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Determining the Ideal Temperature and Fermentation Duration to Enhance Crude Protein Content and Reduce Crude Fiber in Rice Bran Using Solid-State Fermentation with *Aspergillus niger* (USM F4)

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ABSTRACT

Solid-state fermentation (SSF) offers a sustainable method for enhancing the nutritional quality of agricultural residues such as red rice bran. This study aimed to determine the optimal temperature and duration for SSF of red rice bran, focusing specifically on increasing the crude protein (CP) content and reducing the crude fiber (CF) content. SFF of rice bran with *Aspergillus niger* (*A. niger*) USM F4 was conducted over 14 consecutive days at three different temperatures (25°C, 35°C, and 45°C). A total of 63 samples of rice bran were divided into three temperature groups, each containing 21 samples. Three samples per group were collected at 48-hour intervals over the 14-day fermentation period. The fermentation process for the collected samples at 48-hour intervals was halted by oven drying at 60°C for 24 hours. The fermented products were subjected to proximate analysis for crude protein (CP), ash, ether extract (EE), and crude fiber (CF) contents using the methods outlined by the Association of Official Analytical Chemists (AOAC). The results revealed a significant effect of temperature and fermentation duration on CP, ash, EE, and CF content when compared to the unfermented rice bran kept at room temperature (25°C). The peak values of CP and the highest degradation of CF across all temperature levels were observed on day 10 while the maximum increase in ash and EE content occurred on day 8. Among the temperature conditions, the highest CP values and the lowest CF values were recorded at 35°C. Conversely, the lowest improvements in CP and CF degradation were observed at 25°C on day 10. In conclusion, the optimal conditions for SSF of rice bran with *A. niger* to enhance CP content and degrade CF are a temperature of 35°C and a fermentation duration of 10 days.

Keywords: Alternate feed resource, Animal production, *Aspergillus niger*, Proximate component, Solid-state fermentation, Value- addition

INTRODUCTION

Food insecurity in Africa poses a significant threat to the continent's sustainability. Boosting livestock production levels is imperative for augmenting the supply of animal protein for human consumption, thereby contributing to global efforts to address food scarcity, hunger, and food security challenges. The high cost and scarcity of conventional feed ingredients pose a significant challenge to the sustainability of animal production. Consequently, the industry has increasingly focused on identifying alternative feed resources (Akintan et al., 2024; Dou et al., 2024).

Along the same line, agro-industrial by-products have turned into a primary focus of animal scientists seeking potential alternatives to conventional feed resources (Ayojimi et al., 2023). Many of these by-products, including rice bran (RB), are characterized by low crude protein content, high crude fiber levels, and the presence of anti-nutritive factors such as low digestibility and nutrient lock-up, which limit their suitability for livestock production (Obaniyi et al., 2019; Yang et al., 2021).

Fermentation has long served as a method for preserving and enhancing the quality attributes of foods (Siddiqui et al., 2023). Solid-state fermentation (SSF) occurs without free water and presents a technological solution for processing various foods, enhancing their nutritional value, and creating edible products with favorable sensory traits (Senanayake et al., 2023). During SSF, microorganisms metabolize the substrate, producing various metabolites such as organic acids, enzymes, vitamins, antibiotics, and bioactive compounds. These metabolites have potential applications in the food, pharmaceutical, and cosmetics industries, to name a few. The liberated enzymes also help increase the nutritional value of the substrate by increasing the crude protein content and reducing the crude fiber level, thereby transforming the agro-industrial waste into value-added products (Sadh et al., 2018; Senanayake et al., 2023). Yang et al. (2021) assert that the

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SSF of feed represents a promising strategy for bridging the gap between feed supply and demand, enhancing food safety, conserving energy, and lowering emissions.

Fermentation, as proposed by Osemwegie et al. (2020), can enhance the nutritional value of ingredients before they are consumed by animals. Solid-state fermented feed production yields various beneficial components, such as organic acids, enzymes (amylase, cellulase, etc.), vitamins, β-Carotene, γ-linolenic acid, citric acid, peptides, and growth factors, all of which can positively impact animal performance (Yang et al., 2021).

The nutritional characteristics of fermented feed are influenced by several factors, including the fermentation starter (the culture initiating fermentation), the substrate type, the initial moisture content, and the fermentation conditions, such as temperature and duration (Mengesha et al., 2022). Recent research has consistently shown that adjusting the moisture content of fermentation substrates is crucial and should be tailored to the specific properties of the substrates (Sun et al., 2022; Wang et al., 2023), microbial attributes, duration, and temperature (Park et al., 2018).

