Effect of Fermentation on the Proximate and Antinutrient Composition of Banana Peels Fermented with *Aspergillus niger* and *Bacillus subtilis*

RESEARCH ARTICLE

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This article is part of a special issue titled Sustainability, innovation, and development: A Festschrift in honour of Rt. Rev. Prof. Obeka Samuel Sunday.

Abstract

This research investigated the effect of solid-state fermentation on the proximate and antinutrient content of banana peels using pure strains of *Aspergillus niger* and *Bacillus subtilis*, with uninoculated peels as a control. Microbial load, pH, temperature, total titratable acidity, and antinutrient composition were monitored. Fermentation significantly altered proximate composition ($p < 0.05$) compared to unfermented peels. *Bacillus subtilis* increased carbohydrate (34.614%) and protein (15.495%) while decreasing fat (9.420%). Conversely, *Aspergillus niger* fermentation significantly increased fat content (22.823%) and energy value (1256.293 KJ/g), but reduced ash (10.635%), moisture (6.477%), and protein (3.571%). Fermentation significantly decreased ($p < 0.05$) oxalate, phenol, and cyanogenic glycoside content in samples. Phytate and tannin content varied between *Aspergillus niger* and *Bacillus subtilis*-fermented samples. Banana peels fermented with *Bacillus subtilis* exhibited the best nutritional composition, making them suitable as an animal feed substitute. *Aspergillus niger*-fermented banana peels are ideal for animals requiring higher fat and energy, such as pigs.

Methodology

Solid-state fermentation using pure strains of *Aspergillus niger* and *Bacillus subtilis* on banana peels for 72 hours

Key variables

Proximate composition, antinutrient content, microbial load, pH, temperature, and titratable acidity

Main finding

Bacillus subtilis fermentation enhanced nutritional value with increased carbohydrate and protein content

Keywords: Fermentation, banana peels, *Aspergillus niger*, *Bacillus subtilis*, proximate composition, anti-nutrients, animal feed

Introduction

Musa sapientum, commonly known as banana, is a herbaceous plant of the family Musaceae, originating from tropical Southern Asia and now cultivated throughout the tropics (Nayar, 2010). Primarily grown for its fruit, and to a lesser extent for fibre or as an ornamental plant (Nayar, 2010), it typically grows 2-8m tall with 3.5m leaves. Its pseudostem produces a single bunch of fruit in hanging clusters, with twenty fruits per tier and 3-20 tiers per bunch. The fruit's peels are usually discarded as waste after the inner fleshy portion is eaten.

Banana is a major global food resource, ranking fourth among significant foodstuffs after rice, corn, and milk (FAO, 2003; INIBAP, 2002). Grown extensively in tropical and subtropical regions across Asia, South America, and Africa, global *Musa* production in 2003 was estimated at 102 million tons, comprising 68% bananas and 32% plantains (FAO, 2003).

Global Production

World production of *Musa* in 2003 was estimated at 102 million tons, with 68% classified as bananas and 32% as plantains, making it the fourth most significant foodstuff globally.

Waste Generation

Banana peels constitute a significant portion of agricultural waste that is usually discarded after consumption, creating disposal problems in tropical regions.

Potential Utilisation

Recent research focuses on converting banana peel waste into valuable products through fermentation, contributing to sustainable agriculture and waste management.

Given the increasing demand and price of livestock feeds, this study investigates the proximate composition and anti-nutrient content of *Musa sapientum* (banana) peels fermented with *Aspergillus niger* and *Bacillus subtilis*. This research aims to assess the potential of fermented banana peels, usually considered waste, to substitute for the basic nutritional requirements of farm animals.

Rationale for microorganism selection

For banana peel fermentation, this study selects ***Aspergillus niger*** and ***Bacillus subtilis***. Their proven capabilities and established safety profiles are crucial for achieving desired nutritional enhancements and antinutrient reduction through bioconversion.

1

Aspergillus niger

- Proven enzyme production capabilities (pectinase, cellulase, amylase) for lignocellulosic waste breakdown.
- Successful use in banana peel fermentation studies showing enhanced protein and carbohydrate content (Oshoma et al., 2017).
- Ability to produce organic acids that reduce pH and improve nutrient bioavailability.

2

Bacillus subtilis

- Strong proteolytic enzyme production for protein enhancement.
- Proven ability to reduce antinutritional factors in agricultural wastes (Ravindran et al., 2018).
- Safe GRAS (Generally Recognised as Safe) status for food applications.

3

Synergistic advantages

- Complementary enzyme profiles for comprehensive substrate breakdown.
- Different metabolic pathways providing broader nutrient enhancement.
- Established safety profiles for potential food/feed applications, further supported by recent 2024-2025 studies on banana peel valorisation.

This strategic microorganism selection comprehensively optimises banana peel nutritional value for potential animal feed applications.

Objectives of the study

Microbial load assessment

Assess microbial load (bacteria and fungi) during banana peel fermentation at 0, 24, 48, and 72 hours.

Physical parameter monitoring

Monitor fermentation liquid pH, temperature, and total titratable acidity at 0, 24, 48, and 72 hours.

Nutritional analysis

Analyze 72-hour fermentation's effect on banana peel proximate and antinutrient composition.

Nutritional composition of banana peels

Banana peels are nutritionally rich, containing fibre, polyphenols, and antioxidants like dopamine (Hikal & Said-Al Ahl, 2022). Nutritional attributes vary by variety, maturity, and origin (Tartrakoon et al., 1999; Emaga et al., 2008, 2011; Babayemi et al., 2010; Vu et al., 2018). See Tables 1 and 2.

Table 1: Mineral composition of banana peels

Element	Concentration (mg/g)
Potassium	78.10 ± 6.58
Calcium	19.20 ± 0.00
Sodium	24.30 ± 0.12
Iron	0.61 ± 0.22
Manganese	76.20 ± 0.00
Bromine	0.04 ± 0.00
Rubidium	0.21 ± 0.05
Strontium	0.03 ± 0.01
Zirconium	0.02 ± 0.00
Niobium	0.02 ± 0.00

Source: Ranzani et al., 2007

Table 2: Nutrient composition of banana peels

Parameter	Concentration
Moisture	6.70 ± 2.22
Ash (%)	8.50 ± 1.52
Organic matter (%)	91.50 ± 0.05
Protein (%)	0.90 ± 0.25
Crude lipid (%)	1.70 ± 0.10
Carbohydrate (%)	59.00 ± 1.36
Crude fibre (%)	31.70 ± 0.25
Hydrogen cyanide (mg/g)	1.33 ± 0.10
Oxalate (mg/g)	0.51 ± 0.14
Phytate (mg/g)	0.28 ± 0.06
Saponins (mg/g)	24.00 ± 0.27

Source: Ranzani et al., 2007

Maturity Effect on Banana Peels

Banana fruit maturity (index one to seven) involves physical changes from green to dark yellow peels, often with dark spots. Beyond physical changes, maturity stages also alter physicochemical properties and nutritional value; for instance, ripe banana peels exhibit slightly superior nutritive properties compared to green and almost ripe peels, as detailed in Table 3.

Table 3: Nutrient composition in banana peel of three different maturity stages

Nutrient composition (%)	Green banana	Almost ripe banana	Ripe banana
Dry matter	91.62	92.38	95.66
Crude protein	5.19	6.61	4.77
Crude fiber	11.58	11.10	11.95
Ash	16.30	14.27	14.58
Energy (Kcal/kg)	4383	4692	4592

Source: Tartrakoon et al., 2002

Rich nutrient profile

Banana peels contain essential minerals like potassium, calcium, and manganese, along with significant carbohydrate content.

Maturity impact

Nutritional composition varies significantly with maturity stages, affecting protein content and energy values.

Waste utilisation

Converting agricultural waste into valuable animal feed ingredients through fermentation processes.

