Evaluation of Fungal Strains for Phytase Production Using Palm Kernel Expeller in Solid-State Fermentation

Siti Khairunnisa, A.[1], Mohd Noor Hisham, M. N.[1*], Shamarina, S.[1], Nasyatul Ekma, M. H.[2] & Noraini, S.[2]

[1]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]Livestock Science Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
*Corresponding author: mnhisham@upm.edu.my

Received: 1 September 2025
Accepted: 13 November 2025

Abstract

Phytic acid, a major anti-nutritional factor in plant-based poultry feed, chelates essential minerals and reduces phosphorus bioavailability, leading to increased feed costs and environmental phosphorus loading. This study evaluated three fungal strains *Aspergillus niger*, *Aspergillus oryzae*, and *Rhizopus oligosporus* for phytase production through solid-state fermentation (SSF) using palm kernel expeller (PKE) as a low-cost substrate. Among the tested strains, *A. niger* exhibited the highest phytase activity (269.67 ± 13.52 FTU/mL), significantly surpassing *A. oryzae* (145.92 ± 13.55 FTU/mL) and *R. oligosporus* (88.42 ± 27.05 FTU/mL) ($p < 0.05$). Maximum enzyme production occurred between Day 3 and Day 4 of incubation, corresponding with peak fungal biomass (0.0557 ± 0.0054 mg/g). These findings demonstrate that *A. niger* effectively converts PKE, an abundant agro-industrial by-product in Malaysia, into a value-added product for poultry feed applications. Incorporating phytase produced via SSF could reduce reliance on inorganic phosphate supplements, enhance phosphorus digestibility, and mitigate environmental risks associated with phosphorus runoff. This approach supports sustainable poultry production and aligns with global sustainability targets, including SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production). The study highlights *A. niger* as a promising candidate for biotechnological applications in feed formulation, contributing to circular economy practices in the livestock industry.

Keywords: phytase, *Aspergillus niger*, solid-state fermentation, palm kernel expeller, sustainable poultry feed.

Introduction

Phytic acid, also known as inositol hexakisphosphate (InsP6), is a major anti-nutritional factor present in plant-based feed ingredients such as cereals and oilseeds. Its strong chelating ability, due to multiple phosphate groups, enables it to bind essential minerals like calcium, zinc, and phosphorus, forming insoluble complexes that reduce mineral bioavailability and nutrient absorption in monogastric animals such as poultry (Rizwanuddin et al., 2023; Angel et al., 2002). Since poultry have negligible endogenous phytase activity, the addition of exogenous microbial phytase has become a common strategy to enhance phosphorus availability, minimize feed costs, and reduce environmental phosphorus excretion (Martínez-Vallespín et al., 2022; Park et al., 2021).

Among microbial sources, *Aspergillus niger* is widely utilized for phytase production due to its efficiency in degrading phytate and its adaptability to solid-state fermentation (SSF) systems (Puppala et al., 2020; Kumari & Bansal, 2021). SSF is considered an eco-friendly and cost-effective approach for enzyme production as it uses minimal water and agricultural by-products, offering advantages such as lower energy requirements, higher product yields, and reduced wastewater generation compared to submerged fermentation (Cano y Postigo et al., 2021; Jatuwong et al., 2020). This technology supports circular economy principles by recycling agro-industrial residues like wheat bran, rice husk, and palm kernel expeller (PKE) into value-added products (Mahmood et al., 2021).

PKE, a by-product of palm oil extraction, is abundant in Malaysia and represents a low-cost feed ingredient. However, its high crude fiber and the presence of phytate limit nutrient digestibility and phosphorus availability, reducing its utility in poultry diets (Azizi et al., 2021; Fitriyah et al., 2022). Recent SSF studies demonstrated improvements in PKE nutritional quality through increased crude protein and reduced fiber content when fermented with fungi (Mahmood et al., 2021). The Malaysian poultry sector heavily depends on imported maize and soybean meal, with over 5 million tonnes imported in 2022, leading to cost fluctuations (Choy, 2024). Consequently, the Malaysian government promotes the use of local by-products like PKE in feed formulations, offering incentives for incorporating 5–10% palm-based by-products into livestock feed (Choy, 2024). Therefore, this study aimed to screen and compare three fungal strains *Aspergillus niger*, *Aspergillus oryzae*, and *Rhizopus oligosporus* for their phytase production potential under SSF using PKE as the substrate. Additionally, phytase activity profiles and fungal biomass production were assessed for the most promising strain under optimized fermentation conditions.