Optimal temperature is crucial for promoting microorganism growth and metabolism, which in turn reduces the fermentation time and improves the product quality (Yang et al., 2021). Elevated temperatures can accelerate reactions and microbial growth (Li et al., 2024). However, they may also deactivate enzymes and cause excessive heat due to the rapid proliferation of microorganisms (Yang et al., 2021). Moisture content is another key factor that significantly impacts the quality of solid-state fermented feed. Low moisture in solid-state fermentation hampers the movement of nutrients, reduces enzyme activity, and consequently limits the growth of microorganisms, thereby affecting the efficiency of the fermentation process (He et al., 2019). Inadequate water availability within the system can prevent nutrients from spreading effectively throughout the substrate, limiting their availability to microorganisms. The enzymes require water to function optimally, and their activity may be hindered when water is scarce. Also, low moisture conditions impede nutrient diffusion and enzyme activity, creating an unfavorable environment for microbial growth (Yang et al., 2021). As a result, the growth of microorganisms is restricted or slowed down, impacting the overall fermentation process (Cruz-Parede et al., 2021; Gonzalez and Aranda, 2023). Conversely, excessive moisture can reduce substrate porosity, impair oxygen transfer, and increase mycotoxin risk. Improper moisture levels disrupt microbial growth, destabilize pH, and reduce dry matter recovery. Laure et al. (2021) opted that incubation duration is another critical factor in the SSF process, as it influences the quality of the end products. In the early stages of SSF, an ample supply of food supports the growth of microorganisms and the production of the desired end product. However, in case the fermentation process is terminated too soon, the organisms may not have completed the conversion of all available nutrients into an end product, leading to incomplete fermentation and potentially lower concentrations of the desired end products (Yang et al., 2021). Extended fermentation periods, on the contrary, diminish nutrient availability, leading to a decline in bacterial population and the triggering of autophagy (Zhang et al., 2015).

Different microorganisms can be used to valorize various substrates for the production of specific products (Pranay et al., 2019; de Oliveira et al., 2020; Melnichuk et al., 2020; Putri et al., 2020). Filamentous fungi from genera such as *Aspergillus, Fusarium, Penicillium, Rhizopus, and Trichoderma* are particularly favored for solid-state fermentation, as highlighted by Munishamanna et al. (2017). This preference stems from their hyphal structure, which enables them to penetrate solid matrices effectively. Additionally, these fungi exhibit efficient digestion of solid organic substrates even under low moisture conditions, rendering them particularly suitable for solid-state fermentation, as emphasized by Abu Yazid et al. (2017). The selection of microorganisms is influenced by several critical factors, including their growth traits, the yield of the intended product, their ability to metabolize particular substrates, their resilience to temperature and pH fluctuations, their susceptibility to genetic alterations, and the safety of the resulting fermented product for human and animal consumption (Upadhyaya et al., 2016).

Yafetto et al. (2020), for instance, showcased that the protein levels in cassava and yam substrates could be enhanced through the application of mono- and co-cultures involving *Aspergillus niger* and *Trichoderma viride*. Similarly, Aruna et al. (2018) utilized a mono-culture approach using the yeast *Saccharomyces cerevisiae* to increase the protein content of yam peels.

Maximizing the potential of solid-state fermentation in enhancing agro-industrial by-products in the livestock industry requires the determination of optimal conditions. Hence, this study aimed to identify the ideal temperature and duration for solid-state fermentation of rice bran (RB) using *Aspergillus niger* (USM F4).

MATERIALS AND METHODS

Study location

The research was conducted at the Animal Science and Microbiology Laboratories of Landmark University, Omu-Aran, Kwara State, Nigeria.

Source of rice bran

The rice bran was purchased from a feed mill in Omu Aran town, Kwara State, Nigeria.

Source of candidate organism for solid-state fermentation

The fully typed strain of filamentous *Aspergillus niger* (USM F4) was obtained from the stock culture at the Microbiology Laboratory of Landmark University, Omu Aran, Kwara State, Nigeria. The organism was sub-cultured on Potato Dextrose Agar slants and maintained at 4°C for a week (Ogbonna et al., 2015; Ajibo and Said, 2023). To inhibit bacterial growth, the agar was supplemented with 0.5 ml of gentamycin (40 mg/ml) After 7 days, the mycelia had completely covered the surface of the petri dish. Spores of *Aspergillus niger* were harvested by gently tapping and inverting the plate. Subsequently, the spores were washed with sterile saline solution, and their quantification was performed using a hemocytometer (Model No: 8100110, AS ONE Japan) following the Fuchs-Rosenthal technique (Wolk et al., 2000), resulting in an estimated concentration of approximately 2.0×10^7 spore-forming units (sfu) per milliliter

