Optimization of Solid-State Fermentation of *Sorghum bicolor* Using *Aspergillus niger* and Its Impact on Nutritional Composition

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ABSTRACT

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| The production of poultry contributes significantly to global food security by offering a reasonably priced source of protein. However, a major obstacle is the high cost of feed components, especially maize. Because of its high fiber content and anti-nutritional elements like tannins and phytates, sorghum stalk, an abundant agricultural residue, presents a possible substitute but is still neglected. This study aimed to optimize the solid-state fermentation of sorghum stalk using *aspergillus niger* to enhance its nutritional composition. The fermentation process was optimized by varying pH, ranging from pH levels of 5 to 8 and temperature of 200C to 350C together with fermentation duration to maximize enzymatic activity and microbial biomass growth in six days. From the results pH6 and temperature 350C has the highest biomass growth. The proximate composition at pH6 were; moisture (45.63% to 37.37%), crude fat (2.41% to 1.24%), crude fibre (4.41% to 2.91%), Crude protein (2.47% to 2.01%), Ash (5.56% to 3.60%) and Carbohydrate (39.83%). The proximate result at temperature 350C were; moisture (38.83%), crude fat (2.87%), crude fibre (4.27%), Crude protein (2.80%), Ash (5.27%), and Carbohydrate (45.95%). The results obtained highlighted the potential of solid-state fermentation as an effective method for improving the nutritional quality of sorghum stalk enhancing its value as animal feed. |

*Keywords:* *Poultry feed, sorghum stalk, solid-state fermentation, aspergillus niger, Nutritional enhancement.*

1. INTRODUCTION

Poultry production plays a critical role in global food security, providing an affordable source of protein-rich meat and eggs. However, one of the primary challenges in the poultry industry is the high cost of feed ingredients, particularly maize, which accounts for a significant portion of production costs (1). Katsina State in northern Nigeria the study area, has of recent witnessed a rise in the prevalence of banditry and kidnapping that has led to a decrease in agricultural production, leading to a continuous increase in the cost of food stuffs including the raw materials for poultry feed (2). In feed production, maize is the carbohydrate source of choice, but its availability and affordability is usually affected by the supply chain leading to its demand usually exceeding its supply with a resultant increase of above 2000% over the last 2 decades (3). This gloomy scenario has translated to a hike in the cost of poultry feeds and an exponential increase in poultry production cost. Left with no option, feed producers are forced to scout for alternatives that will replace the conventional feed raw materials but at the same time maintaining quality and standards. Several initiatives have been implemented, such as finding cheaper and locally available materials as partially substitutable energy source instead of maize in poultry feed formulations (4). The world generates a large volume of agro-waste byproducts that might be beneficial alternative feedstuffs (5).

Sorghum stalk, an abundant agricultural byproduct, offers a promising alternative to maize but is underutilized due to its high fiber content and the presence of anti-nutritional factors such as tannins and phytates (6). While most are produced for human consumption, the remainder is cropped for industrial applications (7). Sorghum is valued for its nutritional benefits, as it is a good source of carbohydrates, protein, fiber, vitamins (such as niacin, B6, and folic acid), and minerals (like magnesium, phosphorus, and zinc) (8).

Solid-state fermentation (SSF) is a biotechnological approach that utilizes filamentous fungi such as *Aspergillus niger* to improve the digestibility and nutritional value of plant-based feed ingredients (9). The fermentation process enhances protein content, reduces fiber, and mitigates anti-nutritional factors, making sorghum stalk a viable substitute for conventional feed ingredients. The reliance on maize as a primary poultry feed ingredient poses economic and environmental challenges due to price fluctuations and supply chain disruptions (10). Sorghum stalk is readily available in sorghum-producing regions, yet its direct use in poultry diets remains limited. Fermentation using *Aspergillus niger* can significantly improve its nutritional value, thereby providing an economical and sustainable alternative to traditional feedstuffs (11). Furthermore, reducing agricultural waste through bioconversion aligns with sustainable farming practices and the circular economy model, minimizing environmental impact while maximizing resource efficiency (12). By optimizing solid state fermentation conditions, this study aims to enhance the applicability of sorghum stalk as a nutrient-rich feed component.