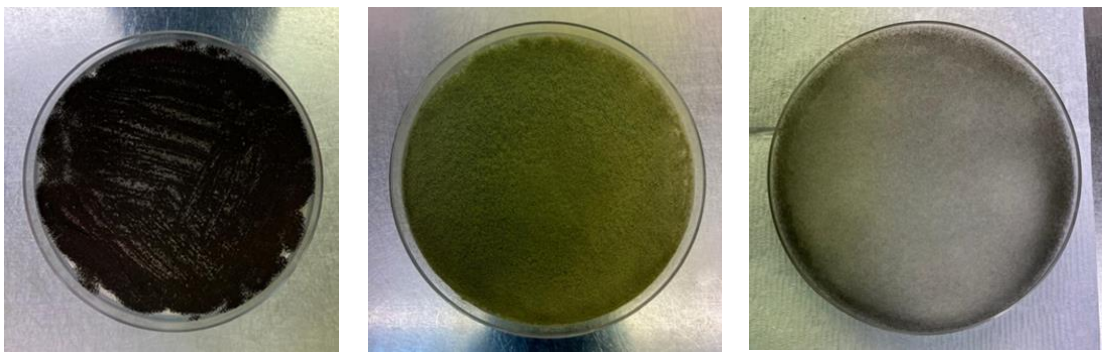
Materials and Methods

Experimental Fungal Strains

Three fungal strains, *Aspergillus niger*, *Aspergillus oryzae*, and *Rhizopus oligosporus*, were used in this study. These cultures were obtained from the Livestock Science Research Centre, MARDI (Malaysia), in the form of agar slants. Stock were subculture onto potato dextrose agar (PDA) plates and incubated at 30°C for three days to promote optimal growth. Subculturing was performed every four weeks, and cultures were stored at 4°C to preserve viability. Routine checks for contamination (e.g., changes in colony color, morphology, or presence of foreign mycelium) were conducted before experimental use (Puppala et al., 2020; Mahmood et al., 2021). Representative colony morphology of the three fungal species is shown in Figure 1.

Preparation Of Spore Suspension

Spore suspensions were prepared from 3-day-old fungal cultures grown on potato dextrose agar (PDA) at 30°C. Sterile distilled water (15 mL) was added to each fully sporulated plate, and spores were gently dislodged using a sterile inoculating loop. The resulting spore suspensions were transferred into sterile 250 mL bottles and stored at 4°C until further use. To ensure homogeneity, suspensions were gently swirled without vortexing or filtration to minimize spore aggregation and loss, as recommended by Kumari and Bansal (2021). The spore concentration was standardized to 1×10^7 spores/mL using a hemocytometer and used as the inoculum for subsequent SSF experiments.



(A) **(B)** **(C)**

Figure 1. Sporulated plates of fungal isolates derived from stock cultures: (A) *Aspergillus niger*, (B) *Aspergillus oryzae*, and (C) *Rhizopus oligosporus*.

Experimental Design and Solid-State Fermentation (SSF)

A completely randomized design (CRD) was implemented, comprising three replicates per treatment. Each replicate contained 10.00 ± 0.01 g of sterilized palm kernel expeller (PKE) in a 250 mL Erlenmeyer flask, inoculated with 1.0 mL of spore suspension (1×10^7 spores/mL). Sterile distilled water (9 mL) was added to achieve a final substrate moisture content of 50% moisture content. Treatments included the three fungal strains (*A. niger, A. oryzae*, and *R. oligosporus*) and an uninoculated control. Flasks were incubated at 30 ± 2°C under static conditions for 3 days for preliminary screening of phytase activity (Objective 1). For enzyme production profiling (Objective 2), the highest-performing strain from Objective 1 was fermented under similar conditions for 7 days, with sampling at 24-hour intervals to assess phytase activity and fungal biomass. All experiments were performed in triplicate to ensure reproducibility (Mahmood et al., 2021; Park et al., 2021).

