Optimization of Solid-State Fermentation Parameters to Enhance Phytase Production by *Aspergillus niger* Using Palm Kernel Expeller

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Abstract

The production of microbial phytase through solid-state fermentation (SSF) offers a sustainable alternative to conventional enzyme production systems. This study aimed to optimize key SSF parameters using the one-factor-at-a-time (OFAT) approach to enhance phytase activity by *Aspergillus niger* cultivated on palm kernel expeller (PKE), a nutrient-rich agro-industrial by-product. Although initial screening included *A. oryzae* and *Rhizopus oligosporus*, only *A. niger* demonstrated the highest phytase activity of 269.67 ± 13.52 FTU/mL under non-optimized conditions and was selected for further optimization. Three critical SSF parameters: incubation temperature (28°C–50°C), moisture content (30%–60% v/w), and inoculum size (1%–15% v/w) were individually evaluated. Maximum phytase activity (299.42 ± 6.64 FTU/mL) was achieved at 28°C, 40% moisture, and 1% inoculum size. Under these optimized conditions, phytase production improved by 23% compared to the unoptimized set-up (116.92 ± 7.57 FTU/mL), confirming the significant influence of physical parameters on fungal enzyme biosynthesis. These findings support the valorization of PKE for local phytase production, contributing to improved phosphorus bioavailability in poultry diets while reducing reliance on imported feed additives. The results provide a foundation for further scale-up studies aligned with Malaysia's circular bioeconomy goals and Sustainable Development Goals (SDGs) 2 and 12.

Keywords: phytase; solid-state fermentation; *Aspergillus niger*; OFAT optimization; palm kernel expeller; poultry feed

Introduction

Phytic acid (myo-inositol hexakisphosphate) is the major storage form of phosphorus in plant-based feed ingredients such as maize, soybean meal, and wheat bran. However, in monogastric animals including poultry, the bioavailability of phosphorus from

phytic acid is limited due to the absence of endogenous phytase enzymes in the digestive tract (Rizwanuddin et al., 2023). Consequently, a substantial portion of dietary phosphorus remains undigested, leading to increased feed formulation costs through the inclusion of inorganic phosphate supplements and contributing to phosphorus pollution via excretion (Mahmood et al., 2021). Supplementation with microbial phytase has become a widely adopted strategy to improve phosphorus digestibility and reduce environmental impact. Among microbial sources, filamentous fungi such as *Aspergillus niger*, *A. oryzae*, and *Rhizopus oligosporus* are well-recognized phytase producers under solid-state fermentation (SSF) conditions, especially when grown on low-cost agricultural residues (Martínez-Vallespín et al., 2022; Elkhateeb & Fadel, 2022). Palm kernel expeller (PKE), a nutrient-rich by-product of Malaysia's palm oil industry, has shown promise as a substrate for fungal enzyme production due to its abundance, affordability, and sustainability profile (Abdul-Sani et al., 2024).

In this study, *A. niger* demonstrated consistent and significantly higher phytase activity under preliminary SSF trials using PKE. Hence, it was selected for downstream optimization. The optimization of fermentation parameters is critical in enhancing enzyme yield. Although multivariate optimization tools like Response Surface Methodology (RSM) and factorial designs are statistically robust, they are complex, time-consuming, and require large numbers of experimental runs. In contrast, the One-Factor-at-a-Time (OFAT) approach, while more limited in interaction analysis, offers simplicity and cost-effectiveness during the early stages of bioprocess development, particularly when resources or substrate volumes are constrained (Kumari & Bansal, 2021; Priya et al., 2023). Despite previous studies reporting the feasibility of SSF for phytase production, few have focused specifically on optimizing OFAT parameters using PKE as substrate with *A. niger*. Furthermore, the interaction between key parameters such as temperature, moisture content, and inoculum size and their impact on fungal biomass and enzyme productivity in this context remains underexplored. Therefore, the present study aimed to (1) optimize critical fermentation parameters (temperature, moisture, and inoculum size) using the OFAT method for maximum phytase yield by *A. niger* and (2) evaluate phytase production under optimized versus unoptimized SSF conditions. These findings contribute to improved feed biotechnology applications and valorization of agro-industrial waste while supporting Malaysia's circular bioeconomy and Sustainable Development Goals (SDG 2 and SDG 12).