2. material and methods

**2.1 Sampling Area**

The study was carried out during 2024 in Katsina State, Nigeria. The State is located between latitude 12015’N and longitude of 7030’E in the North West Zone of Nigeria, with an area of 24,192km2 (9,341 sq meters). Katsina State has two distinct seasons: rainy and dry. The rainy season begins in April and ends in October, while the dry season starts in November and ends in March. The average annual rainfall, temperature, and relative humidity of Katsina State are 1,312 mm, 27.3ºC and 50.2%, respectively (13).

 **2.2 Sample Preparation**

Sorghum stalks were obtained from farms located within Umaru Musa Yar’adua University Katsina, Katsina State, Nigeria. The Sorghum stalks were sorted cleaned to remove any contaminants or foreign materials, they were dried thoroughly to reduce moisture content, which helps prevent mold growth during storage. The dried Sorghum stalks were then grinded into smaller particles which increases the surface area. The grinded sample was treated using concentrated sulphuric acid (H2SO4) to reduce contamination.

**2.3 Preparation of fermentation Medium**

Exactly 60 g each, of the sample were measured and placed into ten different fermentation trays. 140mls of solution containing 28 g of peptone dissolved in 1000mls of distilled water, 0.28 g of Disodium Hydrogen Phosphate, 1.4 g of Sodium Chloride, was measured and placed into each fermentation tray, this mixture was shaken vigorously and was then autoclaved at 121⁰C for 30 minutes.

**2.4 Inoculum Preparation and Fermentation Process**

A pure culture of *Aspergillus niger* was obtained from a previously isolated strain at the laboratory in the department of Microbiology of Umaru Musa Yaradua University, Katsina State. The inoculum was prepared by cultivating the *Aspergillus niger* in potato dextrose broth (PDB) at 30°C for 48 hours (14). The grounded sorghum stalks were placed in sterile fermentation trays to a thickness of about 2-3 cm. The substrate was inoculated with the prepared spore suspension at a rate of 10% (v/w) and thoroughly mixed to ensure even distribution of the inoculum. The trays were incubated at 30°C with 70-80% relative humidity for 7 days (15). The pH levels were adjusted using sodium hydroxide to achieve the desired pH levels of 5, 6, 7, and 8 (16).

The temperature of the fermentation vessels were varied by placing them in separate rooms with temperatures of 20, 25, 30, and 35°C (16). Sampling for biomass measurement were conducted every 24 hours, and the fungal biomass were harvested by filtration, washed, dried, and weighed to determine the dry weight. Biomass growth were quantified based on weight differences of the biomass before and after drying (17 Khan). Proximate analysis was conducted to determine the nutritional contents in fermented sorghum stalks samples using standard methods (18 AOAC, 2012).

3. results and discussion

**3.1 Nutritional Composition of Fermented Sorghum Stalk**

Moisture, ash, crude fiber, crude protein, and carbohydrate content were measured to assess the nutritional makeup of sorghum stalks before and after solid-state fermentation (SSF). The results demonstrate that fermentation considerably changed the sorghum stalk's nutritional composition, increasing its potential as a feed item with added nutrients.

**3.2 Biomass Growth of *Aspergillus niger* at Different pH Levels and Temperatures During Sorghum Stalk Fermentation**

Table 1 illustrates the biomass growth of *Aspergillus niger* under varying pH levels and temperatures during the fermentation of sorghum stalk over a period of 162 hours. *Aspergillus niger*, a filamentous fungus commonly used in industrial applications due to its robust enzyme production, displays optimal growth under specific pH6 conditions that align with findings from other studies (19; 20). The observed trend in the biomass growth of *Aspergillus niger* aligns with existing literature, where acidic to neutral pH levels generally favor fungal growth (21). According to Yin et al. (19) *Aspergillus niger* typically shows enhanced growth in slightly acidic conditions, with decreased biomass as the environment becomes more alkaline. This is likely due to enzyme functionality, as enzymes critical to fungal metabolism perform optimally within specific pH6 ranges (19).

The fungal development at pH 6, rose from 0.15 at 24 hours to 0.77 at 162 hours. Similar to pH 7, while somewhat lower, pH 5 and pH 8 also promoted notable development. The lowest growth was seen under the control pH condition, indicating that fermentation conditions have a major impact on fungal proliferation. The results may also reflect the cellular adaptability of *Aspergillus niger* in acidic environments, supporting findings by Zhou *et al.* (20) that show metabolic processes in fungi, including nutrient uptake and enzymatic activity, are optimized under these conditions.