Enzyme extraction

The entire wet mass of the fermented PKE was homogenized using a sterile spatula to ensure even distribution. Approximately 5.0 ± 0.5 g of the homogenized sample was collected from each flask and stored in labeled bags for subsequent glucosamine analysis. Crude phytase extraction was performed by mixing the fermented substrate with 50 mL of distilled water in an Erlenmeyer flask. The mixture was manually agitated for uniform contact and filtered through Whatman No. 1 filter paper. The resulting filtrate served as the crude enzyme extract for phytase activity assay (Ibrahim & Lim, 2014; Mahmood et al., 2021).

Phytase assay calculation

Phytase activity was quantified using a modified colorimetric method adapted from Mohammadi-Kouchesfahani et al. (2019). One phytase unit (FTU) was defined as the amount of enzyme required to liberate 1 µmol of inorganic phosphate per minute from 5 mM sodium phytate at pH 5.15 and 40°C under assay conditions optimized to preserve enzymatic stability (Park et al., 2021). To establish the calibration curve, a series of phosphate standards were prepared using sodium phosphate in sodium acetate buffer (pH 5.15). Absorbance values were recorded at 660 nm using a microplate spectrophotometer. A linear regression equation was derived from the standard curve ($R^2 > 0.98$), enabling conversion of absorbance readings to phosphate concentrations. Phytase activity in each sample was expressed as FTU/mL based on the following formula:

$$y = mx + c$$

Where:

- y is the absorbance at 660 nm,
- x is the phytase activity (FTU/mL),
- m is the slope of the regression line, and
- c is the y-intercept.

All assays were conducted in triplicate, and enzyme activity values were calculated using the standard curve

equation. This approach enabled reliable estimation of phytase activity across different experimental conditions.

Determination of fungal biomass (Glucosamine assay)

Fungal biomass was estimated indirectly by quantifying N-acetyl glucosamine released from chitin after acid hydrolysis, following Sakurai et al. (1977) with modifications. Approximately 0.5 g of dried fermented substrate was hydrolyzed in concentrated sulfuric acid for 24 hours, diluted to 1 N, and autoclaved at 121°C for 15 minutes. The solution was neutralized to pH 7 using NaOH, and glucosamine was measured colorimetrically at 530 nm using Ehrlich's reagent. Glucosamine concentration (mg/g) was calculated against a standard curve prepared from commercial glucosamine (Sigma-Aldrich, Germany) (Ibrahim & Lim, 2014; Mahmood et al., 2021).

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Data distribution was evaluated using the Shapiro–Wilk test to determine normality. For datasets meeting normal distribution assumptions, one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test was used to assess differences among treatment means. In cases where normality assumptions were not met, the Kruskal–Wallis test was employed, followed by pairwise comparisons using the Mann–Whitney U test. Bonferroni adjustment was applied to account for multiple comparisons. Statistical significance was accepted at $p < 0.05$ (Park et al., 2021).

Results and Discussion

Screening of Fungal Strains for Phytase

The initial screening (Table 1) demonstrated that *Aspergillus niger* produced significantly higher phytase activity (269.67 ± 13.52 FTU/mL, $p < 0.05$) compared to *A. oryzae* (145.92 ± 13.55 FTU/mL) and *R. oligosporus* (88.42 ± 27.05 FTU/mL). No enzyme activity was detected in the uninoculated control, confirming that the observed phytase activity was a direct result of fungal metabolism under solid-state fermentation (SSF) using palm kernel expeller (PKE) as substrate. These results reaffirm the superior phytase-producing potential of *A. niger*, which is widely recognized for its robust enzyme secretion system and adaptability to SSF conditions. Similar trends have been reported by Puppala et al. (2020) and Mahmood et al. (2021), where *A. niger* outperformed other fungal strains in phytase yield when cultivated on agro-industrial substrates such as wheat bran, rice husk, and oilseed cakes.