Substrate preparation and inoculation for solid-state fermentation

Thirty grams of rice bran (RB) were weighed and added to each of the sixty-three 250 ml Erlenmeyer flasks. Subsequently, the moisture content was adjusted to 80%, and the pH was set to 5.0, as described by Teixeira da Silva et al. (2016). The mouths of the flasks were sealed with water-resistant cotton wool and covered with aluminum foil. All the samples were then autoclaved for 15 minutes at a pressure of 103.421 KPa and a temperature of 121°C. After autoclaving, the samples were cooled at 25°C before inoculation with the fungus. In each flask, 0.1 g of urea [$CO(NH_2)_{/2}$], serving as a nitrogen supplement, was added per gram of the sample. After sterilization, rice bran samples in each flask were aseptically inoculated with 4 ml of the prepared *A. niger* (USM F4), containing $2.0 \text{ x } 10^7$ spore-forming units per milliliter, within a laminar flow chamber. The incubation temperature varied at 25°C, 35°C, and 45°C, with 24 inoculated samples incubated at each temperature.

Triplicate samples were collected at 2-day intervals over 14 days (specifically on days 2, 4, 6, 8, 10, 12, and 14) at each temperature to determine the optimal fermentation period. The fermentation process was terminated by oven drying the samples at 60°C for 24 hours, following the method outlined by Lasekan and Shittu (2019).

Determination of proximate values

The unfermented RB and the fermented samples were analyzed for crude protein (CP), ether extract (EE), ash, and crude fiber (CF) using standard procedures outlined by the Association of Official Analytical Chemists (AOAC, 2012). Crude protein content was analyzed by the Kjeldahl Method (AOAC Method 968.06). It was then calculated by multiplying the value of nitrogen by a conversion factor of 6.25.

Crude protein (%) = N X 6.25 (Formula 1)

Ether extract (EE) analysis was performed using Soxhlet Extraction (AOAC Method 920.39). Lipids in the samples were extracted utilizing petroleum ether as the solvent. The samples were placed in an extraction thimble and introduced into the Soxhlet apparatus. As the ether was heated to its boiling point, it passed through the samples, dissolving the lipids, which were then transported into a collection flask. The extraction continued until all fat was thoroughly removed. Following this, the lipid residue underwent drying to eliminate any lingering traces of ether. The weight of the dried residue was measured to determine the quantity of lipids extracted from the samples using Formula 2.

Ether extract (%) = (initial weight – final weight) ÷ initial weight X 100 (Formula 2)

Ash analysis was conducted using the Dry Ashing method (AOAC Method 942.05), where the organic material underwent complete combustion, leaving behind inorganic residue (ash). One gram of the sample was heated in a muffle furnace at 550°C until thorough combustion occurred. After cooling, the ash was weighed using a sensitive scale (Chaux Corp. Pine Brook, NJ USA. Model PAS 12).

The analysis of crude fiber (CF) was conducted using AOAC method 978.10. It involved sequential acid and alkali extractions to eliminate protein, sugar, starch, lipids, and portions of structural carbohydrates and lignin. Initially, the sample underwent digestion with sulfuric acid to extract sugar and starch. Subsequently, secondary digestion in sodium hydroxide was aimed at removing proteins, hemicellulose, and lignin. The determination of crude fiber was achieved gravimetrically by examining the residue remaining after the completion of acid and alkaline digestions.

Statistical analysis

All experiments were conducted in triplicate, with the proximate parameters expressed as the mean ± standard deviation of three measurements. Statistical analysis was performed using SPSS version 18, employing analysis of

variance (ANOVA) to compare the data. Mean values were compared using the Duncan multiple range test, with significance set at $p < 0.05$. The optimization of temperature and fermentation period was performed using a one-factor-at-a-time design. This approach is straightforward to implement, making it suitable for preliminary or exploratory studies where simplicity is prioritized over complexity.

RESULTS AND DISCUSSION

Table 1 presents the proximate analysis of the unfermented rice bran. The values obtained are similar to those in previous studies on the proximate composition of rice-bran meal (Mishra, 2017; Devi et al., 2021).

Table 1. Proximate components of unfermented rice bran

Parameters	Percentage
Moisture	9.45
Crude protein	13.48
Ether extract	12.35
Ash	10.50
Crude fiber	27.50
Nitrogen free extract	29.72

Effect of temperature and fermentation period on proximate components of rice bran

The findings of this study indicated that the proximate components of rice bran were significantly influenced ($p < 0.05$) by varying solid-state fermentation temperatures and durations, as compared to unfermented rice bran.