Temperature also had a significant impact on biomass accumulation; at 35°C, biomass increased the most, from 0.17 at 24 hours to 0.83 at 162 hours. This observation is consistent with research indicating that *Aspergillus niger* has optimal growth and metabolic activity within a mesophilic temperature range, typically between 30–37°C, where enzymatic reactions are highly efficient (22; 14). According to these results, *Aspergillus niger* grows most effectively at a relatively high temperature and a neutral pH, which increases microbial activity and fermentation efficiency. Studies showed that growth at 35°C aligns with the thermal stability of key enzymes in *Aspergillus niger*, such as proteases and amylases, which facilitate rapid biomass accumulation under optimal conditions (22). Li *et al*. (14) explain that maintaining such temperatures can support the high metabolic rate needed for industrial applications, such as citric acid production, as *Aspergillus niger* exhibits increased synthesis of organic acids and secondary metabolites at this temperature.

 **Table 1: Biomass Growth of *Aspergillus niger* at Different pH Levels and Temperatures During Sorghum Stalk Fermentation**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **24hrs** | **48hrs** | **72hrs** | **96hrs** | **138hrs** | **162hrs** |
| pH 8 | 0.08 | 0.10 | 0.13 | 0.16 | 0.21 | 0.32 |
| pH 7 | 0.13 | 0.25 | 0.37 | 0.53 | 0.61 | 0.73 |
| pH 6 | 0.15 | 0.19 | 0.32 | 0.56 | 0.64 | 0.77 |
| pH5 | 0.08 | 0.12 | 0.25 | 0.44 | 0.67 | 0.71 |
| pH Control | 0.08 | 0.13 | 0.19 | 0.27 | 0.47 | 0.54 |
| T20°C | 0.04 | 0.09 | 0.16 | 0.21 | 0.23 | 0.37 |
| T25°C | 0.06 | 0.13 | 0.17 | 0.23 | 0.31 | 0.44 |
| T30°C | 0.13 | 0.27 | 0.29 | 0.36 | 0.57 | 0.67 |
| T 35°C | 0.17 | 0.29 | 0.36 | 0.59 | 0.72 | 0.83 |
| T Control | 0.13 | 0.23 | 0.32 | 0.41 | 0.53 | 0.59 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

Table 2 details the nutritional makeup of the fermented sorghum stalk. Temperature and pH had a major impact on the nutritional makeup of the fermented sorghum stalk, according to the proximate analysis. Out of all the pH levels that were examined, pH 6 had the lowest carbohydrate content (39.83%) and the greatest ash content (5.56%), crude fiber (4.41%), moisture (45.63%), and crude fat (2.41%). This implies that the sorghum stalk's total nutritional content was raised and nutrient retention was increased by fermentation at pH 6. This finding is consistent with research by Mukumbo *et al*. (23), which highlights that fermentation often results in reduced moisture content, thereby enhancing the shelf life of the feed material and which could improve feed palatability.

 On the other hand, pH 7 had the greatest crude protein concentration (2.47%) and lowest in the pH 5 (2.01%), suggesting that *Aspergillus niger* may benefit from a neutral pH for protein synthesis. This significant increase in protein at pH 7 can be attributed to the enhanced microbial synthesis of proteins during fermentation, which is crucial for improving the nutritional value of the Sorghum stalk as animal feed. According to Ma *et al.* (24), fermentation can enhance protein content through microbial biomass accumulation, especially at optimal pH levels conducive to microbial growth.