Uses of Banana Fruit and its Peels

Banana is a high-calorie tropical fruit, with 100 grams providing 90 calories. It contains beneficial antioxidants, minerals, and vitamins. The fruit's soft, easily digestible flesh, composed of simple sugars like fructose and sucrose, instantly replenishes energy and revitalises the body, making it a common choice for athletes and a supplement for underweight children. It also provides a good amount of soluble dietary fibre (7% of DRA per 100g), aiding normal bowel movements and reducing constipation. Additionally, bananas contain health-promoting flavonoid polyphenolic antioxidants such as lutein, zeaxanthin, AY, and α-carotenes (albeit in small amounts), which act as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) involved in ageing and various disease processes.

Banana is a good source of vitamin B6 (pyridoxine), offering about 28% of the daily recommended allowance. Pyridoxine, an important B-complex vitamin, is beneficial in the treatment of neuritis and anaemia and helps decrease homocysteine levels, a factor in coronary artery disease (CHD) and stroke. The fruit is also a moderate source of vitamin C (about 8.7mg per 100g), which helps the body resist infectious agents and scavenge harmful oxygen-free radicals. Fresh bananas provide adequate levels of minerals like copper, magnesium, and manganese. Magnesium is essential for bone strengthening and has a cardioprotective role; manganese acts as a cofactor for the antioxidant enzyme superoxide dismutase; and copper is required for red blood cell production. Fresh banana is a very rich source of potassium, with 100g providing 358 mg. Potassium is a vital component of cell and body fluids, helping control heart rate and blood pressure by countering the adverse effects of sodium.

While the biggest risk of banana peels is slipping, they are not poisonous; in fact, they are edible and packed with nutrients. Banana peels are consumed in many parts of the world, though less commonly in the West (Flores-Galarza *et al.*, 2000). They contain high amounts of vitamin B6 and B12, magnesium, potassium, some fibre, protein, and various bioactive compounds like polyphenols and carotenoids. However, peels must be thoroughly washed before eating due to potential pesticide residues. Furthermore, banana peels can serve as animal feed, enhancing nutrient intake and reducing agricultural waste.

Antinutritional Factors of Banana Peels

Antinutrients are natural or synthetic compounds, found in many foods (e.g., grains, legumes, vegetables, fruits, nuts), that hinder the absorption of vitamins, minerals, and other nutrients by interfering with digestive enzymes. Examples include tannins, oxalates, phenols, saponins, and cyanogenic glycosides.

Tannins affect a food's nutritive value by forming protein complexes (with both substrate and enzyme), inhibiting protein digestibility and reducing protein biological value. Recent studies (Sahoo & Lenka, 2024) show banana peels contain varying antinutrient levels, which are significantly affected by processing methods. Fermentation effectively reduces these antinutrient levels, enhancing suitability for consumption (Ozabor et al., 2020).

Phytate is naturally present in many foods, and high concentrations can adversely affect digestibility. When above a certain level, phytate reduces mineral availability, as well as protein solubility, functionality, and digestibility (Akubo and Badifu, 2004). Phytate chelates divalent cationic minerals like Ca, Fe, Mg, and Zn, thereby reducing their dietary availability.

Tannins

Form protein complexes, inhibiting digestion and absorption; bind iron, making it unavailable; and are the main antinutritional factor in banana peels.

Phytates

Reduce mineral availability and protein digestibility by chelating divalent cationic minerals like Ca, Fe, Mg, and Zn.

Oxalates

Are associated with kidney disease and decrease the absorption of essential minerals like calcium by binding with them.

Cyanogenic Glycosides

Are the most poisonous antinutrients that can cause immediate death in high dosages and various health issues in small dosages.

Oxalates, from plant sources and ingested in large doses, can cause irreversible oxalate nephrosis and are associated with kidney diseases, decreasing the availability of essential minerals like calcium. Cyanogenic glycosides (which release hydrogen cyanide) are the most poisonous antinutrients, capable of causing various health issues in small dosages, including chest pain, heart palpitations, and weak muscles, and even immediate death in high dosages.

Banana Peels as Agricultural Waste

Agro-industrial wastes, including peels from fruit, are generated during the industrial processing of agricultural products. These wastes are an inherent consequence of the food industry. With increasing environmental concerns, protecting the environment necessitates understanding activities that compromise its aesthetic quality. Environmental resources are now considered an economic variable, leading to the redesign of production processes with environmental technology. Fruit are highly perishable, with significant losses during the agri-food chain due to spillage, decay, water loss, and mechanical damage. Consequently, recent efforts focus on utilising cheap, renewable agricultural sources like banana peel waste as alternative substrates, for example, in animal feeds (FAO, 2003).

Materials and Methods

Materials Used

Nutrient agar, Potato Dextrose agar, pressure pot, autoclave, inoculating loop, conical flasks, petri dishes, Bunsen burner, weighing balance, measuring cylinder, cotton wool, 70% ethanol, stirring rod, phenolphthalein, sodium hydroxide, distilled water, spatula, syringe, nose mask, hand glove, test tubes, tube rack, aluminium foil, incubator, refrigerator, nutrient broth, Potato Dextrose broth, muslin cloth, filter paper, plastic white buckets, pH meter, thermometer, knife, stainless tray, paper tapes, marker, and tissue paper.

Collection of the sample

Banana fruit (*Musa sapientum*) was purchased from a local retail outlet in Ilara-Mokin market, Akure Metropolis, Ondo State, Nigeria, in April 2016. The sample was identified by Dr. A.O. Ojokoh of the Department of Microbiology, Federal University of Technology, Akure.

Source of microorganisms

Isolation

For fermentation, *Aspergillus niger* and *Bacillus subtilis* were obtained from the Department of Microbiology, having been previously isolated from traditional banana peel fermentation.

Biochemical characterisation and identification of isolates

Bacterial and fungal isolates were confirmed by morphological characterisation. Test microorganisms were cultured on media and observed after 24 hours (37°C) and 72 hours (25°C) of incubation, respectively. Biochemical characterisation, including Gram staining, Catalase, Motility, Oxidase, Coagulase tests, and Lactophenol blue, followed the method of Olutiola *et al*. (2000).

Gram staining

A distinct 24-hour-old colony was aseptically emulsified in a sterile water drop on a clean, grease-free slide using an inoculating loop to create a smear. The smear was air-dried and heat-fixed gently by passing it through a flame. It was flooded with crystal violet for 30 seconds and rinsed with distilled water. Lugol's iodine was then applied for 30 seconds and washed with water. 70% alcohol was applied and drained until no more colour was seen. Safranin was added for 30 seconds, then washed off. The air-dried smear was examined under an oil immersion microscope. Gram-positive microorganisms appeared purple, while Gram-negative appeared pink (Cheesbrough, 2006).

Catalase test

Three millilitres (3 mL) of 3% hydrogen peroxide were added to a loopful of the bacterial isolate on a clean glass slide. Effervescence (bubble formation) indicated a positive catalase test (Cheesbrough, 2006).

Sugar Fermentation

Sugar fermentation tests determined the organisms' ability to ferment sugars, producing acid or gas. To prepare the sugar indicator broth, 1g each of glucose, maltose, fructose, lactose, and sucrose, along with 1g of peptone water medium, was added to individual conical flasks, each containing 100ml of distilled water. The mixtures were stirred until dissolved. 1-2 drops of methyl red indicator were added to each mixture. Then, 10ml of each sample was dispensed into sterilised test tubes using a syringe. Durham tubes were inserted to check for gas formation. Test tube mouths were sealed with foil paper and cotton wool. The mixtures were sterilised in an autoclave at 121°C for 15 minutes, cooled to room temperature, inoculated with bacterial isolates, and incubated at 37°C for 24 hours (Fawole and Oso, 2001).