Materials and Methods

Experimental Fungal Strains

Three filamentous fungal strains; *Aspergillus niger*, *Aspergillus oryzae*, and *Rhizopus oligosporus* were selected for this study based on their historical use in enzyme production, their Generally Recognized as Safe (GRAS) status, and

previous reports of phytase synthesis under solid-state fermentation (SSF) conditions. These strains were procured in the form of slant cultures from the Livestock Science Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Malaysia.

To ensure viability and optimal sporulation, each fungal strain was subcultured onto freshly prepared potato dextrose agar (PDA) medium (composition: 200 g potato extract, 20 g dextrose, 15 g agar per liter) and incubated at 30°C for three days under sterile conditions. This incubation temperature is ideal for promoting robust hyphal growth and maximizing spore formation in mesophilic fungi (Elkhateeb & Fadel, 2022). Subcultures were routinely transferred onto new PDA plates every four weeks to maintain metabolic activity and minimize genetic drift due to prolonged storage (Jatuwong et al., 2020).

The maintenance of fungal cultures was carried out under refrigerated conditions (4°C) to prolong viability and reduce metabolic degradation. Prior to each experimental use, culture integrity was verified through morphological assessment under a stereomicroscope, focusing on colony texture, pigmentation, and sporulation patterns. Indicators of contamination such as atypical colony coloration, altered margin morphology, or the presence of foreign hyphae prompted immediate discard and re-culturing of affected stock. This routine quality control is essential to ensure the purity of monocultures used in SSF trials, especially when evaluating enzyme production profiles (Puppala et al., 2020; Mahmood et al., 2021).

Strain Selection Justification

Among the three fungal strains tested; *Aspergillus niger*, *Aspergillus oryzae*, and *Rhizopus oligosporus*, *A. niger* was selected for further study based on its superior phytase production under solid-state fermentation (SSF) using palm kernel expeller (PKE) as substrate. Preliminary screening showed that A. niger produced the highest phytase activity (269.67 ± 13.52 FTU/mL), significantly higher than A. oryzae and R. oligosporus ($p < 0.001$) (Siti Khairunisa et al., 2025). This strain is widely known for its strong enzyme-secreting ability, acid-tolerant phytase, and fast colonization in SSF environments. It also has Generally Recognized as Safe (GRAS) status, making it suitable for enzyme applications in animal feed. Therefore, *A. niger* was chosen as the most promising candidate for parameter optimization in this study.

Microbial Strain and Culture Maintenance

A pure stock culture of *Aspergillus niger* was obtained from the Food Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI). Subculturing was performed on Potato Dextrose Agar (PDA; Himedia, India) prepared using 39 g/L PDA powder, sterilized at 121°C for 15 minutes. The cultures were incubated at 30°C for 3 days to ensure optimal sporulation and maintained at 4°C for short-term use, following standard

fungal culture maintenance protocols (Elkhateeb & Fadel, 2022).

Preparation of Spore Suspension

Spores from sporulated cultures were harvested by gently scraping the surface of PDA plates with 15 mL sterile distilled water using an inoculating loop. The suspension was filtered through sterile gauze, transferred to 250 mL sterile bottles, and stored at 4°C. Spore concentration was determined using a Fuchs-Rosenthal haemocytometer and adjusted to 8.5×10^7 spores/mL, with inoculum volume standardized to 1%–15% (v/w) based on substrate weight. Homogeneity was maintained by gentle swirling (Kumari & Bansal, 2021).

Substrate Preparation and Moisture Calculation

Palm kernel expeller (PKE) with <5% shell content was sourced from Felda Kernel Product Sdn. Bhd., Negeri Sembilan, Malaysia. Substrates were air-dried, ground, and stored at ambient conditions (28 ± 1.5°C). For SSF, 10.00 ± 0.01 g of PKE was weighed into 250 mL Erlenmeyer flasks and autoclaved at 121°C for 20 min.

Moisture content was adjusted using the formula:

$V = [(M \times W)/(100 - M)]$

where V is the volume of water added (mL), M is desired moisture content (%), and W is dry substrate weight (g) (Zulfiqar et al., 2023).