Effect of temperature and fermentation period on crude protein

The crude protein (CP) content is a crucial parameter affecting the overall quality of animal feed products. The CP level can vary depending on factors such as the microorganism used, their access to carbon and nutrients, and cultivation conditions (Shi et al., 2021). Figure 1 illustrates the changes in the CP content of RB following SSF at three temperature levels and with different durations. In this study, the CP content exhibited a progressive increase with fermentation duration across all temperature levels, albeit at varying rates. Various authors have reported comparable results using rice bran as a substrate with different fungal species. Specifically, Omarini et al. (2019) utilized *Pleurotus sapidus*, Wolayan and Mandey (2019) employed *A. niger*, Sukma et al. (2021) utilized *Rhizopus oryzae*, and Huervana et al. (2024) employed *Tichoderma harzianum.*

In SSF, the fungus releases a range of extracellular enzymes, enabling it to acquire nutrients while altering the chemical composition of the substrate and producing various metabolites (El-Gendi et al., 2021). The rise in protein levels may stem from the proliferation of fungus mycelium driven by microbial growth and reproduction throughout fermentation (Shi et al., 2021). The increased CP content of the RB suggests that the treated substrate may serve as a valuable protein source for livestock (Huervana et al., 2024). The greatest enhancements in CP content occurred on the 10th day across all temperature levels, followed by a decline. Specifically, CP values reached 18.23%, 25.34%, and 22.35% at 25°C, 35°C, and 45°C, respectively, representing respective improvements of 35.23%, 87.98%, and 65.80% compared to the 13.48% CP content in unfermented RB. The notable 87.98% improvement observed at 35°C suggests that *A. niger* USM F4 thrives best at this temperature. During fermentation, the fungus may release proteins from the substrate to utilize it as a carbon source, thereby increasing the CP of the fermented product. Variations in CP among different temperatures may be attributed to *A. niger* USM F4 releasing a higher number of enzymes during fermentation at 35°C.

Huervana et al. (2024) had earlier reported a 169.2% enhancement of CP in rice bran fermented with *Tichoderma harzianum* at 30°C for 4 days under solid-state fermentation. Wolayan and Mandey (2019) conducted a similar study, fermenting RB with *A. niger* at 30°C for 10 days, and reported a crude protein enhancement of 41.24%. This enhancement is notably lower than the values observed at 35°C on the 10th day in the current study. Similarly, Sukma et al. (2021) conducted an SSF experiment using the fungus *Rhizopus oryzae* (*R. oryzae*) at varying incubation temperatures (28°C, 30°C, and 32°C) for 5 days and achieved the highest percentage increase in CP content of 62.51% at 30°C. Also, Oliveira et al. (2010) reported a 40% enhancement in CP using the same fungus (*R. oryzae)* at 30°C. Omarini et al. (2019) reported a 72.97% increment in crude protein when RB underwent 10 days of solid-state fermentation assisted by *Pleurotus sapidus* at 25°C for 5 days.

The choice of variables, such as the fermentation temperature, the duration, and the microorganism used for the fermentation process significantly influenced the crude protein enrichment in RB in this study (Sukma et al., 2021).

Furthermore, differences between the values obtained in this study and those in the previous studies may be attributed to various factors, including the origin of *A. niger*, its enzyme production capacity, the amount of inoculum, the duration and conditions of fermentation, the pretreatment method, the origin of the substrate, etc. (Altop et al., 2018).