Coagulase Test

A distinct bacterial colony was emulsified in normal saline on a grease-free slide. An equal volume of plasma was added and mixed aseptically. The formation of clumps or precipitates indicated a positive coagulase test; their absence showed a negative result.

Motility Test

Motility was determined by placing a drop of normal saline on a sterile slide, suspending and emulsifying an isolate colony, then covering it with a coverslip. The slide was examined microscopically using a X10 objective lens. Movement in different directions indicated a positive test.

Oxidase Test

Whatman No. 1 filter paper was placed in a sterile petri dish, and 2-3 drops of freshly prepared oxidase reagent were added. Using a sterile inoculating loop, a colony of the isolates was smeared on the filter paper and observed after 3 minutes. A purple-blue colouration indicated a positive test; its absence indicated a negative test.

Simmons Citrate Agar Test

1.2g of Simmons citrate agar was weighed, dissolved in 50ml of distilled water according to manufacturer specifications, and dispensed into sterilised test tubes (10ml per tube). The tubes were sealed with foil paper and cotton wool, then sterilised at 121°C for 15 minutes in an autoclave. After sterilisation, the agar was immediately slanted and allowed to gel. Bacterial isolates were inoculated and incubated at 37°C for 24 hours before checking the result.

Lactophenol cotton blue staining

For fungal identification, a drop of Lactophenol blue stain was placed on a sterile glass slide. Hyphae were picked from the culture plate with a sterile needle, placed on the stain, and viewed under a microscope at a x40 objective lens.

Sample preparation

Banana fruits were washed, peeled, and cut into smaller pieces. 300g of banana peels were weighed into three conical flasks and labelled: SPX (uninoculated), SPA (with *Aspergillus niger*), and SPB (with *Bacillus subtilis*). All flasks containing banana peels were wrapped in aluminium foil and sterilised in an autoclave at 121°C for 15 minutes.

Preparation of the microorganisms in broth

2.8g of Nutrient Agar and 3.9g of Potato Dextrose Agar were weighed into separate conical flasks and dissolved in 100ml of distilled water, respectively. The thoroughly mixed media were filtered using Whatman filter paper into beakers. Subsequently, 15ml of each broth (nutrient and potato dextrose) was dispensed into test tubes, corked with cotton wool and foil, and **sterilised** at 121°C for 15 minutes. After cooling, pure isolates of *Aspergillus niger* and *Bacillus subtilis* were aseptically inoculated into the potato dextrose broth and nutrient broth, respectively. They were then incubated at 25°C for 72 hours and 37°C for 24 hours, respectively, prior to fermentation.

Fermentation of banana peels

300g of sterile banana peels were transferred into separate, **labelled**, sterile transparent buckets. 600ml of sterile distilled water was added to each sample. For fermentation, 15ml of 18-hour-old *Bacillus subtilis* inoculum from the nutrient broth and 72-hour-old *Aspergillus niger* inoculum from the potato dextrose broth were added to their respective samples. One bucket was left uninoculated, serving as the control.

Determination of Microbial Population

Serial Dilution and Pour Plating of the Samples

Microbial population (CFU/mL total viable count, SFU/mL fungi count) was determined daily at 0, 24, 48, and 72 hours. Fermentation liquid samples (1 mL each) from uninoculated, *Aspergillus niger*, and *Bacillus subtilis* setups were collected at these intervals and serially diluted to the fifth dilution using 9 mL of sterile distilled water. Dilution factors of 0.1-3 were aseptically pour-plated with 15 mL molten nutrient agar (total viable count) or PDA agar (fungi count). Nutrient agar plates were incubated at 37°C for 24 hours, while PDA plates were incubated at 28°C for 72 hours, per manufacturer's instructions.

PHYSICO-CHEMICAL PROPERTIES

Determination of the pH of the Fermenting Banana Peels

The pH of samples was measured at 24-hour intervals during fermentation using the AOAC (2005) method. Samples were thoroughly stirred for homogenisation. The HANNA pH metre 209 was calibrated with sterile distilled water before dipping the electrode into each sample for measurement.

Determination of the Total Titratable Acidity (TTA) of the Fermenting Banana Peels

TTA was determined at 0, 24, 48, and 72 hours of fermentation using the AOAC method (2005). Approximately 20 mL of each liquid sample was pipetted into sterile conical flasks, and 2 drops of phenolphthalein indicator were added. Titration was performed using 0.1M NaOH to a faint pink colour, stable for at least 1 minute, against a white background. The titre volume was recorded to calculate TTA, expressed as percentage lactic acid produced.

$$TTA = \frac{\text{Titre value} * 9mg}{100}$$

Determination of the temperature of the fermenting banana peels

Temperature of fermenting samples was determined at 0, 24, 48, and 72 hours using the AOAC (2012) method. Sterile thermometers were aseptically placed into each sample for 3-5 minutes, then removed and the temperature recorded.

Termination of the fermentation

At the end of the 72-hour fermentation, water was gently drained from all samples (banana peels with *Bacillus subtilis*, *Aspergillus niger*, and uninoculated peels). The banana peels were then placed on foil, labelled, and air-dried for subsequent proximate and antinutrient analysis.

Proximate analysis of the sample

Proximate composition of samples, including moisture, carbohydrate, protein, fat, crude fibre, and ash contents, was determined using the AOAC (2012) method.

Determination of moisture content

Moisture content was determined by the gravimetric method according to AOAC (2005). An empty, pre-washed and dried evaporating dish was weighed (W1). 10g of sample was added to the dish and weighed (W2). The dish with sample was then oven-dried at 105°C for 8 hours. After drying, it was cooled in a desiccator to room temperature and reweighed. This drying, cooling, and weighing process was repeated at 30-minute intervals until a constant weight (W3) was obtained. Moisture weight was calculated and expressed as a percentage of the sample analysed, using the expression below:

$$\% \text{ Moisture content} = \frac{W_2 - W_1 \text{ x } 100}{W_2 - W_1}$$

Where:
W1 = initial weight of evaporating dish;
W2 = weight of evaporating dish + weight of sample before drying;
W3 = final weight of evaporating dish after drying at 105°C + weight of sample after drying

Determination of Ash Content

The ash content was determined by the gravimetric method according to AOAC (2005). An empty, pre-washed, and dried crucible was weighed (W1). A 5g sample was then weighed into the crucible (W2). The crucible with the sample was placed in a muffle furnace at 550°C for 4 hours. After ashing, the crucible was cooled in a desiccator to room temperature and reweighed (W3). Percentage ash content was calculated as:

$$\% \text{ Ash content} = \frac{W_2 - W_1 \text{ x } 100}{W_2 - W_1}$$

Where:
W1 = weight of empty crucible;
W2 = weight of crucible + sample before ashing;
W3 = final weight of crucible + sample after ashing at 550°C.

Determination of Fat Content

Fat content was determined using the Soxhlet direct solvent extraction method. An empty, dried extraction thimble was weighed (W1). A 3g sample was weighed into the thimble, then placed in a 500ml Soxhlet extractor apparatus (W2). The apparatus, including a condenser and a 500ml round bottom flask, was assembled and mounted on a heating mantle. Light petroleum ether (boiling range 40-60°C) was added to the flask. Extraction continued for 6 hours until the sample was defatted. The thimble was then removed, dried in a hot-air oven at 105°C for 1 hour, cooled in a desiccator to room temperature, and weighed (W3). Crude fat content was calculated as:

$$\% \text{ Fat} = \frac{W_2 - W_1 \times 100}{W_2 - W_1}$$

Where:

W1 = weight of empty extraction thimble;
W2 = weight of sample + extraction thimble;
W3 = dried weight of defatted sample + extraction thimble.