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

Group	Average Phytase Activity (FTU/mL)	p-value
Uninoculated (Control)*	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 presented as mean ± standard error (SE).
[a, b, c] within the same column indicates statistically significant differences among means ($p < 0.05$).
*Control group was replicated nine times (n = 9).

A. niger's enzymatic efficiency is attributed to its well-developed secretory machinery and expression of multiple isoforms of phytase, which enhance its ability to hydrolyze phytic acid even in low-moisture environments typical of SSF (Priya et al., 2023; Kumari & Bansal, 2021). Furthermore, it possesses a suite of lignocellulolytic enzymes, including cellulases, xylanases, and proteases, which enable it to break down complex carbon sources in lignocellulosic residues such as PKE, thereby enhancing its metabolic access to phytate (Jatuwong et al., 2020; Rizwanuddin et al., 2023). In contrast, *A. oryzae* and *R. oligosporus* produced significantly lower levels of phytase, likely due to differences in gene expression regulation, enzyme stability, and substrate specificity. Studies have shown that *R. oligosporus*, although commonly used in traditional fermentation, has limited phytase gene expression compared to *A. niger*, and its enzyme is less active under SSF conditions (Gomez-Osorio et al., 2022; Gong et al., 2023).

Moreover, the nutrient profile of PKE is rich in fiber and low in readily available phosphorus demands fungi with strong phytate hydrolyzing ability. *A. niger's* acid-stable phytase, with optimal activity at low pH, is particularly suited for such conditions (Martínez-Vallespín et al., 2022), which may further explain its superior performance. Additionally, its shorter lag phase and faster mycelial colonization may contribute to earlier and more abundant enzyme secretion compared to other species (El-Gendi et al., 2021). Overall, these findings support the selection of *A. niger* as the most effective strain for phytase production under SSF using PKE, with direct implications for enhancing phosphorus availability in poultry feed and reducing environmental phosphorus runoff through biotechnological valorization of agro-industrial waste.

Table 2. Mean phytase activity (FTU/mL) of *Aspergillus niger* from Day 0 to Day 7 of incubation (n = 12)

Days (hours)	Average phytase activity (FTU/mL)	p-value
Day 0 (0 hour)	0.00 ± 0.00[a]	<0.0018
Day 1 (24 hours) *	8.00 ± 1.51[b]	
Day 2 (48 hours)	19.25 ± 2.83[c]	
Day 3 (72 hours)	165.50 ± 10.07[d]	
Day 4 (96 hours)	182.58 ± 4.24[de]	
Day 5 (120 hours)	136.75 ± 2.39[f]	
Day 6 (144 hours)	134.25 ± 5.88[fg]	
Day 7 (168 hours)	125.50 ± 13.43[fgh]	

Note: Values are expressed as mean ± standard error (SE).
Means within the same column followed by different superscript letters (a–h) are significantly different ($p < 0.05$), as determined by the Mann–Whitney U test.
*Day 1 data was based on n = 11 replicates.

Phytase Production Profiles by Aspergillus niger

Daily analysis from Day 0 to Day 7 (Table 2) revealed that *Aspergillus niger* reached peak phytase activity on Day 4 (182.58 ± 4.24 FTU/mL), with a substantial increase from Day 2 (48 hours) (19.25 ± 2.83 FTU/mL) to Day 4 ($p < 0.05$), followed by a gradual decline from Day 5 onwards. This production profile is characteristic of fungal enzyme expression under solid-state fermentation (SSF), where enzyme secretion is tightly linked to the active growth phase and substrate utilization dynamics. The initial lag phase (Day 0–1) reflects the period of fungal adaptation to the PKE substrate. The exponential rise in phytase activity between Day 2 and Day 4 correlates with rapid mycelial expansion and the biosynthetic phase of extracellular enzyme secretion. This phase is supported by the upregulation of phytase-encoding genes (phyA, phyB, etc.), nutrient assimilation, and enhanced metabolic turnover (Kumari & Bansal, 2021; Priya et al., 2023).

