Optimization of Lipase Synthesis by Native Fungal Strains via Solid-State Fermentation of Shea Nut Cake

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ABSTRACT

Lipases are commercially valuable enzymes, recognized for their broad versatility and substrate specificity. Solid-state fermentation (SSF) offers a cost-effective and environmentally sustainable strategy for enzyme production, leveraging locally sourced agro-industrial residues. This study aims to identify and screen indigenous fungal strains with high lipase-producing potential and to evaluate shea nut cake as an alternative substrate for lipase production. A full factorial 323^2 experimental design was employed to systematically optimize SSF process parameters, including temperature (30°C, 35°C, 40°C), incubation time (72 h, 96 h, 120 h), and nutrient supplementation (glucose and yeast extract) as potential inducers or inhibitors. The objective of this optimization was to enhance lipase yield while minimizing production costs and environmental impact. Fungal isolates exhibited variable lipase production under different conditions. *Aspergillus niger* (C1) demonstrated the highest lipase yield (9.22 U/gds) at 40°C, 96 hours, with yeast extract supplementation, while *Saccharomyces sp.* achieved a maximum yield of 7.88 U/gds at 30°C, 120 hours, with glucose supplementation. Using indigenous fungal strains for lipase production through SSF of shea nut cake promotes sustainable agricultural practices, reduces agro-industrial waste, and offers economic benefits.

Keywords: Agro-industrial residues, *Aspergillus niger,* Lypase production, Indigenous fungal strains, and *Saccharomyces sp.*

INTRODUCTION

Microbial enzyme production is gaining prominence in biotechnology due to its shorter processing time and minimal energy requirements. Enzyme-based biocatalysis is a key subfield of biotechnology, offering a sustainable alternative to conventional chemical processes (Jonathan *et al.,*2017). Enzymes enhance industrial manufacturing by reducing energy consumption, lowering raw material costs, and minimizing harmful byproducts, aligning with the principles of green chemistry (Brzeska & Agnieszka, 2021). Among industrial enzymes, lipases have emerged as crucial biocatalysts, constituting over 10% of the global enzyme market (Jonathan & Adeoyo 2011a, Dutra *et al.,* 2022).

Lipases are serine hydrolase enzymes that mediate the hydrolysis of triacylglycerols, diacylglycerols, and monoacylglycerols, yielding free fatty acids and glycerol. These enzymes are naturally present in plants, animals,

and microorganisms (Chandra *et al.,* 2020). Bacterial enzymes are often preferred over plant- or animal-derived counterparts due to their higher stability and ease of large-scale cultivation (Meenakshi *et al.,* 2021). Lipases have broad industrial applications, including pharmaceuticals, detergents, flavor modification, paper production, cosmetics, leather processing, wastewater treatment, and biodiesel synthesis. Their role in polymer biodegradation and fatty waste decomposition highlights their significance in environmental sustainability. Due to their exceptional catalytic efficiency, lipases are considered one of the most important industrial enzymes (Nisar *et al.,* 2021).

Solid-state fermentation (SSF) is a fermentation process in which microorganisms grow on a solid substrate with little to no free liquid phase (Chaitanya *et al.,* 2022). Unlike submerged fermentation (SmF), SSF provides a unique environment for microbial metabolism and growth. Filamentous fungi and yeasts, which can thrive under low-moisture conditions, are particularly well suited for SSF (Banat *et al.,* 2021). SSF offers several technological advantages over SmF, including enhanced oxygen transfer due to the higher surface area of the solid substrate, which facilitates mass transfer (Ramos-Sánchez *et al.,* 2015). Additionally, the direct exposure of microbial cells, particularly aerial hyphae, to the gas phase enhances overall oxygen availability. The low water activity in SSF prevents bacterial contamination, which typically requires high water activity for growth (Brück *et al.,* 2022). Moreover, optimizing water activity and selecting an appropriate fungal inoculum can minimize contamination from competing fungal species. Despite these advantages, SSF has some limitations, such as heat accumulation from microbial metabolism, which can impact fermentation efficiency.

Shea nut cake (SNC) is a brown amorphous byproduct generated during shea butter production. Despite its composition of minerals, phytochemicals, and proximate elements, SNC remains largely underutilized and is often discarded as waste. Given its nutrient-rich composition, SNC presents a promising alternative substrate for enzyme production, particularly in SSF systems.

This study aims to optimize the conditions for lipase production by Indigenous fungal strains through SSF using shea nut cake as a substrate. The research explores the potential of Indigenous fungi in utilizing SNC for enzyme biosynthesis while also evaluating the economic feasibility and sustainability of SNC as a resource for large-scale lipase production.