Solid-State Fermentation (SSF)

After cooling, sterilized PKE was inoculated with spore suspensions at 1%, 5%, 10%, or 15% (v/w) based on dry substrate weight. The moistened substrate was mixed thoroughly with sterile spatulas to ensure uniform fungal distribution. The flasks were sealed with cotton plugs and incubated in static conditions at the specified temperatures (28–50°C) for 3 days. No external aeration was supplied; passive oxygen diffusion occurred via flask neck, consistent with standard SSF practice (Chowdhury et al., 2021).

Experimental Design and Optimization

A One-Factor-at-a-Time approach (OFAT) was adopted to optimize key parameters affecting phytase production, including temperature, moisture content, and inoculum size:

Temperature (°C): 28, 30, 40, 50

Moisture content (% v/w): 30, 40, 50, 60

Inoculum size (% v/w): 1, 5, 10, 15

Control treatments (uninoculated PKE) were included in each batch to account for baseline phosphate levels and rule out abiotic release of phosphorus.

Fermentation Under Optimized Conditions

The best conditions 28°C, 40% moisture content, and 1% inoculum size were used to carry out optimized SSF in triplicate. Incubation was carried out for 3 days, after which samples were harvested for enzyme extraction.

Enzyme Extraction

Fifty mL of sterile distilled water was added to each flask post-incubation. The mixture was agitated on an orbital

shaker at 150 rpm for 60 min, then filtered using Whatman No. 1 filter paper. The supernatant was collected as the crude enzyme extract and stored at 4°C (Ibrahim & Lim, 2014).

Phytase Activity Assay

Phytase activity was determined by measuring the release of inorganic phosphate from 5 mM sodium phytate in 0.2 M sodium acetate buffer (pH 5.15). The reaction mixture (500 µL enzyme, 250 µL buffer, 500 µL phytate) was incubated at 40°C for 45 min and stopped with 1 mL 15% TCA. Absorbance was measured at 660 nm using an Epoch microplate reader. One phytase unit (FTU) was defined as the amount of enzyme liberating 1 µmol of inorganic phosphate per minute (Park et al., 2021; Mohammadi-Kouchesfahani et al., 2019).

Statistical Analysis

Data were tested for normality using the Shapiro–Wilk test. Parametric data were analyzed by one-way ANOVA with Tukey's HSD post hoc. Non-parametric data were assessed using the Kruskal–Wallis test with Mann–Whitney U and Bonferroni correction. Differences were considered significant at $p < 0.05$ (Gomez-Osorio et al., 2022; Gong et al., 2023).

Results and Discussion

Phytase Production under Varying SSF Parameters Using Aspergillus niger

Rationale for Fungal Strain Selection

In the initial screening phase, three fungal strains *Aspergillus niger*, *A. oryzae*, and *Rhizopus oligosporus* were evaluated for their phytase-producing potential under standardized solid-state fermentation (SSF) conditions using palm kernel expeller (PKE) as the sole carbon substrate. As shown in Table 1, *A. niger* exhibited significantly higher phytase activity (269.67 ± 13.52 FTU/mL) than *A. oryzae* (145.92 ± 13.55 FTU/mL) and *R. oligosporus* (88.42 ± 27.05 FTU/mL), with all differences being statistically significant ($p < 0.001$). No enzyme activity was detected in the uninoculated control, confirming that the observed phytase production was directly attributable to fungal metabolism and not substrate autolysis. The superior enzymatic yield of *A. niger* can be attributed to its robust secretory apparatus and high expression levels of phytase isoenzymes, which enhance its ability to hydrolyze phytate even in low-moisture and nutrient-limited environments typical of SSF systems (Priya et al., 2023; Gong et al., 2023). This strain also exhibits enhanced tolerance to acidic pH and produces thermostable phytase, which is advantageous for poultry feed applications where the enzyme must survive pelleting and gastrointestinal transit (Martínez-Vallespín et al., 2022; Rizwanuddin et al., 2023).