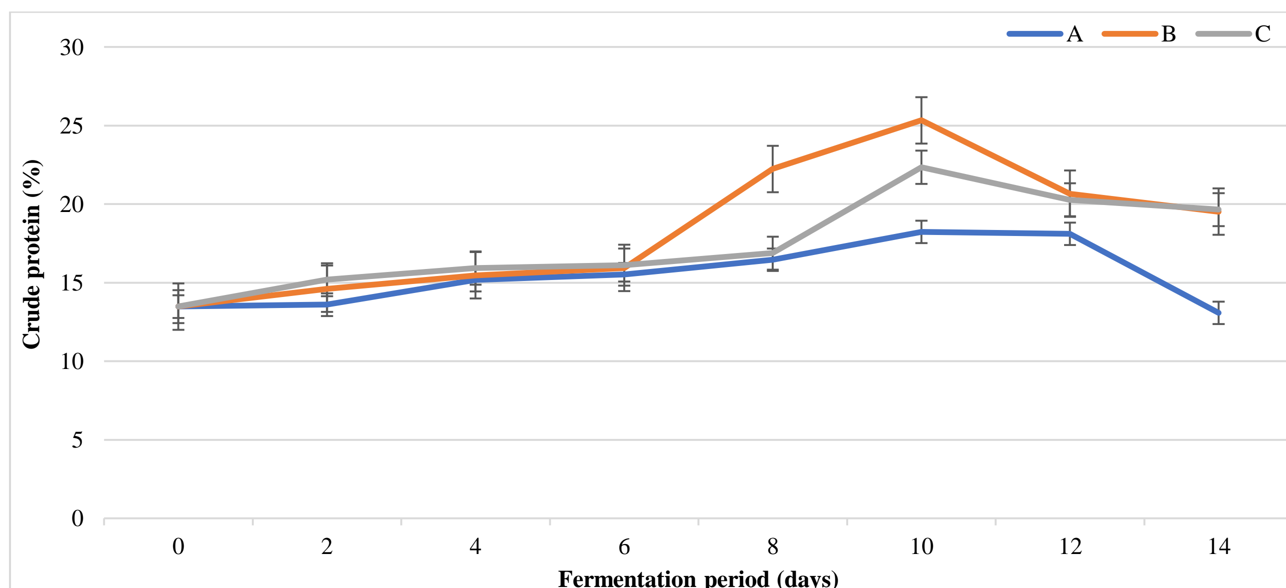

Figure 1. Influence of temperature and fermentation period on the crude protein of rice bran .**A**: 25°C, **B**: 35°C, **C**: 45°C.

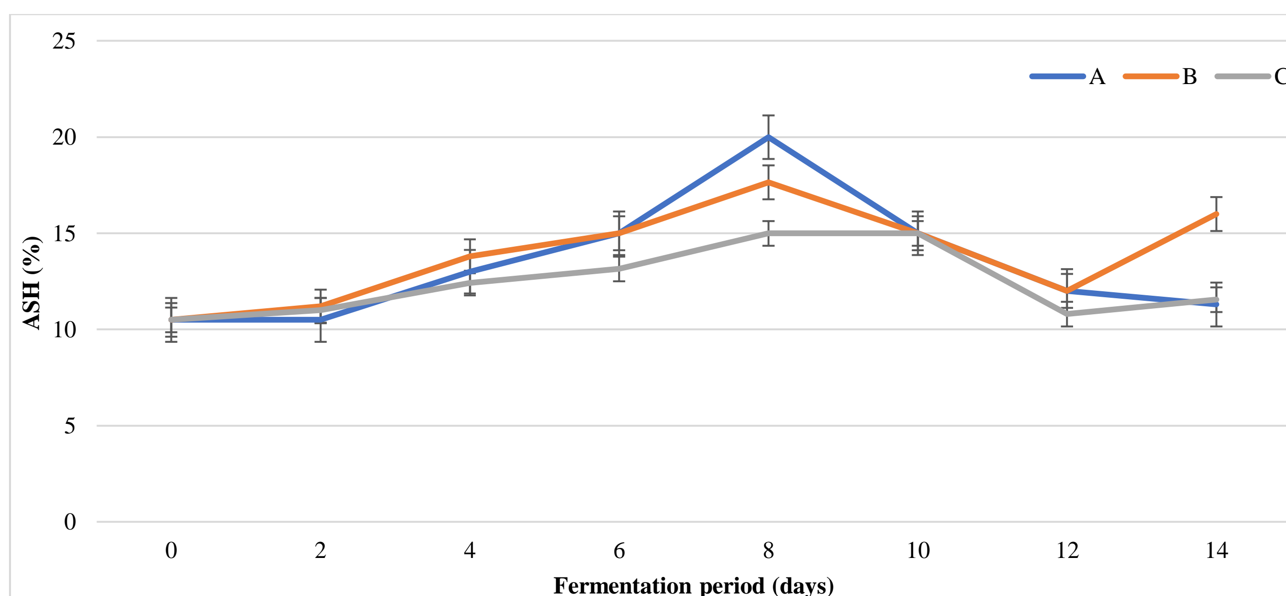

Figure 2. Effect of temperature and fermentation period on the ash content of rice bran .A: 25°C; B: 35°C; C: 45°C

Effect of temperature and fermentation period on ash component of rice bran

It was noted that the solid-state fermentation process led to an increase in the ash value of rice bran. As illustrated in Figure 2, both temperature and fermentation duration exerted significant effects on the ash content of RB ($p < 0.05$). Across all temperature levels (25°C, 35°C, and 45°C), the ash content exhibited a consistent rise from 10.50% in unfermented rice bran until day 8, followed by a gradual decline. The maximum ash contents recorded were 20.00% (25°C), 17.65% (35°C), and 15.00% (45°C), representing enhancements of 90.48%, 68.10%, and 42.10% of the ash value in unfermented RB, respectively.