Determination of Crude Fibre

Crude fibre was analysed using the AOAC (2005) method. Approximately 2g of each defatted sample was weighed into a 1-litre conical flask (W1). 200ml of 1.25% sulfuric acid was added, and the content was boiled for 30 minutes, then filtered under vacuum and washed repeatedly with distilled water. The sample was returned to the flask, 200ml of 1.25% NaOH solution was added, and it was boiled for another 30 minutes before filtering. The sample was thoroughly washed with distilled water, then 10% HCl solution, and further washed with distilled water to remove residual acid. It was then treated with approximately 10ml of light petroleum ether and 10ml of absolute ethanol. The sample was transferred to an empty crucible and dried in a hot-air oven at 105°C for about 1 hour. It was then placed in a desiccator and allowed to cool to constant weight (W2). It was then ashed in a muffle furnace at 550°C for 90 minutes, cooled in a desiccator, and reweighed (W3). The percentage crude fibre was calculated from the loss of weight on incineration:

$$\% \text{ Crude fibre} = \frac{W_2 - W_3 \times 100}{W_1}$$

Where:
W1 = weight of defatted sample
W2 = weight of sample at 105°C
W3 = weight of sample at 550°C

Determination of Crude Protein

Protein content was determined by the Micro-Kjeldahl method. Approximately 0.30g of each sample was weighed into a 50ml Micro-Kjeldahl digestion flask. A copper sulphate catalyst tablet and 5ml of concentrated tetraoxosulphate (VI) acid (H_2SO_4) were added. The flasks were digested on a block, starting at low temperature for 30 minutes, then increased to red hot in a fume cupboard for 2 hours until the samples cleared. The digests were transferred to volumetric flasks, each diluted to 50ml with distilled water.

10ml of each digested sample was measured into the distillation apparatus, gradually introducing 10ml of 40% NaOH solution. The mixture was steam-distilled, and the distillate collected into 5ml of 2% boric acid solution containing 3 drops of mixed indicator. 50ml of distillate from each duplicate was titrated with 0.01M HCl solution to a pink endpoint. The percentage nitrogen content calculated for each sample was multiplied by a factor of 6.25 to obtain the percentage protein content.

$$\% \text{ Protein} = \frac{N.F * M * V1 * T * PF * 100}{V2 * W}$$

Where:

N.F = Nitrogen factor (0.014)

M = Molarity of HCl (0.01)

V1 = final volume of digest (50ml)

V2 = Volume of digest (10ml)

T = Titre volume of distillate

W = Weight of sample used

PF = Protein multiplication factor (6.25)

Determination of Carbohydrate

Total carbohydrate content was estimated by "difference" by subtracting the sum of the percentage concentrations of other proximate compositions from 100:

Total carbohydrate = 100 – (% moisture + % ash + % fat + % protein + % crude fibre)

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Antinutrient Analysis of the Sample

Determination of Phytate

Phytate content was determined using the method of Young and Greaves (2000). 4 g of finely ground sample was soaked in 100 ml of 2% HCl for 3 hours and then filtered. 25.0 ml of the filtrate was placed in a 100 ml conical flask, 5.0 ml of 0.03% NH_4SCN solution was added as indicator, and 50 ml of distilled water was added for proper acidity. This was titrated with $FeCl_3$ solution (containing ~0.005 mg Fe/ml). The equivalent was obtained, and phytate content in mg/100g was calculated:

Phytic acid = Titre * 195 * 1.19 * 3.55 mg/g.

Determination of Oxalate Content

Oxalate content was determined by the titrimetric method (AOAC, 2005). 1 g of the sample was weighed into a 100 ml conical flask. 75 ml of 1.5 N sulphuric acid was added, and the solution was intermittently stirred with a magnetic stirrer for ~1 hour before filtering with Whatman No. 1 filter paper. 25 ml of the filtrate was collected, heated to ~80°C on a heating mantle, and then titrated against 0.1 N $KMnO_4$ solution to a faint pink colour persisting for ~30 seconds.

Determination of Total Phenol

Total phenol content was determined by the method of AOAC (2005). 0.20 ml of the sample was mixed with 2.5 ml of 10% Folin-Ciocalteu's reagent and 2 ml of 7.5% sodium carbonate solution. The mixture was incubated at 45°C for 40 minutes, and the absorbance of the coloured mixture was read at 700 nm using a UV-visible spectrophotometer. Garlic served as the standard phenol.

Determination of tannin content

0.2 g of sample was weighed into a 50 mL bottle. 10 mL of 70% aqueous acetone was added, and the bottle was covered. The bottle was shaken in an ice bath shaker for 2 hours at 30°C. Each solution was then centrifuged, and the supernatant stored on ice. 0.2 mL of each solution was pipetted into a test tube, and 0.8 mL of distilled water was added. Standard tannic acid solutions were prepared from a 0.5 mg/mL stock, diluted to 1 mL with distilled water. To both sample and standard, 0.5 mL of Folin Ciocalteu reagent was added, followed by 2.5 mL of 20% Na_2CO_3. The

solutions were vortexed, incubated for 40 minutes at room temperature, and absorbance was read at 725 nm against a reagent blank. Concentration was determined from a standard tannic acid curve (Makkar *et al.*, 2000).

Determination of cyanogenic glycosides

4 g of sample was weighed into a 250 mL conical flask. 50 mL of distilled water, then 2 mL of orthophosphoric acid, was added. The sample was stirred magnetically for 30 minutes, then covered with aluminium foil and left overnight at room temperature to liberate bound hydrocyanic acid. The mixture was transferred to a 250 mL distillation flask with a drop of paraffin oil and anti-bumping chips. After fitting the distillation setup, approximately 45 mL of distillate was collected in a receiving flask containing 4 mL of distilled water and 1 g of NaOH pellet. The distillate was transferred to a 50 mL volumetric flask and made up to the mark with distilled water. 20 mL of this solution was measured into an Erlenmeyer flask with 1.60 mL of 5% KI solution. The solution was then titrated against 0.10 M $AgNO_3$ solution, along with a blank, until a faint, permanent turbidity indicated the endpoint.

Statistical analysis

All determinations were carried out in triplicate. Results were subjected to statistical analysis using SPSS Version 20. Descriptive statistics were used to interpret the results.

RESULTS AND DISCUSSION

Microbial load of the sample

For uninoculated banana peel samples, no growth was observed on PDA plates throughout 72 hours of fermentation. NA plates, however, showed 3×10^{-3} CFU/ml colonies at 72 hours, characterised by round edges, flat elevation, and cream colour. Microbial count (total viable count) on NA plates for peels fermented with *Bacillus subtilis* increased from 0 to 72 hours (6, 25, 41, and 63 $\times10^{-3}$ CFU/ml, respectively), exhibiting cream colour, flat elevation, round edges, and undulate margins, consistent with *Bacillus subtilis* morphology. Fungi count for banana peels fermented with *Aspergillus niger* increased after 24 hours of fermentation (0, 1, 3, and 4 $\times10^{-3}$ SFU/ml), showing white felt-like fluffy growth that turned black with age and had a cream reverse, consistent with *Aspergillus niger* morphology. These results are represented in Table 6.

Changes in the Physicochemical properties

pH

As shown in Figure 1, pH results for uninoculated, *Aspergillus niger*, and *Bacillus subtilis* fermented banana peels over 24-hour intervals averaged 6.0275 (uninoculated), 5.8775 (*A. niger*), and 6.1225 (*B. subtilis*).

Total titratable acidity (TTA)

Figure 2 presents Total Titratable Acidity (TTA) results for uninoculated, *Aspergillus niger*, and *Bacillus subtilis* fermented banana peels at 24-hour intervals. Mean TTA values were 0.01425 for both uninoculated and *A. niger*, and 0.02450 for *B. subtilis*.