Day 4 appears to be the optimal harvest time for phytase yield, as also observed by Mahmood et al. (2021), who reported peak enzyme titres *by A. niger* on Days 3–5 depending on substrate and moisture conditions. Similar profiles have been documented in studies using SSF with wheat bran, coconut cake, and cassava peel, where enzyme production coincided with rapid fungal biomass accumulation (Jatuwong et al., 2020;

Gong et al., 2023). The post-Day 4 decline in phytase activity may result from several factors. First, nutrient depletion, especially carbon, nitrogen, and phosphorus may limit further growth and enzyme biosynthesis. Second, the accumulation of secondary metabolites such as oxalate, fumarate, or organic acids can inhibit enzymatic stability or denature the enzyme at the microenvironmental level (Singh et al., 2011; Gómez-Osorio et al., 2022).

Additionally, feedback inhibition and proteolytic degradation of extracellular enzymes during the stationary phase are known constraints in fungal fermentations (Martínez-Vallespín et al., 2022).

Environmental parameters such as pH drift, moisture reduction, and oxygen limitation also contribute to the enzymatic decline in prolonged SSF. As the substrate dries or compacts, the oxygen gradient becomes restricted, affecting aerobic metabolism required for sustained phytase expression (Pandey et al., 2001; Gong et al., 2023). Thus, determining the precise harvesting window between Day 3 and Day 4 is critical for industrial phytase production to maximize yield and reduce enzyme loss. This aligns with current optimization strategies in enzyme biotechnology, where fermentation time is a key determinant of bioprocess efficiency and economic feasibility (Park et al., 2021).

Fungal Biomass (Glucosamine) by Aspergillus niger

Fungal biomass, estimated through glucosamine quantification (Table 3), showed a pronounced increase from Day 1 to Day 4, with a peak concentration of 0.0557 ± 0.0054 mg/g on Day 4. This trend closely mirrors the phytase activity profile of *Aspergillus niger*, reinforcing the well-documented correlation between mycelial growth and extracellular enzyme production in filamentous fungi under solid-state fermentation (SSF) conditions. The use of glucosamine as a biomarker for fungal biomass is particularly suitable for SSF systems, where direct biomass measurement is challenging due to solid substrate interference. Glucosamine is derived from chitin, a key structural component of fungal cell walls, and its concentration serves as an indirect yet reliable indicator of fungal growth (Gong et al., 2023; Sakurai et al., 1977). In SSF, where moisture content is low and spatial growth is heterogeneous, glucosamine-based assays have been shown to correlate well with hyphal density and metabolic activity (Priya et al., 2023).

The synchronized peak of glucosamine content and phytase activity on Day 4 supports the hypothesis that enzyme production is tightly coupled with active mycelial proliferation. During this exponential phase, fungal cells exhibit maximal transcriptional activity, including upregulation of genes responsible for phytase synthesis, nutrient assimilation, and secretion (Kumari & Bansal, 2021; Gong et al., 2023). Beyond Day 4, a plateau and subsequent decline in glucosamine concentration were observed, likely due to a combination of factors including substrate nutrient depletion (carbon, nitrogen, phosphorus), decreased water activity, and the accumulation of metabolic inhibitors. Oxygen limitation is another critical factor affecting fungal growth in SSF. As hyphae penetrate deeper into the substrate matrix, oxygen diffusion becomes restricted, resulting in hypoxic microenvironments that suppress aerobic metabolism and biomass accumulation (Gomez-Osorio et al., 2022; Pandey et al., 2001). Additionally,

shifts in pH caused by organic acid production (e.g., oxalic or citric acid) during fermentation may disrupt intracellular enzyme stability and inhibit further hyphal elongation (Singh et al., 2011; Gong et al., 2023).

Table 3. Fungal biomass production by *Aspergillus niger* quantified as glucosamine content (n = 12)

Days (hours)	Average mycelial glucosamine content (mg/g)	p-value
Day 0 (0 hour)	0.0000 ± 0.0000^{ab}	<0.0018
Day 1 (24 hours) *	0.0003 ± 0.0013^{b}	
Day 2 (48 hours)	0.0104 ± 0.0013^{c}	
Day 3 (72 hours)	0.0167 ± 0.0015^{dfgh}	
Day 4 (96 hours)	0.0557 ± 0.0016^{e}	
Day 5 (120 hours)	0.0202 ± 0.0031^{dfgh}	
Day 6 (144 hours)	0.0180 ± 0.0025^{fgh}	
Day 7 (168 hours)	0.0172 ± 0.0018^{dfgh}	

Note: Values are presented as mean ± standard error (SE).
Initial comparisons were performed using the Kruskal–Wallis test.
Means within the same column bearing different superscript letters (a–h) indicate significant differences ($p < 0.05$), as determined by the Mann–Whitney U test.