Crude fat content ranged from 1.24% in the control to 2.41% at pH 6. Higher fat content at elevated pH levels could be due to microbial synthesis of lipids under acidic conditions, as supported by Alokika *et al*. (25), who noted that fermentation can increase fat content by microbial biosynthesis of fatty acids. The decline in crude fat at lower pH levels suggests that acidic conditions may inhibit lipid accumulation. Ash content ranged from 3.60% in the control to 5.56% at pH 6. The increased ash content at pH 6 levels indicates a higher concentration of minerals, possibly due to enhanced breakdown and release of mineral components from the stalk matrix. This is consistent with findings by Kimarai (26), who noted that fermentation, can increase the bioavailability of minerals in feed substrates

 With the carbohydrate content remaining at its greatest (52.09%), the control pH condition had the lowest values for the majority of proximate metrics. This suggests that fermentation was less successful in increasing nutrient bioavailability in the absence of pH optimization This reduction in carbohydrates during fermentation reflects the utilization of sugars by fermenting microorganisms, as documented by Alokika *et al*. (25). However, Crude fiber content was notably higher at pH 6 compared to other pH levels, reinforcing the importance of optimized fermentation conditions in breaking down fiber while preserving essential nutrients.

**Table 2: Proximate Composition of Fermented Sorghum Stalk at Varying pH Levels**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **parameters** | **% Moisture** | **% Crude fat** | **% Crude Fibre** | **% Crude Protein** | **% Ash** | **% Crude Carbohydrate** |
| pH 8 | 43.20 | 1.81 | 3.95 | 2.31 | 3.76 | 44.97 |
| pH 7 | 42.10 | 1.70 | 3.62 | 2.47 | 4.80 | 45.31 |
| pH 6 | 45.63 | 2.41 | 4.41 | 2.16 | 5.56 | 39.83 |
| pH5 |  44.12 | 1.30 | 3.26 | 2.01 | 4.21 | 45.10 |
| pH Control |  37.74 | 1.24 |  2.97 | 2.36 | 3.60 | 52.09 |

The nutritional composition of fermented sorghum stalk at various temperatures varied significantly and where presented in Table 3, according to proximate analysis. Of the temperatures studied, fermentation at 35°C produced the lowest carbohydrate content (45.95%) and the highest values for crude protein (2.91%), crude fat (2.87%), crude fiber (4.27%), and ash content (5.27%). According to this, fermentation of sorghum stalk at 35°C improves its quality as a feed component and increases nutrient retention. The high content at 35ºC indicates that elevated temperatures can enhance the nutritional value of fermented sorghum stalk by promoting microbial protein synthesis. This observation is supported by Ma *et al.* (24), who noted that higher fermentation temperatures boost protein content through increased microbial activity. Ash content peaked at 35ºC, suggesting that this temperature may be optimal for mineral retention. Kimarai (26), emphasize the importance of fermentation temperature in determining mineral bioavailability, and the current study’s results align with these observation.

*Aspergillus niger* restricted nutritional conversion was demonstrated by the control temperature (unregulated fermentation), which had the greatest carbohydrate content (71.30%) but the lowest crude protein and ash content. Temperature-induced increases in moisture content peaked at 35°C (38.73%), which may enhance digestion but may potentially compromise storage stability as observed by Olukomaiya *et al*. (27), where lower temperatures slowed down microbial metabolism and preserved the carbohydrate content of the substrate.

**Table 3: Proximate Composition of Fermented Sorghum Stalk at Varying Temperature Levels**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameters0C** | **% Moisture** | **% Crude fat** | **% Crude Fibre** | **% Crude Protein** | **% Ash** | **% Crude Carbohydrate** |
| T20°C | 24.41 | 2.07 | 3.42 | 2.18 | 3.68 | 64.24 |
| T25°C | 26.71 | 1.71 | 3.49 | 1.97 | 2.54 | 63.58 |
| T30°C | 33.64 | 1.63 | 2.98 | 2.34 | 2.78 | 56.63 |
| T 35°C | 38.73 | 2.87 | 4.27 | 2.91 | 5.27 | 45.95 |
| TControl | 20.48 | 1.39 | 2.89 | 2.16 | 1.78 | 71.30 |

4. Conclusion

The optimization of solid state fermentation using *Aspergillus niger* significantly enhances the nutritional value of sorghum stalk which showed more productivity at an Optimum temperature of T35 and optimum pH of 6. It also shows that proximate composition of fermented sorghum stalk is influenced by the pH level during fermentation, optimal fermentation at pH 6 appears to enhance protein content while moderating fiber and carbohydrate levels. Temperature treatment significantly influences the proximate composition of feed, with higher temperatures leading to increased crude fiber and protein content, while decrease in temperature lowers carbohydrate content and favor moisture retention.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models and text-to-image generators have been used during the writing or editing of this manuscript.

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