The rise in ash content indicates the potential release of enzymes such as phytase by the organism during fermentation, contributing to the breakdown of antinutrients present in the unfermented substrate. This release may have liberated minerals previously bound or chelated by these antinutrients (Altop et al., 2018). *A. niger* USM F4 may induce the production of enzymes such as hydrolase, hemicellulase, lipase, and pectinase during SSF, potentially altering the structure of anti-nutritional factors (Kaur et al., 2020). Simultaneously, SSF led to an augmentation in substrate ash content, likely attributed to microbial metabolic activity or dry matter loss. Similar results have been reported in

previous studies, where SSF led to increased ash content in various substrates. Instances of such studies include a 43.90% increase in ash content of RB after 4 days of SSF with *A. niger* (Ribeiro et al., 2017), a 50.34% rise in ash content of olive leaves following 2 days of SSF with *A. niger* (Altop et al., 2018), and a 51.31% increment in ash content of rice bran after 10 days of SSF with *Pleurotus sapidus* (Omarini et al., 2019).

The percentages of enhancement in ash content observed in this study at both 25°C and 35°C surpass the 51.31% reported by Omarini (2019) after 10 days of SSF of RB at 25°C with *Pleurotus sapidus* Dk3174. Likewise, the ash improvement in this study exceeds the findings of Oliveira et al. (2010), who observed an enhancement in ash content when RB was fermented for 5 days using *Rhizopus oryzae* at 30°C. However, Bonilla Loaiza et al. (2022) documented a reduction in ash content during solid-state fermentation, noting that fermentation at 25°C yielded the highest ash improvement.

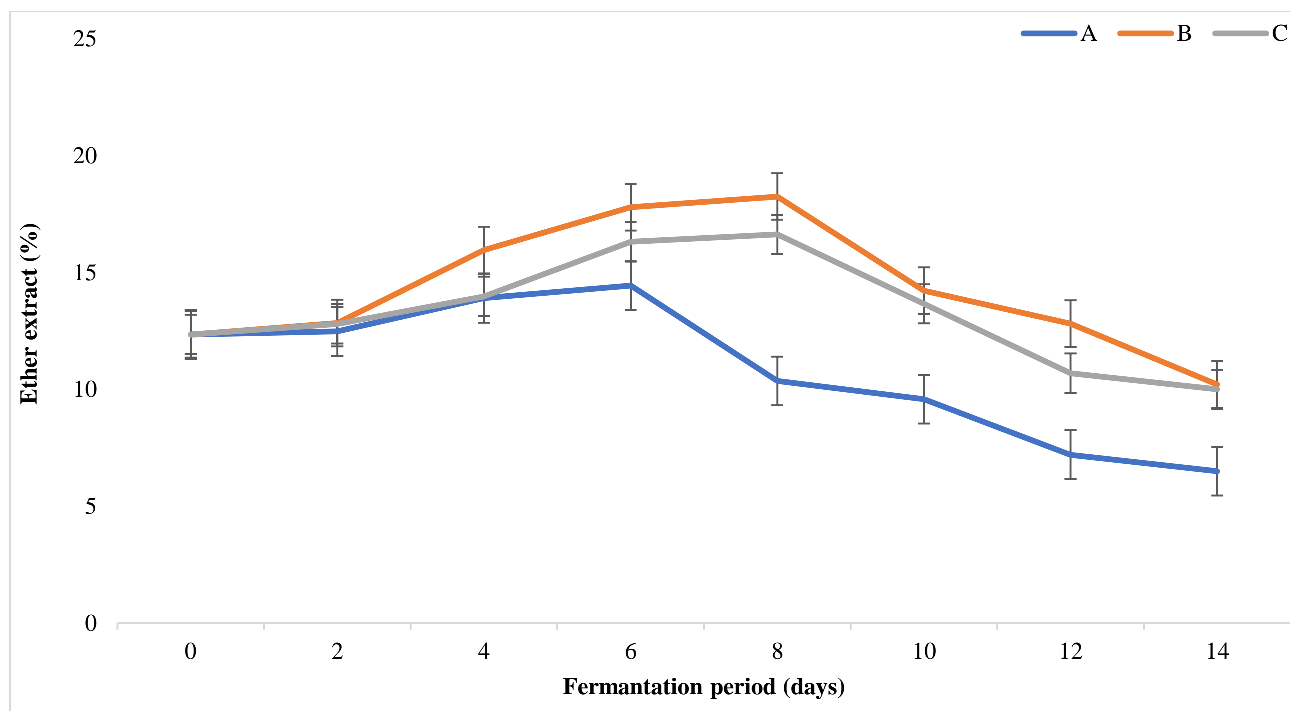

Figure 3. Effect of temperature and fermentation period on the ether extract content of rice bran. **A**: 25°C, **B**: 35°C, **C**: 45°C.