Temperature

Table 7 displays temperature results for uninoculated, *Aspergillus niger*, and *Bacillus subtilis* fermented banana peels over 24-hour intervals. Mean temperatures were 35.9°C (uninoculated), 35.225°C (*A. niger*), and 30.575°C (*B. subtilis*).

Table 4: Biochemical test and morphological characterisation of the bacterial isolate

Biochemical test	*Bacillus subtilis*
Shape	Rod
Color	Cream
Edges	Entire
Elevation	Flat
Surface	Smooth
Gram staining	+
Catalase	+
Citrate	+
Coagulase	_
Oxidase	+
Motility	+
Acid from:	
Fructose	A
Mannitol	NA
Lactose	A
Maltose	A
Sucrose	A
Glucose	A

KEY: (+) = positive result; (-) = negative result; A = Acid produced, NA = No acid produced

Table 5: Table 5: Morphological characterization of the fungi isolate

Characterization	*Aspergillus niger*
Colonial morphology	White felt-like fluffy, turning black with age
Colour	Black
Reverse colour	Cream
Lactophenol cotton blue stain	Septate hyphae with smooth walls

Table 6: Microbial count of the banana peels fermented uninoculated, with *Aspergillus niger* and with *Bacillus subtilis*

Time	Uninoculated		*Aspergillus niger*	*Bacillus subtilis*
(Hr)	NA	PDA	PDA	NA
0	-	-	$0*10^{-3}$	$6*10^{-3}$
24	-	-	$1*10^{-3}$	$25*10^{-3}$
48	-	-	$3*10^{-3}$	$41*10^{-3}$
72	$3*10^{-3}$	-	$4*10^{-3}$	$63*10^{-3}$

KEY: - No growth

Table 7: Temperature of fermented and unfermented banana peels

Time (Hr)	Uninoculated sample	Sample inoculated with *Aspergillus niger*	*Sample inoculated with Bacillus subtilis*
0	52.0	49.0	29.9
24	29.6	29.9	31.7
48	31.3	31.3	30.7
72	30.7	30.7	30.0

6.12

Mean pH

Bacillus subtilis fermented samples

5.88

Mean pH

Aspergillus niger fermented samples

0.024

Mean TTA

Bacillus subtilis samples

Mean pH values were 6.0275 for uninoculated, 5.8775 for *Aspergillus niger*, and 6.1225 for *Bacillus subtilis*-fermented samples. Mean TTA values were 0.01425 for uninoculated, 0.01425 for *Aspergillus niger*, and 0.02450 for *Bacillus subtilis*-fermented samples. Mean temperatures were 35.9°C for uninoculated, 35.225°C for *Aspergillus niger*, and 30.575°C for *Bacillus subtilis*-fermented samples.

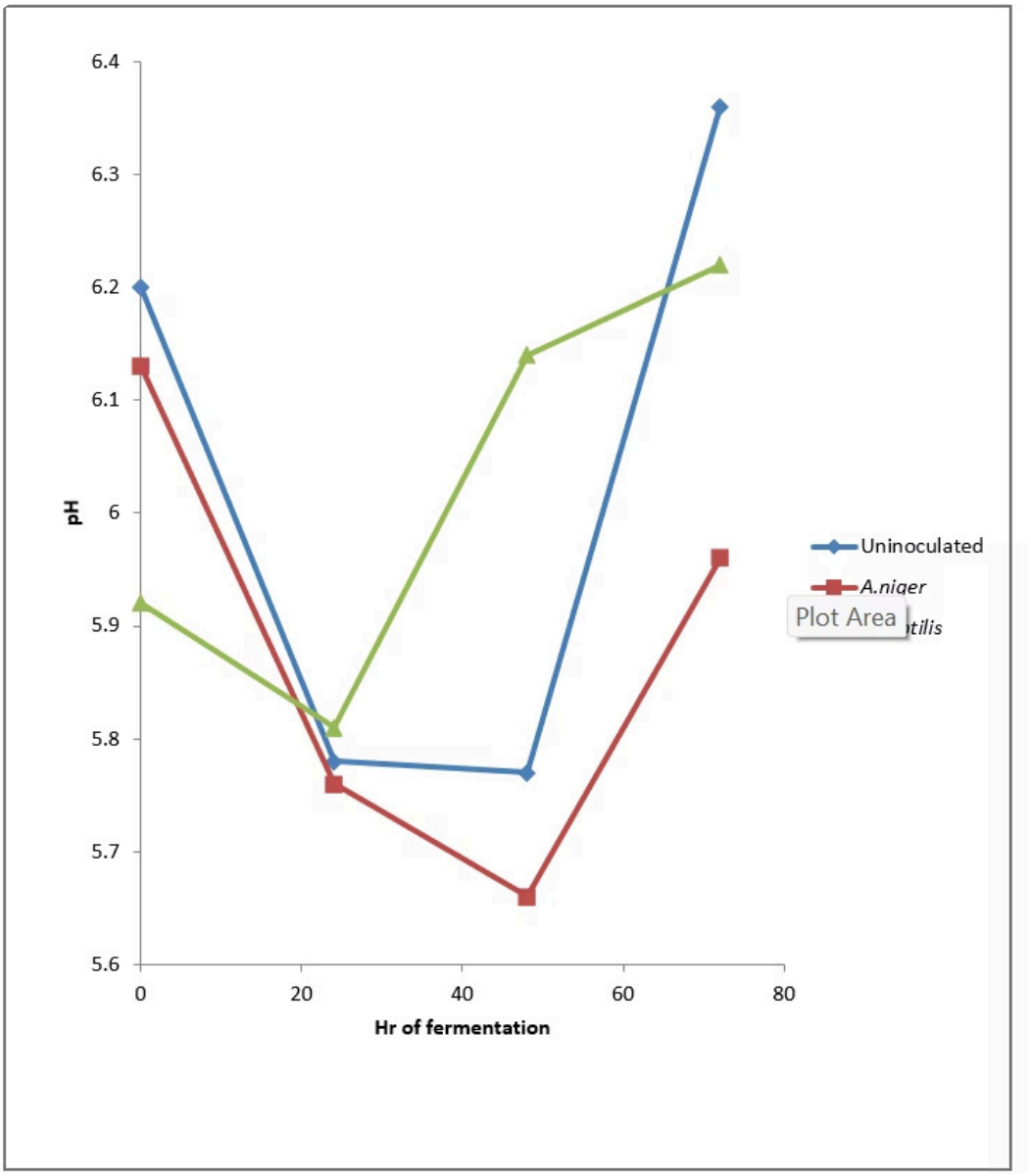

Figure 1: Changes in pH of the uninoculated, *Aspergillus niger* and *Bacillus subtilis* fermented banana peels during the fermentation period.

Bars are presented as Mean ±S.E of replicates (n=3).