The reduction in biomass post-Day 4 could also be linked to a metabolic transition from primary to secondary metabolism, wherein fungi reduce vegetative growth and begin producing sporulation-related compounds or stress-response proteins (Mahmood et al., 2021). This physiological shift is commonly observed in nutrient-limited SSF systems and signifies the end of the active growth phase. Understanding the timing and pattern of biomass accumulation is critical for optimizing enzyme production at industrial scale. Recognizing Day 4 as the turning point for maximal enzyme yield and fungal growth enables efficient scheduling of harvest, maximizing product output and cost-effectiveness in commercial bioprocesses (Park et al., 2021; Jatuwong et al., 2020). Future studies should investigate the possibility of metabolic engineering or co-culture strategies to prolong the exponential phase and enhance biomass-linked phytase secretion.

Implications and Sustainability Outlook

This study highlights the potential of palm kernel expeller (PKE) a readily available agro-industrial by-product in Malaysia as a low-cost and effective

substrate for phytase production via solid-state fermentation (SSF). The ability of *Aspergillus niger* to efficiently convert PKE into a high-value functional enzyme such as phytase holds strong promise for enhancing feed quality, reducing production costs, and advancing sustainable livestock nutrition. In poultry production, phosphorus is a critical yet costly nutrient. However, much of the phosphorus in plant-based feedstuffs is bound in phytic acid, making it unavailable to monogastric animals. Supplementation with microbial phytase improves phosphorus digestibility, thereby reducing the need for inorganic phosphate additives such as dicalcium phosphate, which are increasingly expensive and derived from non-renewable mineral sources (Martínez-Vallespín et al., 2022; Rizwanuddin et al., 2023). By incorporating SSF-derived phytase from *A. niger* into poultry diets, feed manufacturers can improve nutrient bioefficacy while decreasing dependency on imported feed additives.

The adoption of PKE-based SSF phytase also addresses significant environmental concerns. Unutilized phytate in poultry feed is excreted and contributes to phosphorus-rich manure runoff, which is a leading cause of freshwater eutrophication in intensive animal farming zones (Kumari & Bansal, 2021; Priya et al., 2023). Phytase supplementation has been shown to reduce phosphorus excretion by up to 40%, directly supporting more eco-efficient animal production systems (Gong et al., 2023). From an economic standpoint, this approach aligns with Malaysia's national feed security agenda, which aims to reduce reliance on imported feed raw materials such as corn and soybean meal. As of 2022, Malaysia imported more than 5 million tonnes of feed ingredients annually, contributing to high production costs and price volatility (Choy, 2024). Utilizing locally available by-products like PKE not only supports circular bioeconomy principles but also reduces foreign exchange outflow and enhances farmer profitability through reduced input costs (Gomez-Osorio et al., 2022).

In a broader regional context, implementing SSF-based enzyme production supports ASEAN's collective goal of sustainable agri-food systems and bio-based circularity (ASEAN Bioeconomy Framework, 2022). The feasibility of decentralizing such bioprocessing technologies in rural or feed-mill clusters offers further socio-economic benefits, especially to smallholder poultry producers. Overall, the integration of microbial phytase production using PKE into Malaysia's animal feed sector provides a multifaceted solution enhancing nutritional quality, minimizing waste, lowering feed costs, and reducing environmental impact. Future policy should incentivize on-farm bioconversion systems, fund pilot-scale fermenter adoption, and develop guidelines for quality assurance of enzyme-rich fermented feed supplements.