Furthermore, *A. niger* possesses a versatile enzymatic toolkit including cellulases, xylanases, and proteases which facilitates the degradation of complex lignocellulosic substrates like PKE. This contributes to more efficient nutrient assimilation and enhanced phytase biosynthesis (Jatuwong et al., 2020; El-Gendi et al., 2021). In contrast, although *R. oligosporus* and *A. oryzae* are

traditionally used in food fermentations, they exhibited markedly lower phytase activity, likely due to limited gene expression, lower phytase secretion, and substrate incompatibility under SSF conditions (Gomez-Osorio et al., 2022; Saldaña et al., 2022). Additionally, the shorter lag phase and faster hyphal colonization of *A. niger* likely contribute to early enzyme induction, enabling more sustained phytase production throughout fermentation. It's Generally Recognized as Safe (GRAS) status further supports its use in feed enzyme applications (Kumari & Bansal, 2021), making it the most suitable candidate for subsequent parameter optimization. These findings are consistent with those reported by Puppala et al. (2020), Mahmood et al. (2021) and Siti Khairunisa et al. (2025), who identified *A. niger* as the highest phytase producer among multiple fungal strains cultivated on agro-industrial residues. Hence, based on enzymatic performance, physiological adaptability, and industrial relevance, *A. niger* was selected for subsequent optimization studies to enhance phytase production using PKE in SSF systems.

Table 1. Mean phytase activity (FTU/mL) of selected fungal strains at 1×10^7 spores/mL (n = 12)

Treatment	Mean Phytase Activity (FTU/mL)	p-value
Control (uninoculated)*	0.00 ± 0.00^{a}	< 0.001
Aspergillus niger	269.67 ± 13.52^{c}	
Aspergillus oryzae	145.92 ± 13.55^{b}	
Rhizopus oligosporus	88.42 ± 27.05^{b}	

*Note: Values are expressed as mean ± standard error (SE). Superscripts (a–c) indicate significant differences between treatment means at $p < 0.05$. The control group was replicated nine times (n = 9)

Optimization Of Solid Substrate Fermentation Parameters by OFAT (One Factor at a Time) For Production of Phytase by Aspergillus niger

Effect of Temperature on Phytase Production

Temperature strongly influences fungal physiology, enzyme secretion, and substrate utilization efficiency in solid-state fermentation (SSF). In this study, *Aspergillus niger* exhibited statistically significant differences in phytase production across the tested temperature range ($p < 0.005$). As shown in Table 2, the highest phytase activity was recorded at 28°C (134.00 ± 2.13 FTU/mL), followed by 30°C (116.92 ± 7.57 FTU/mL). These values were significantly higher than those observed at 40°C (49.42 ± 3.71 FTU/mL) and 50°C (32.13 ± 5.97 FTU/mL). The superior enzyme output at 28–30°C highlights the mesophilic nature of *A. niger*, whose

metabolic machinery including protein synthesis, hyphal extension, and secretion of extracellular hydrolases is optimized within this range (Tu et al., 2024). SSF systems rely heavily on fungal adaptation to microenvironmental conditions; thus, temperatures above 35°C tend to induce metabolic stress, reduce enzyme folding efficiency, and impair sporulation (Arcus & Mulholland, 2020).

Similar studies have reported comparable findings. Gong et al. (2023) demonstrated that phytase secretion by filamentous fungi peaked between 27°C and 32°C, with higher temperatures suppressing transcription of phytase-encoding genes. Likewise, Pires et al. (2019) observed that phytase activity declined sharply above 35°C in several *Aspergillus* strains, attributing the reduction to partial denaturation of secreted enzymes and reduced oxygen diffusion efficiency in SSF matrices at elevated temperatures.

The significant decline in enzymatic activity at 40°C and 50°C in the present study may be explained by (i) heat-induced enzyme denaturation, (ii) lower fungal growth rates due to thermal inhibition, and (iii) reduced moisture retention, which exacerbates substrate drying during incubation (Gomez-Osorio et al., 2022). Enzymes such as phytase are particularly sensitive to temperature fluctuations because catalytic residues in the active site undergo conformational changes when exceeding thermal stability limits (Tu et al., 2024). Collectively, the results demonstrate that maintaining SSF incubation between 28°C and 30°C is critical for maximizing phytase yield in PKE-based fermentation systems using *A. niger*. This temperature range supports robust fungal colonization and enzymatic secretion, reinforcing its suitability for industrial bioprocessing operations employing mesophilic fungal strains.

