{a}	2.14±0.01^{a}	2.75±0.01^{b}
Cysteine	1.03±0.02^{a}	2.03±0.01^{c}	1.42±0.01^{b}	2.73±0.01^{d}
Proline	1.16±0.02^{a}	1.85±0.01^{b}	1.78±0.01^{b}	2.05±0.01^{c}

SPPC=Unfermented Sweet potato peels**, SPPS**=Spontaneously fermented sweet potato peels**, SPPB**=Sweet potato peels fermented using Bacteria isolates**, SPPY**=Sweet potato peels fermented using *Saccharomyces cerevisiae*

Amino acids analysis

After 3 weeks of fermentation, Table 2 shows the differences in the Amino acid composition across the groups, with only 17 Amino Acids found which are as shown in the Table.

Amino acid profiling indicated that fermentation improved the amino acid composition of sweet potato peels. The most notable improvements were observed in essential amino acids such as lysine, threonine, and leucine. SPPB showed the highest lysine content (2.01%), which is crucial for animal and human nutrition [16]. Similarly, leucine levels peaked in SPPY at 2.82%, reinforcing the effectiveness of fermentation in enhancing protein quality [13].

Methionine content, however, showed slight reductions in all fermented samples, which could be due to microbial degradation or metabolic conversion into other sulfur-containing compounds [20]. Despite these variations, the overall improvement in amino acid profiles confirms the efficacy of fermentation as a strategy for enhancing the nutritional profile of agro-waste materials [21].

Conclusion

This study demonstrates that submerged fermentation using bacterial isolates from pineapple peels effectively enhances the protein content and amino acid profile of sweet potato peels. The process not only enriches the protein composition but also optimizes essential amino acid levels, making the fermented product a viable alternative protein source for animal feed and potentially human consumption [22]. These findings suggest that microbial fermentation is a cost-effective and sustainable method for improving the nutritional quality of agro-waste materials. By leveraging naturally occurring bacterial isolates, this approach aligns with global efforts to reduce food waste and enhance food security [23].

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