Effect of temperature and fermentation duration on the ether extract of rice bran

Figure 3 depicts how temperature and fermentation duration influence the EE component of RB during SSF. Significant enhancements in the EE component of RB were observed under various temperatures and fermentation durations ($p < 0.05$), as compared to the unfermented RB (Table 1). The highest enhancement occurred on day 8, with EE peaking at 15.25% and a 63.10% increase recorded at 35°C.

The rise in EE content is influenced by various factors, such as the production of lipolytic enzymes by *A. niger* in the RB matrix, microbial growth utilizing nutrients, and enzymatic breakdown of cell walls releasing trapped lipids. Moreover, the SSF process can modify RB's physicochemical properties, making lipids more soluble (Christ-Ribeiro et al., 2021; Spaggiari et al., 2021). *A. niger* demonstrates a remarkable ability to transform substrates into value-added compounds, thereby significantly augmenting the lipid content in fermented rice bran.

Previous studies have reported varying effects of SSF on EE content. Ribeiro et al. (2017) conducted a study where rice bran was fermented with *A. niger* at 30°C for 4 days, while Altop et al. (2018) investigated the fermentation of olive leaves with *A. niger* at the same temperature for 2 days. Both studies reported an increase in EE. Conversely, Oliveira et al. (2010) and Omarini et al. (2019) observed a reduction in EE during SSF. In Omarini et al.'s (2019) study, a substantial 74.45% decrease in the EE content was observed when rice bran underwent solid-state fermentation for 10 days using *Pleurotus sapidus* at 25°C. Similarly, Shi et al. (2021) documented a 30% reduction in EE when Moringa oleifera leaves were subjected to solid-state fermentation by *A. niger* at 32°C for 7 days.

Effect of temperature and fermentation period on crude fiber degradation

In the present study, the crude fiber content of the unfermented RB (27.50%) significantly decreased by solid-state fermentation (SSF) using *A. niger* USM F4 ($p < 0.05$) (Figure 4). The lowest CF values were observed on the 10th day

across all temperature levels. Specifically, CF values were 12.45 (25°C), 8.36 (35°C), and 10.33 (45°C), indicating CF degradation by 54.73%, 69.60%, and 62.44% respectively. The highest degradation of CF (69.60%) occurred at 35°C on the 10th day.

A. niger, known for its cellulase production (Altop et al., 2018), shows promising prospects for single-cell protein production. The breakdown of crude fiber (CF) during fermentation by *A. niger* USM F4 is believed to entail the secretion of enzymes like cellulases, hemicellulases, and ligninases. The degradation of crude fiber (CF) during fermentation by *A. niger* USM F4 likely involves the secretion of enzymes such as cellulases, hemicellulases, and ligninases. These enzymes break down the complex polysaccharides in CF into simpler sugars for fungal growth. Additionally, the metabolic activities and by-products of *A. niger* may directly or indirectly degrade CF (Cui et al., 2021; El-Gendi et al., 2021). Fermentation alters substrate pH, moisture, and other parameters, making CF more susceptible to enzymatic breakdown (Siddiqui et al., 2023; Kasprowicz-Potocka et al., 2024). Physical disruption of rice bran during fermentation enhances CF degradation by increasing enzyme accessibility (Dilaga et al., 2022; Ibrahim et al., 2023). Moreover, *A. niger* has the potential to selectively utilize various components of rice bran, such as CF, for its growth (Yu et al., 2021). This preference often leads to the breakdown of more fermentable CF components into degradation products. In a similar study, Shi et al. (2021) conducted SSF of *Moringa oleifera* leaf using *A. niger* GIM 3.576 at 32°C for 7 days and reported a 70% degradation of CF. Oliveira et al. (2010) fermented RB with *Rhizopus oryzae* in a solid-state setting at 30°C over 5 days, noting a 50% reduction in CF. Similarly, Wolayan and Mandey (2019) fermented RB with *A. niger* for 10 days at 30°C, yielding a 47.81% reduction in CF.