Conclusion

This study successfully identified *Aspergillus niger* as the most effective phytase-producing strain under solid-state fermentation (SSF) using palm kernel expeller (PKE) as substrate. This study demonstrated that *Aspergillus niger* produced the highest phytase activity (269.67 ± 13.52 FTU/mL) among the fungal strains tested under solid-state fermentation (SSF) using palm kernel expeller (PKE) as substrate. Peak phytase production (182.58 ± 4.24 FTU/mL) and fungal biomass (0.0557 ± 0.0054 mg/g glucosamine) were recorded on Day 4 of fermentation, confirming that enzyme secretion was closely linked to mycelial growth. These findings validate the bioconversion potential of PKE as a suitable substrate and confirm *A. niger* as an efficient strain for microbial phytase production under SSF. The use of glucosamine as an indirect biomass marker effectively captured fungal growth dynamics in SSF systems. These outcomes support the application of *A. niger*-derived phytase in improving phosphorus bioavailability in poultry feed while reducing environmental phosphorus losses.

Acknowledgement

The authors gratefully acknowledge the Malaysia Agricultural Research and Development Institute (MARDI), Livestock Science Research Centre, and the Faculty of Science, Universiti Putra Malaysia (UPM) for their support in providing laboratory facilities and technical assistance throughout the experimental work. The collaboration and institutional commitment from both MARDI and UPM were instrumental to the successful completion of this study.

Conflict of Interest

The authors declare no conflict of interest associated with this publication

References

Ahmed, S.A. & Abood, N.H. 2017. Effect of some growth factors on protease production by *Rhizopus oryzae*. *J. Al-Nahrain Univ. Sci.* 20(2): 90–95.

Angel, R., Tamim, N., Applegate, T., Dhandu, A. & Ellestad, L. 2002. Phytic acid chemistry: Influence on phytin-phosphorus availability and phytase efficacy. *J. Appl. Poult. Res.* 11(4): 471–480.

ASEAN Bioeconomy Framework. 2022. *Strategic Framework for Bio-Based Circular Economy in Southeast Asia*. https://asean.org/bioeconomy-framework

Azizi, M.N., Loh, T.C., Foo, H.L. & Chung, E.L.T. 2021. Is palm kernel expeller cake a suitable alternative feed ingredient for poultry? *Animals* 11(2): 338.

Cano y Postigo, L.O., Jacobo-Velázquez, D.A., Guajardo-Flores, D., Garcia Amezquita, L.E. & García-Cayuela, T. 2021. Solid-state fermentation for enhancing the nutraceutical content of agrifood by-products: Recent advances and industrial feasibility. *Food Biosci.* 41: 100926.

Casey, A. & Walsh, G. 2004. Identification and characterization of fungal phytases—opportunities and

challenges. *Enzyme Microb. Technol.* 37(2): 195–204.

Choy, N.Y. 2024. Malaysia's feed industry outlook: Localizing raw materials for sustainability. *Malays. J. Anim. Sci.* 27(1): 45–58.

Choy, N.Y. 2024. Plantation ministry mulling incentives for using palm oil waste in poultry feed. *The Edge Malays.* https://theedgemalaysia.com/node/731834

Dave, G. & Modi, H. 2019. Phytase-Fe_3O_4 nanoparticles-loaded microcosms of silica for catalytic remediation of phytate-phosphorous from eutrophic water bodies. *Environ. Sci. Pollut. Res.* 26(15): 14988–15000.

El-Gendi, H., et al. 2021. Biotechnological applications of fungal phytase: A review. *Biotechnol. Rep.* 29: e00622.

Elson, L.A. & Morgan, W.T.J. 1933. A colorimetric method for the determination of glucosamine and chondrosamine. *Biochem. J.* 27(6): 1824–1828.

Fitriyah, F., Aziz, M.A., Wahyuni, S., Fadila, H., Permana, I.G., Priyono, N. & Siswanto, N. 2022. Nutritional improvement of oil palm and sugarcane plantation waste by solid-state fermentation of *Marasmiellus palmivorus*. *IOP Conf. Ser. Earth Environ. Sci.* 974(1): 012121.

Gerin, P., Bellon-Fontaine, M.N., Asther, M. & Rouxhet, P.G. 1995. Immobilization of fungal spores by adhesion. *Biotechnol. Bioeng.* 47(6): 677–687.