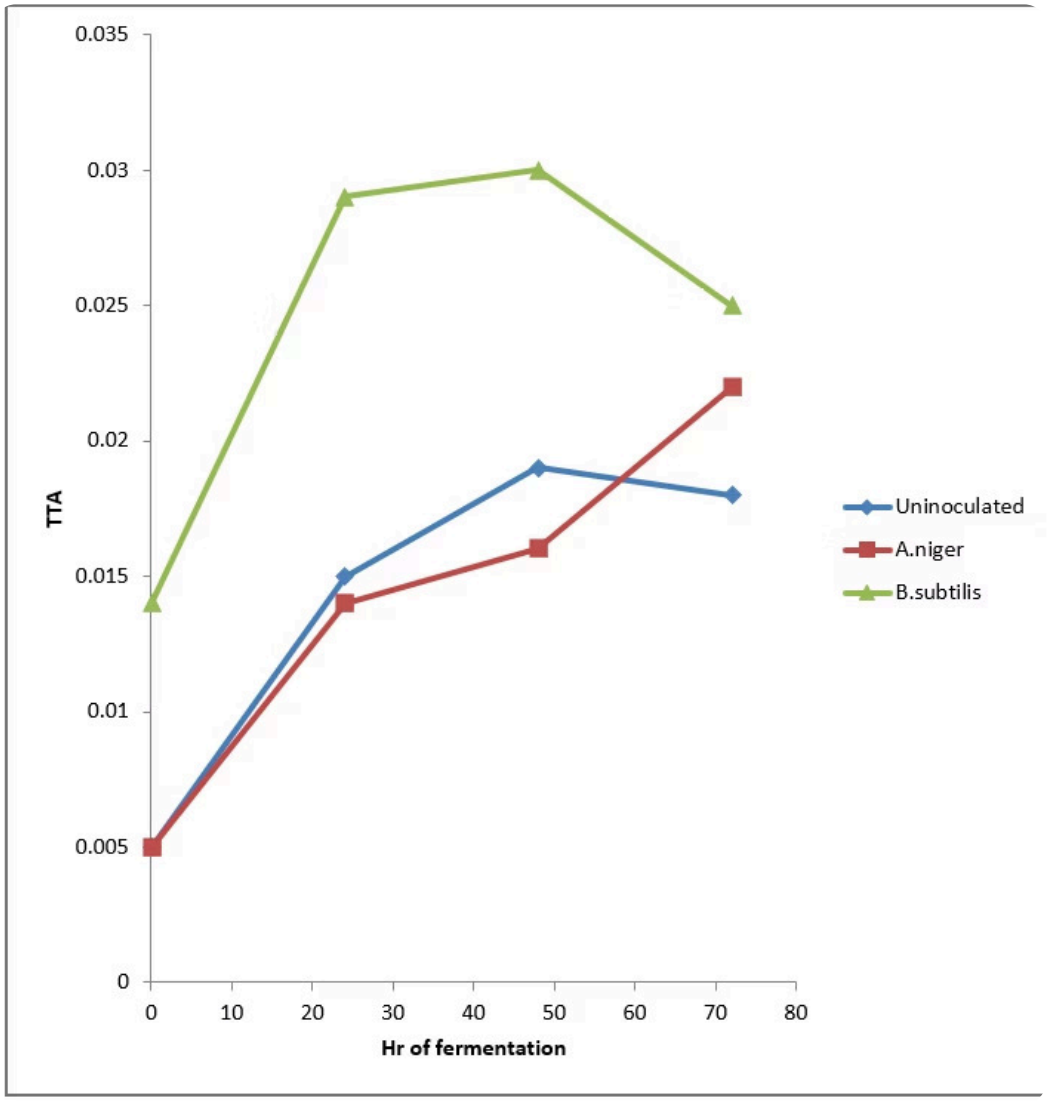

Figure 2: Changes in Total Titratable Acidity (TTA) of the uninoculated, *Aspergillus niger* and *Bacillus subtilis* fermented banana peels during the fermentation period.

Bars represent Mean ±S.E of replicates (n=3).

Proximate analysis of the unfermented and fermented banana peel samples after 72 hours of fermentation.

The changes in the proximate composition of banana peel samples are represented in Figure 3. Moisture content decreased in all samples: the unfermented sample had 13.284%, while fermented samples were 8.815% (uninoculated), 6.477% (*Aspergillus niger*), and 8.880% (*Bacillus subtilis*). Crude protein for the unfermented peel was 13.725%. Fermented samples showed 13.672% (uninoculated), 3.571% (*Aspergillus niger*), and 15.495% (*Bacillus subtilis*). Crude fat values were 13.152% for the unfermented sample, and 12.492% (uninoculated), 22.823% (*Aspergillus niger*), and 9.420% (*Bacillus subtilis*) for fermented samples. Ash content was 15.302% for the unfermented sample, and 12.529% (uninoculated), 10.635% (*Aspergillus niger*), and 13.743% (*Bacillus subtilis*) for fermented samples. Crude fibre increased from 15.290% in the unfermented sample to 38.951% (uninoculated), 35.839% (*Aspergillus niger*), and 17.848% (*Bacillus subtilis*) in fermented samples. Carbohydrate levels increased in the *Bacillus subtilis* fermented sample (34.614%) but decreased in uninoculated (13.541%) and *Aspergillus niger* (20.655%) fermented samples, compared to 29.247% in the unfermented sample. Energy value increased in the *Aspergillus niger* fermented sample (1256.293 KJ/g) but decreased in uninoculated (924.825 KJ/g) and *Bacillus subtilis* (1200.393 KJ/g) fermented samples, relative to 1217.148 KJ/g in the unfermented sample. These changes are also shown in Figure 4.

Antinutrient content analysis of the unfermented and fermented banana peel samples after 72 hours of fermentation.

Observed antinutrient changes are recorded in Figure 5. Oxalate content decreased in all samples: from 33.315 mg/g in the unfermented sample to 14.226 mg/g (uninoculated), 17.468 mg/g (*Aspergillus niger*), and 14.406 mg/g (*Bacillus subtilis*) in fermented samples. Tannin content decreased in uninoculated (3.739 mg/g) and *Bacillus subtilis* (4.303 mg/g) fermented samples, but increased in *Aspergillus niger* fermented samples (7.377 mg/g) compared to the unfermented sample (7.018 mg/g). All fermented samples showed a progressive increase in phytate content (uninoculated: 16.892 mg/g, *Aspergillus niger*: 30.282 mg/g, *Bacillus subtilis*: 19.158 mg/g) from the unfermented value of 12.669 mg/g. Similarly, phytic acid increased in all fermented samples (uninoculated: 4.758 mg/g, *Aspergillus niger*: 8.435 mg/g, *Bacillus subtilis*: 5.474 mg/g) from the unfermented value of 3.529 mg/g.

Phenol contents also decreased in all samples (Figure 6): from 8.067% in the unfermented sample to 4.099% (uninoculated), 8.018% (*Aspergillus niger*), and 4.718% (*Bacillus subtilis*) in fermented samples. Cyanogenic glycosides decreased across all samples (Figure 7): from 256.196 mg/kg in the unfermented sample to 128.091 mg/kg (uninoculated), 142.120 mg/kg (*Aspergillus niger*), and 88.901 mg/kg (*Bacillus subtilis*) in fermented samples.