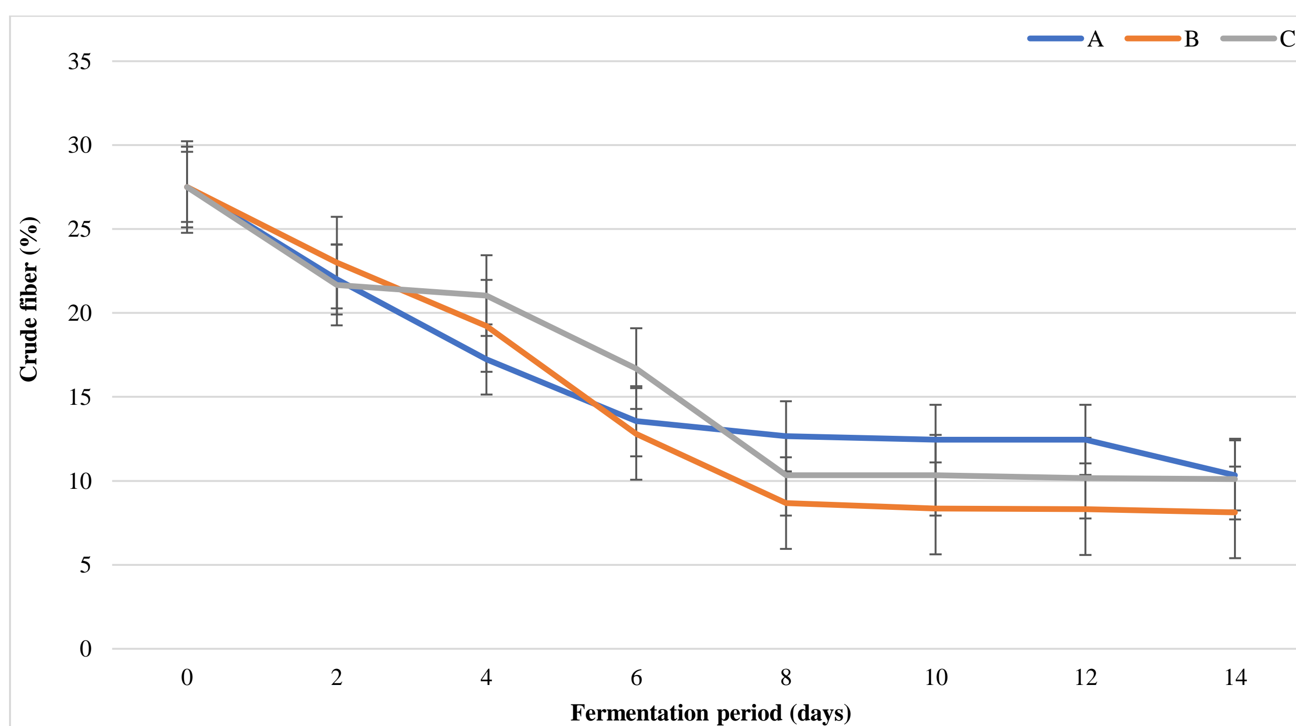

Figure 4. Effect of temperature and fermentation period on the crude fiber of rice bran. **A**: 25ºC, **B**: 35ºC, **C**: 45ºC.

CONCLUSION

The current study demonstrated that solid-state fermentation can enhance the bioavailability of nutrients in rice bran, potentially improving its digestibility in animals. The optimal media temperature and duration for solid-state fermentation using *A. niger* USM F4 to boost crude protein levels and degrade crude fiber in rice bran are determined to be 35°C and 10 days, respectively. Moreover, the utilization of SSF assisted with *A. niger* USM F4 can elevate the quality of agro-industrial by-products by enhancing crude protein content and degrading crude fiber. This, in turn, facilitates increased utilization of rice bran in livestock feed, particularly in monogastric animals. Further research is recommended to explore optimal fermentation conditions for *A. niger* USM F4 in rice bran, enzyme characterization for industrial applications, nutritional evaluations for livestock feed, confirmation of antinutrient reduction, and pilot-scale trials for industrial feasibility.

DECLARATIONS

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Authors' contributions

Animashahun Razaq Adekunle and Akpor Oghenerobor Benjamin conceptualized the study, while Olamide Musa, Oluwafemi Precious, Animashahun Adedeji Peculiar and Idowu Abiodun experimented. Alabi Olayinka Olubunmi, Oyawoye Enoch Olayiwola, and Animashahun Razaq Adekunle provided supervision and data curation. Laboratory analyses were performed by Animashahun Razaq, Akpor Oghenerobor Benjamin, and Olamide Musa, with data analysis conducted by Oghenerobor Benjamin, Okocha Reuben, and Olamide Musa. Olamide Musa and Oluwafemi Precious drafted the manuscript, which was revised by Animashahun Razaq Adekunle, Akpor Oghenerobor, Oyawoye Enoch Olayiwola, and Alabi Olayinka Olubunmi. All authors reviewed and approved the final version of the manuscript for publication in the current journal.

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Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon request.

Competing interests

No conflicts of interest have been disclosed by the authors.

Ethical considerations

Every author has reviewed ethical considerations such as plagiarism, duplicate publication or submission, redundancy, data fabrication or falsification, consent for publication, and misconduct.

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