Table 2. Effect of incubation temperature on phytase activity (FTU/mL) during SSF by *Aspergillus niger*

Temperature (°C)	Phytase Activity (FTU/mL)	p-value
Control (uninoculated) (n = 10)	0.00 ± 0.00^{a}	< 0.005
28 (n = 12)	134.00 ± 2.13^{b}	
30 (n = 12)	116.92 ± 7.57^{b}	
40 (n = 12)	49.42 ± 3.71^{cd}	
50 (n = 8)	32.13 ± 5.97^{d}	

Note: Values expressed as mean ± standard error (SE). Statistical comparisons applied the Kruskal–Wallis test. Different superscripts within a column indicate significant differences at $p < 0.05$ (Mann–Whitney U test).

Effect of Moisture Content on Phytase Production

Moisture content is a critical variable in solid-state fermentation (SSF), as it directly modulates the physicochemical environment of the substrate, influencing fungal colonization, enzymatic diffusion, and metabolic activity. In SSF systems, water functions as a medium for nutrient solubilization, gas diffusion, and metabolic waste transport. However, maintaining an appropriate balance is crucial; excessive moisture can lead to reduced porosity and oxygen availability, while insufficient moisture restricts fungal growth due to osmotic stress and poor substrate penetration (Saldaña et al., 2022; Puri et al., 2021).

In the current study, phytase production by *Aspergillus niger* was assessed at varying moisture contents (30%, 40%, 50%, and 60% v/w), with results summarized in Table 3. The maximum phytase activity was recorded at 40% moisture content (231.50 ± 7.03 FTU/mL), which was significantly higher than that at 30% (155.67 ± 7.70 FTU/mL), 50% (148.17 ± 7.20 FTU/mL), and 60% (9.00 ± 2.67 FTU/mL) ($p < 0.005$).

Table 3. Effect of moisture content on phytase activity (FTU/mL) in SSF by *Aspergillus niger*

Moisture Content (%)	Phytase Activity (FTU/mL)	p-value
Control (uninoculated) (n = 10)	0.00 ± 0.00^{a}	< 0.005
30 (n = 12)	155.67 ± 7.70^{b}	
40 (n = 12)	231.50 ± 7.03^{c}	
50 (n = 12)	148.17 ± 7.20^{bd}	
60 (n = 7)	9.00 ± 2.67^{e}	

Note: Values are expressed as mean ± standard error (SE). Data were analyzed using the Kruskal–Wallis test. Superscripts (a–e) within the same column denote significant differences ($p < 0.05$), as determined by the Mann–Whitney U test.

These results suggest that 40% moisture content provides an optimal hydration level, ensuring sufficient water availability for nutrient diffusion and enzyme secretion, while maintaining substrate porosity for aerobic fungal metabolism. Excess moisture (60%) likely resulted in reduced oxygen diffusion, which can impede oxidative metabolism and phytase gene expression (Elkhateeb & Fadel, 2022; Wang et al., 2023). Conversely, lower moisture levels (30% and 50%) may not have provided adequate water for solubilizing substrate-bound phytate, thereby reducing enzyme-substrate interaction (Gomez-Osorio et al., 2022). Interestingly, several prior studies reported contrasting optimal moisture levels for phytase production,

underscoring the substrate- and strain-specific nature of SSF optimization. Shivanna and Venkateswaran (2014) observed maximal phytase yield at 60% moisture using *A. niger* CFR 335 on wheat bran, while Elkhateeb and Fadel (2022) noted optimum activity at 53–60% moisture for *A. carbonarius* cultured on canola meal. Additionally, *Penicillium* species showed peak activity at 60% when cultivated on fava bean particles (Elkhateeb & Fadel, 2022). These variations highlight the importance of empirical optimization tailored to the specific fungal strain and substrate matrix used.

The findings of the present study reaffirm the suitability of palm kernel expeller (PKE) as a cost-effective SSF substrate when maintained at 40% moisture, offering a balance between fungal growth support and process efficiency. Furthermore, the results are consistent with industrial fermentation parameters where water activity between 0.6 and 0.8 is often targeted to suppress bacterial contamination while sustaining filamentous fungal viability (Benedetti et al., 2023).