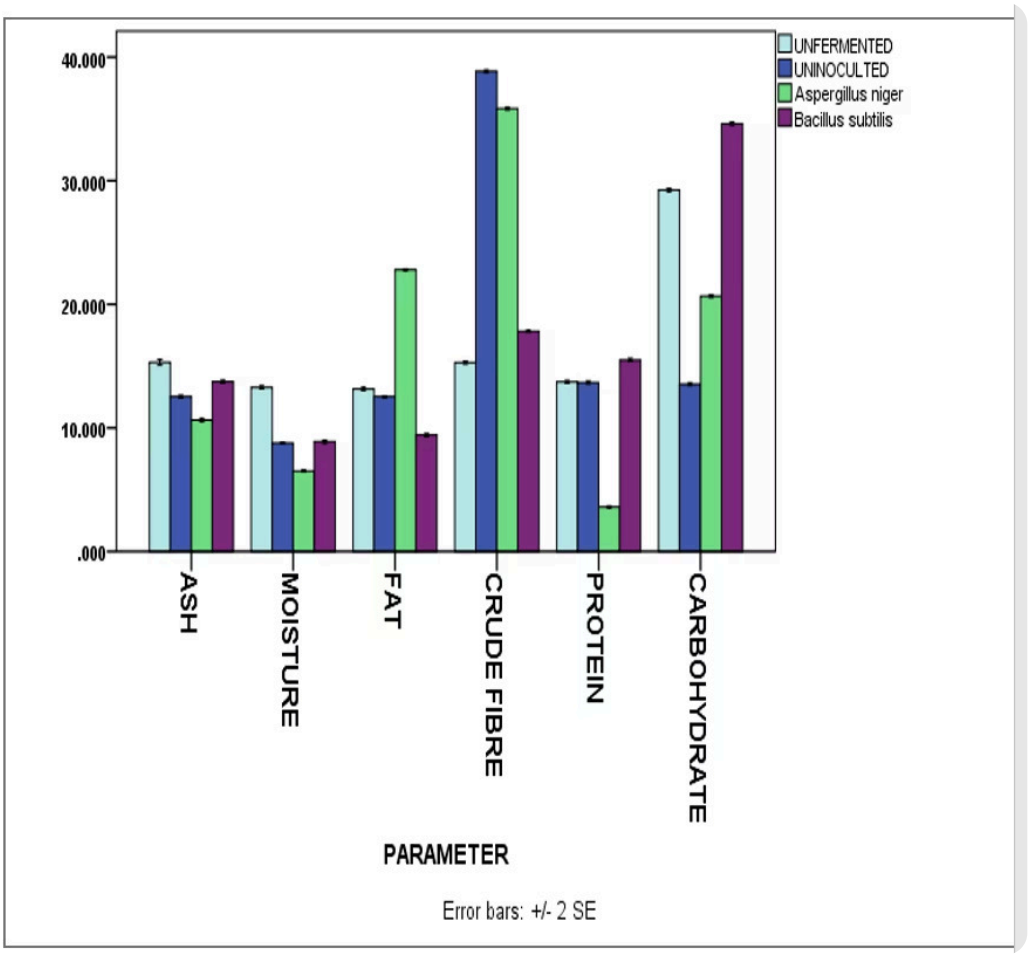

Figure 3: Proximate composition (%) of the unfermented banana peel and fermented banana peel after fermentation (72hrs)

Bars are presented as Mean ± S.E. of replicates (n=3)

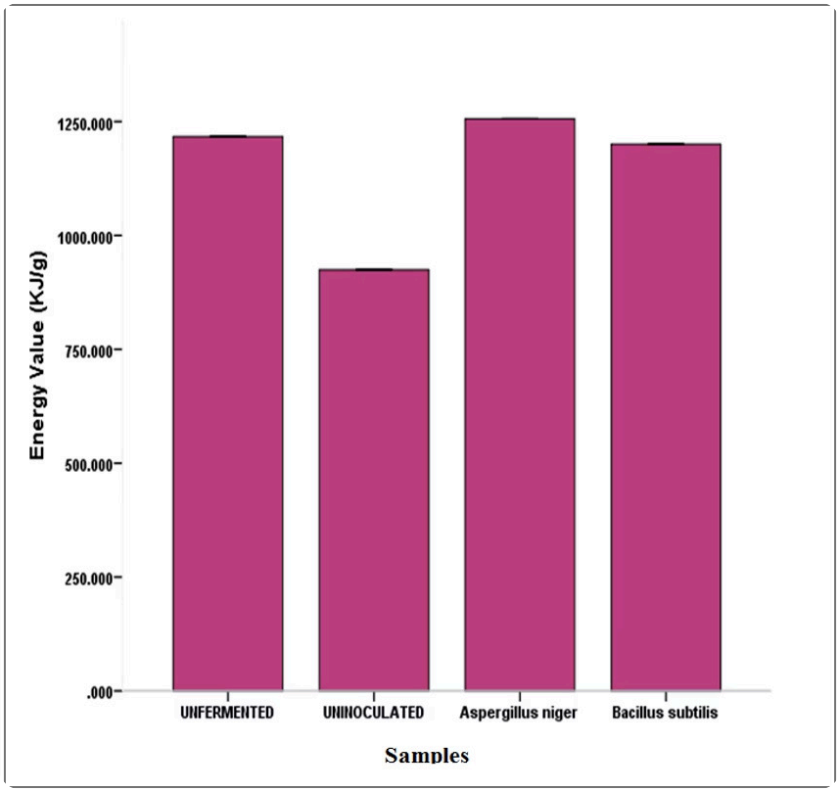

Figure 4: Energy value (kJ/g) of the unfermented banana peels and fermented banana peels after fermentation (72 hrs)

Bars are presented as Mean ±S.E. of replicates (n=3).

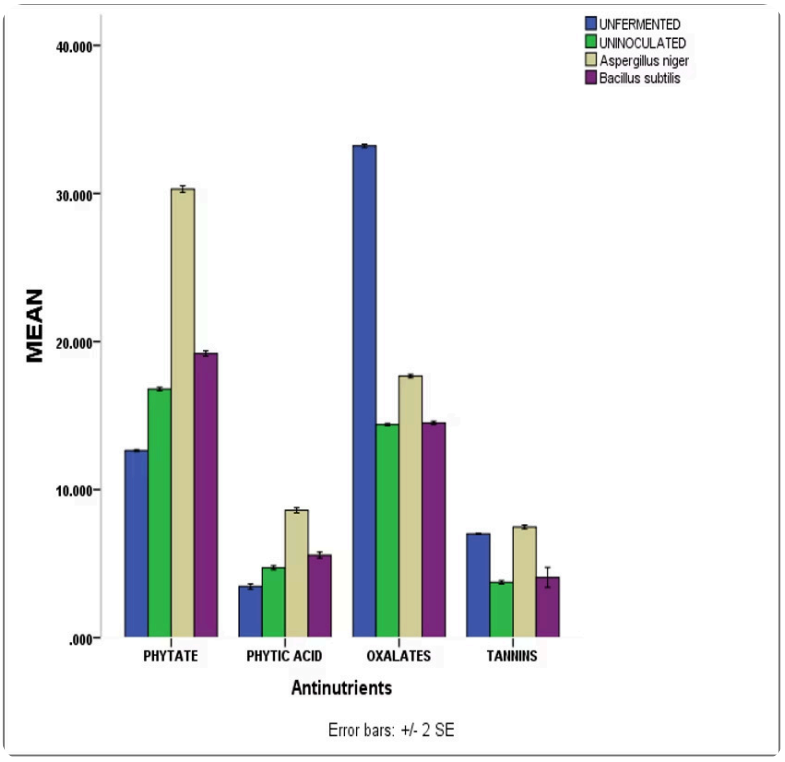

Figure 5: Antinutrient content (mg/g) of the unfermented banana peels and uninoculated, *Aspergillus niger* and *Bacillus subtilis* fermented banana peels after fermentation (72 hrs)

Bars are presented as Mean ±S.E. of replicates (n=3).

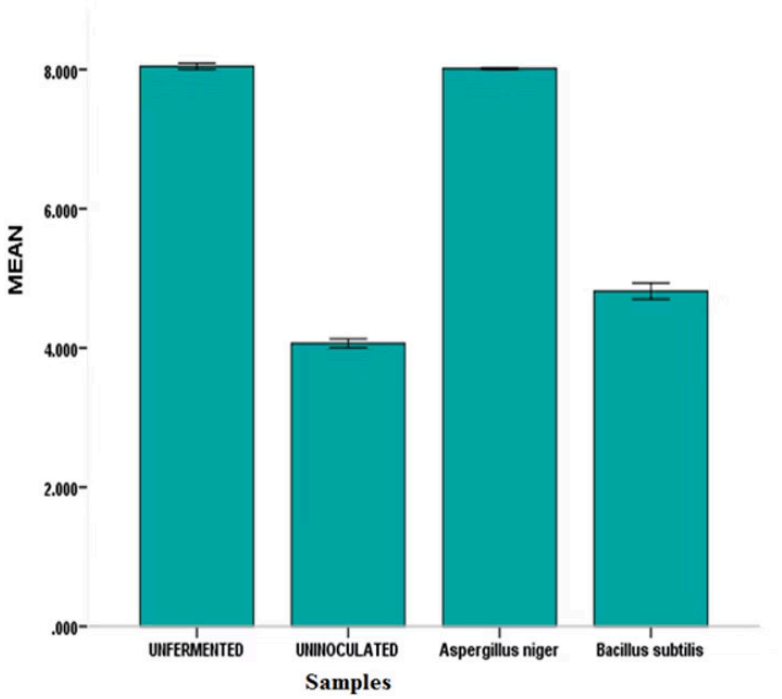

Figure 6: Phenol content (%) of unfermented banana peels and uninoculated, *Aspergillus niger*, and *Bacillus subtilis* fermented banana peels after fermentation (72 h).