Gomez-Osorio, L., Nielsen, J.U., Martens, H.J. & Wimmer, R. 2022. Upgrading the nutritional value of PKE using a *Bacillus subtilis*-derived monocomponent β-mannanase. *Molecules* 27(2): 563.

Gong, Z., Zhang, S. & Liu, J. 2023. Recent advances in chitin biosynthesis associated with the morphology and secondary metabolite synthesis of filamentous fungi in submerged fermentation. *J. Fungi* 9(2): 205.

Ibrahim, D. & Lim, S.H. 2014. Utilization of palm kernel expeller cake as a novel substrate for phytase production by *Aspergillus niger* USM AI1. *BioResources* 9(1): 1488–1497.

Jatuwong, K., Suwannarach, N., KumLa, J., Penkhrue, W., Kakumyan, P. & Lumyong, S. 2020. Bioprocess for production, characteristics, and biotechnological applications of fungal phytases. *Front. Microbiol.* 11: 188.

Kumari, N. & Bansal, S. 2021. Statistical modeling and optimization of microbial phytase production towards utilization as a feed supplement. *Biomass Convers. Biorefin.* 13(9): 8339–8349.

Mahmood, S., Shahid, M.G., Nadeem, M. & Haq, I.U. 2021. Screening of phytate-degrading fungi and optimization of culture conditions for phytase synthesis. *Pak. J. Bot.* 53(2): 703–709.

Martínez-Vallespín, B., Männer, K., Ader, P. & Zentek, J. 2022. Evaluation of

high doses of phytase in a low-phosphorus diet in comparison to a phytate-free diet in broiler chickens. *Animals* 12(15): 1955.

Mohammadi-Kouchesfahani, M., Hamidi-Esfahani, Z. & Azizi, M.H. 2019. Isolation and identification of lactic acid bacteria with phytase activity from sourdough. *Food Sci. Nutr.* 7(11): 3700–3708.

Newmeyer, D. 1990. Filtering small quantities of conidial suspensions to remove mycelial fragments. *Fungal Genet. Rep.* 37(1): Article 1480.

Pandey, A., Selvakumar, P., Soccol, C.R. & Nigam, P. 2001. Solid state fermentation for the production of industrial enzymes. *Curr. Sci.* 77(1): 149–162.

Park, S.Y., et al. 2021. Phytase application in poultry nutrition: An updated review. *Anim. Biosci.* 34(1): 1–12.

Priya, N., Singh, B., Sharma, J.G. & Giri, B. 2023. Optimization of phytase production by *Penicillium oxalicum* in solid-state fermentation for potential as a feed additive. *Prep. Biochem. Biotechnol.* 54(6): 819–829.

Puppala, K.R., Buddhiwant, P.G., Agawane, S.B., Kadam, A.S., Mote, C.S., Lonkar, V.D., Khire, J.M. & Dharne, M.S. 2020. Performance of *Aspergillus niger* (NCIM 563) phytase-based feed supplement for broiler growth and phosphorus excretion. *Biocatal. Agric. Biotechnol.* 31: 101887.

Rizwanuddin, S., Kumar, V., Naik, B., Singh, P., Mishra, S., Rustagi, S. & Kumar, V. 2023. Microbial phytase: Sources, production, and role in the enhancement of nutritional aspects of food and feed. *J. Agric. Food Res.* 12: 100559.

Sakurai, Y., Lee, T.H. & Shiota, H. 1977. On the convenient method for glucosamine estimation in *koji*. *Agric. Biol. Chem.* 41(4): 619–624.

Samat, N. 2011. Peningkatan skala sistem bioreaktor dulang untuk penghasilan hampas isirong kelapa sawit (PKE) terfermentasi. *MSc. Thesis*, Universiti Kebangsaan Malaysia, Bangi, Malaysia.

Singh, B., Satyanarayana, T. & Goel, V. 2011. Improved phytase production by a mutant of *Aspergillus niger* in submerged fermentation using statistical methods. *J. Ind. Microbiol. Biotechnol.* 38(6): 791–797.