Effect of Inoculum Size on Phytase Production

Inoculum size is a pivotal factor in solid-state fermentation (SSF) that determines the initial fungal biomass, colonization rate, and metabolic dynamics of the fermentation process. It must be carefully optimized to strike a balance between effective substrate colonization and resource allocation. An inadequate inoculum may result in slow substrate colonization and increased susceptibility to microbial contamination. Conversely, excessive inoculum can lead to nutrient competition, oxygen depletion, and the accumulation of toxic metabolites, ultimately inhibiting enzyme synthesis (Mahmood et al., 2021; Saldaña et al., 2022). The findings of the present study revealed a statistically significant effect of inoculum size on phytase activity ($p < 0.005$), as shown in Table 4. The highest phytase yield (299.42 ± 6.64 FTU/mL) was obtained at an inoculum size of 1% (v/w). A declining trend in enzyme activity was observed with increasing inoculum levels: 5% (268.58 ± 13.11 FTU/mL), 10% (235.25 ± 5.12 FTU/mL), and 15% (226.08 ± 8.84 FTU/mL), with all differences being statistically significant ($p < 0.05$).

The superior performance at 1% inoculum size could be attributed to optimal spore dispersion and nutrient accessibility. At this level, the fungal mycelia likely encountered minimal competition for resources and oxygen, facilitating effective hyphal extension and maximal enzyme secretion. These results are consistent with the observations by Gomes et al. (2018), who reported enhanced phytase productivity at lower inoculum levels due to better substrate colonization and reduced biomass-induced metabolic stress. Lower inoculum levels also mitigate overgrowth, which can cause substrate aggregation, reduce interstitial spaces, and limit oxygen diffusion (Benedetti et al., 2023). In contrast, previous reports demonstrate that optimal inoculum sizes are strain- and substrate-specific. Dailin et al. (2019) showed that *A. niger* CFR 335 achieved maximum phytase activity

at 1.0 mL, while *A. ficuum* SGA 01 performed best at 1.5 mL under similar SSF conditions using agricultural residues.

Table 4. Effect of inoculum size on phytase activity (FTU/mL) during SSF by *Aspergillus niger*

Inoculum Size (% v/w)	Phytase Activity (FTU/mL)	p-value
Control (uninoculated) (n = 10)	0.00 ± 0.00^{a}	< 0.005
1% (n = 12)	299.42 ± 6.64^{b}	
5% (n = 12)	268.58 ± 13.11^{c}	
10% (n = 12)	235.25 ± 5.12^{de}	
15% (n = 12)	226.08 ± 8.84^{e}	

Note: Values are expressed as mean ± standard error (SE). Data were analyzed using the Kruskal–Wallis test. Superscripts (a–e) within the same column denote significant differences ($p < 0.05$), as determined by the Mann–Whitney U test.

Likewise, Mahmood et al. (2021) found optimal phytase expression at 10% (v/w) inoculum using a wheat bran-based matrix. These discrepancies underscore the importance of tailored inoculum optimization based on fungal physiology, substrate characteristics, and fermentation design (Elkhateeb & Fadel, 2022). From an industrial standpoint, minimizing inoculum size without compromising enzyme yield presents a cost-effective strategy for large-scale phytase production. Lower inoculum requirements reduce spore production costs and inoculum preparation time, enhancing the scalability and economic viability of SSF-based enzyme manufacturing systems (Tu et al., 2024).

Evaluation of Phytase Production by Aspergillus niger Using Optimized vs Unoptimized Fermentation Conditions

The second objective of this study was to evaluate the effectiveness of optimized solid-state fermentation (SSF) conditions in enhancing phytase production by *Aspergillus niger*. Optimization parameters were previously established using a one-factor-at-a-time (OFAT) approach, identifying 28°C incubation temperature, 40% (v/w) moisture content, and 1% (v/w) inoculum size as the most favorable combination. These conditions were hypothesized to synergistically enhance fungal metabolism, nutrient assimilation, and enzymatic productivity.