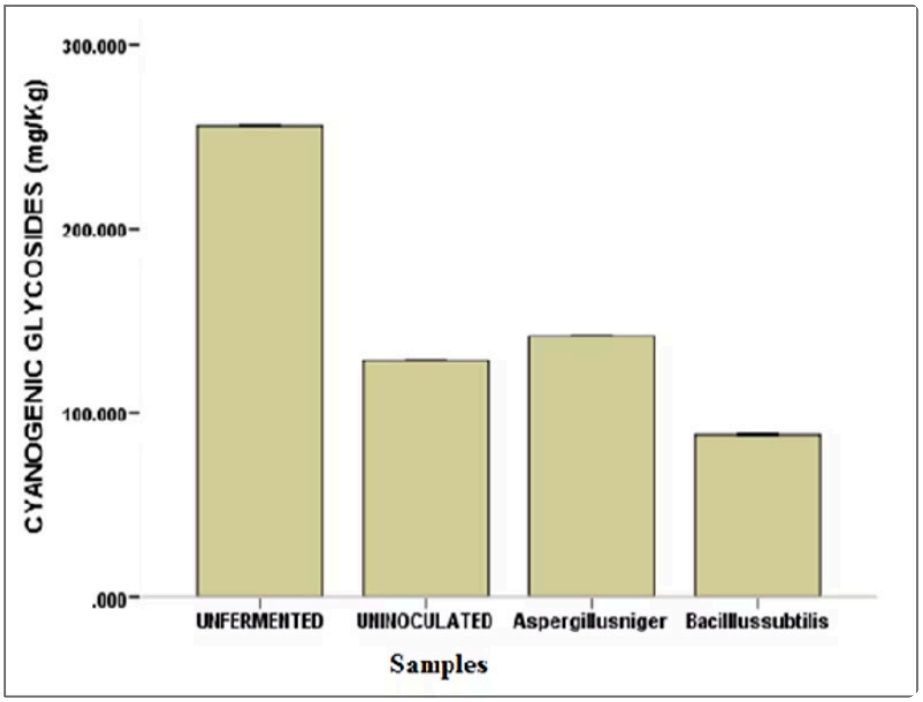

Figure 7: Cyanogenic glycoside content (mg/kg) of unfermented banana peels and uninoculated, *Aspergillus niger*, and *Bacillus subtilis* fermented banana peels after fermentation (72 h).

Bars are presented as Mean ± S.E. of replicates (n=3) for both figures.

DiSCUSSION

Fermentation plays a crucial role in improving food nutrient content. As fermentation progressed, pH fluctuated and TTA increased due to the production of organic acids by fermenting micro-organisms (Ojokoh, 2007). These changes in pH are consistent with metabolic activities (Adams and Nicolaides, 1997).

The moisture content of banana peels fermented with *Aspergillus niger*, *Bacillus subtilis*, and uninoculated samples surprisingly decreased compared to unfermented peels. This indicates improved shelf life and reduced risk of microbial deterioration or mould growth in the fermented banana peels.

The increase in protein content of samples fermented with *Bacillus subtilis* may stem from the micro-organism's efficient degradation of the sample and secretion of extracellular enzymes (Odetokun, 2000; Ojokoh, 2005b). Conversely, the decrease in protein for banana peels fermented with *Aspergillus niger* aligns with findings by Oladele and Oshodi (2008) for fermented *Jatropha cathartica* and *Jatropha curcas*.

01

Moisture Reduction

All fermented samples showed decreased moisture content, indicating improved shelf life characteristics and reduced microbial deterioration risk.

02

Protein Enhancement

Bacillus subtilis fermentation increased protein content through the secretion of extracellular enzymes and sample degradation processes.

03

Anti-nutrient Elimination

Fermentation significantly reduced harmful anti-nutrients, particularly oxalate, phenol, and cyanogenic glycosides, improving safety for consumption.

An increase in the fat content of samples fermented with *Aspergillus niger* may be due to microbial oil production during fermentation (Ojokoh, 2005a). Conversely, ash content, a measure of total inorganic compounds and minerals, decreased in all fermented banana peels compared to unfermented samples.

Anti-nutritive factors limit the use of plants in food due to their deleterious effects on humans and animals (Kubmarawa et al., 2008). Fortunately, fermentation significantly reduced anti-nutrient levels–specifically oxalate, phenol, and cyanogenic glycosides–in both uninoculated and inoculated banana peels (*Aspergillus niger* and *Bacillus subtilis*) compared to unfermented peels. This indicates that fermented banana peels are safe for both human and livestock consumption.

CONCLUSION

This study revealed variations in the proximate and anti-nutrient composition of banana peels after fermentation with pure strains of *Aspergillus niger*, *Bacillus subtilis*, and uninoculated processes, likely due to microbial enzyme activities. *Bacillus subtilis* fermentation resulted in the highest increase in carbohydrate and protein contents, with the lowest fat content and cyanogenic glycosides. *Aspergillus niger* fermentation showed different anti-nutrient reduction patterns. These findings align with research demonstrating fermentation improves banana peel nutritional profiles and reduces anti-nutrient content (Ozabor et al., 2020; Sahoo & Lenka, 2024), representing a sustainable approach to waste management and food security (Hikal & Said-Al Ahl, 2022).

01

Use *Bacillus subtilis* for basic animal feed

Banana peels fermented with *Bacillus subtilis* can be formulated as food for farm animals that require less fat but basic nutritional requirements (carbohydrate and protein).

02

Use *Aspergillus niger* for high-fat animal feed

Fermented banana peels with *Aspergillus niger* can be formulated for animals that require significant fat and energy, such as pigs.

03

Utilise uninoculated fermented peels

Uninoculated fermented banana peels can also be formulated as food for animals due to their high fibre content that aids digestion.

04

Conduct further research

Further research should examine other banana varieties, different fermenting organisms, and conduct in-vivo assays to determine their effects on animals.

High fibre content, as found in uninoculated fermented banana peels, promotes health benefits for livestock and fish farming by aiding digestion through increased removal of potential mutagens, steroids, and xenobiotics.

RECOMMENDATION

I recommend the use of banana peels fermented with *Bacillus subtilis* and uninoculated as part of animal feed formulations, given their ability to increase carbohydrate, protein, and fibre content. Further research should analyse other banana varieties and plant wastes using different fermenting organisms and conduct in-vivo assays to determine their effects on animals.

ACKNOWLEDGEMENT

Not Applicable

CONFLICTS OF INTEREST

The author declares no conflict of interest.

FUNDING

This research received no funding from any agency.

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Received: June 30, 2025

Accepted: August 22, 2025

Published: November 19, 2025

Citation:

Yinka D. O., Ojoko, A. O., & Adeoye, O. M. (2025). Effect of Fermentation on the Proximate and Antinutrient Composition of Banana Peels Fermented with *Aspergillus niger* and *Bacillus subtilis*. *SustainE, 3*(3), 210 - 240. In A. A. Atowoju, E. O. Oyekanmi, A. A. Akinsemolu, & D. M. Duyile (Eds.), *Sustainability, Innovation, and Development: A Festschrift in Honour of Rt. Rev. Prof. Obeka Samuel Sunday* [Special issue]. https://doi.org/10.55366/suse.v3i3.11