As illustrated in Table 5, phytase activity under optimized SSF conditions reached 143.58 ± 3.45 FTU/mL, significantly higher than the 116.92 ± 7.57 FTU/mL recorded under unoptimized settings ($p < 0.0167$). This 27 FTU/mL difference corresponds to an approximate 23% enhancement, or a 1.23-fold increase in enzymatic yield. Such improvement confirms the importance of carefully modulated SSF conditions in maximizing microbial

enzyme production. The increase is likely a result of the integrated effects of the three optimized variables.

At 28°C, the metabolic efficiency of *A. niger* is maintained without triggering heat stress or protein denaturation, ensuring the structural integrity and catalytic activity of the phytase enzyme (Arcus & Mulholland, 2020). The 40% substrate moisture provided a conducive microenvironment for both oxygen transfer and solubilization of macro- and micronutrients, promoting uniform colonization and active enzyme secretion (Saldaña et al., 2022). Moreover, the 1% (v/w) inoculum level ensured sufficient spore density for effective colonization without inducing inter-mycelial competition for nutrients and space (Abdul-Sani et al., 2024). These results are consistent with studies emphasizing the interplay between SSF variables in enzyme expression. For example, Elkhateeb and Fadel (2022) found that even small changes in substrate hydration or inoculum levels could significantly alter enzymatic outputs. Similarly, Benedetti et al. (2023) highlighted that precise aeration and moisture control are crucial in fungal fermentation systems for achieving reproducible enzyme yields.

Table 5. Comparison of phytase production by *Aspergillus niger* under optimized and unoptimized SSF parameters

Treatment	Mean Phytase Activity (FTU/mL)	p-value
Control (uninoculated) (n = 12)	0.00 ± 0.00^{a}	< 0.0167
Optimized parameters (n = 12)	143.58 ± 3.45^{b}	
Unoptimized parameters (n = 12)	116.92 ± 7.57^{c}	

Note: Values are presented as mean ± standard error (SE). Data were analyzed using the Kruskal–Wallis test. Superscripts (a–c) indicate significant differences ($p < 0.05$) based on Mann–Whitney U test. Optimized parameters: 28°C, 40% (v/w) moisture, 1% (v/w) inoculum. Unoptimized parameters: 30°C, 50% (v/w) moisture, 10% (v/w) inoculum.

From a broader application standpoint, the 23% increase in phytase activity holds economic and ecological relevance. Higher enzymatic output implies more efficient phytate hydrolysis, resulting in greater phosphorus bioavailability for monogastric animals such as poultry. This can reduce the need for costly inorganic phosphate supplementation, thereby lowering feed production costs and minimizing phosphorus discharge into the environment (Rizwanuddin et al., 2023). Given that phosphorus runoff from poultry farms contributes to eutrophication and aquatic ecosystem degradation, the adoption of optimized microbial phytase systems aligns with sustainable livestock production goals and environmental stewardship. However, despite the encouraging laboratory-scale results, further feasibility studies are needed before commercial application. Factors such as fermentation scalability, substrate loading limits, moisture regulation under

industrial conditions, and cost-benefit analysis of inoculum preparation must be addressed. Integration of scale-up designs such as rotating drum fermenters or modular tray systems should also be explored to maintain uniform aeration and temperature control during extended SSF operations (Mahmood et al., 2021).

Conclusion

This study demonstrated that phytase production by *Aspergillus niger* under solid-state fermentation (SSF) can be significantly improved through systematic optimization of three critical parameters: incubation temperature, substrate moisture content, and inoculum size. Among the tested conditions, an incubation temperature of 28°C, moisture content of 40% (v/w), and inoculum size of 1% (v/w) collectively yielded the highest phytase activity at 143.58 ± 3.45 FTU/mL, representing a 23% increase compared to unoptimized conditions. These findings highlight the importance of parameter-specific refinement in enhancing fungal enzymatic output under SSF conditions. The improved enzyme yield achieved under these optimized conditions reinforces the feasibility of utilizing agro-industrial residues such as palm kernel expeller (PKE) as cost-effective substrates for microbial phytase production. This strategy supports sustainable animal nutrition by reducing dependence on inorganic phosphate supplements and aligning with environmental conservation goals (Elkhateeb & Fadel, 2022; Rizwanuddin et al., 2023).

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Conflict of Interest

The authors declare that there are no conflicts of interest associated with this study.

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