MATERIALS & METHODS

Shea Nut Cake (SNC) Procurement.

Shea nut cake was sourced from local fufu producers in Idofian, Ifelodun, Kwara State, Nigeria (8° 23' 0" N, 4° 43' 0" E).

Isolation of Indigenous Fungal Strains

Fungal strains were isolated from soil samples collected at the shea nut cake sourcing site by inoculating them onto potato dextrose agar (PDA). Successive subculturing was performed to obtain pure cultures of the fungal isolates.

Screening for lipase-producing fungal strains

Lipase production by the fungal strains was assessed by supplementing potato dextrose agar (PDA) with 5% (v/v) lipid-containing substrate (shea butter) and observing the formation of clear zones, indicative of lipase activity (Mobarak-Qamsari *et al.,* 2011).

Substrate Preparation

Shea nut cake was collected, manually screened to remove debris and extraneous materials, and subsequently ground into a fine powder using a laboratory mortar and pestle. The substrate was then sterilized by autoclaving at 121°C for 15 minutes under a pressure of 15 psi to eliminate potential microbial contaminants

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Inoculum Preparation

The identified lipase-producing microorganism was cultivated on potato dextrose agar (PDA) and incubated at its optimal growth temperature to ensure robust proliferation and availability for solid-state fermentation (SSF). Following incubation, fungal spores were aseptically harvested, and spore suspensions were prepared using sterile distilled water.

Solid State Fermentation (SSF) and Optimization design factors

A 5 g portion of the shea nut cake substrate was placed into a 250-mL Erlenmeyer flask and moistened with 0.05 M citrate buffer to adjust the initial moisture content and pH. In selected experiments, the substrate was additionally supplemented with glucose and yeast extract to investigate the influence of nutrient enrichment on fermentation performance. The flasks were sterilized by autoclaving at 121°C and 15 psi for 15 minutes. After cooling, 1 mL of the spore suspension of the lipase-producing fungal strain was aseptically inoculated and thoroughly mixed into the substrate to achieve uniform distribution. The inoculated flasks were incubated at 30°C, 35°C, and 40°C for 72, 96, and 120 hours. Fungal growth and substrate alterations were monitored at regular intervals.

Lipase Extraction

At specified time intervals, the solid substrate bearing fungal biomass was aseptically collected. The fermented material was homogenized to achieve sample uniformity. A 0.5 g portion of the moldy substrate was mixed with 10 mL of an extraction buffer containing 1% (w/v) NaCl and 1% (v/v) Tween-80. The mixture was manually ground using a mortar and pestle and then filtered through Whatman No. 1 filter paper. The retained residue was dried at 70°C for 24 hours to determine the dry weight of the solids. The filtrate obtained was centrifuged at 4,000 rpm for 20 minutes, and the resulting supernatant was collected and used as the crude enzyme extract for subsequent lipase activity assays.

Assay of Lipase

Lipase activity was determined using a modified titrimetric procedure based on the method of Arzoglou *et al.* (1994). The reaction mixture included 5 mL of an olive oil emulsion substrate, 20 mL of 0.1 M citrate buffer, and 1 mL of enzyme extract. After homogenization, the mixture was incubated at 30°C on a rotary shaker at 130 rpm for 30 minutes. The reaction was stopped by adding 15 mL of an acetone-ethanol solution (1:1, v/v). The amount of free fatty acids released was quantified by titration with 0.05 M NaOH, using phenolphthalein as an indicator until a stable pink endpoint was reached. A blank control was conducted by introducing the enzyme just before titration. One unit (U) of lipase activity was defined as the amount of enzyme releasing one micromole of fatty acids per minute under the assay conditions. Results were expressed as units per gram of dry substrate or per milliliter of enzyme extract.

Lipase activity (U/L) = ACV106

A = the amount of NaOH in mL used each minute;

U = fatty acid in micro-moles emitted every minute

C = NaOH concentration in moles per liter;

V = volume of enzyme sample in microliters

RESULTS

The microbial isolates were subsequently sub-cultured on Potato Dextrose Agar (PDA) to obtain pure cultures of each fungal strain, as illustrated in Plates 1–4. Five morphologically distinct fungal isolates, presumed to possess lipase-producing capabilities, were selected for preliminary screening and designated as SNC/B1, SNC/B2, SNC/C1, SNC/D2, and SNC/E1. Among these, isolates SNC/C1 and SNC/D2 demonstrated the highest

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lipolytic activity, evidenced by prominent zones of clearance on lipid-supplemented agar. Consequently, these two isolates were selected for subsequent solid-state fermentation studies and optimization of lipase production parameters.

Plate 1: Macroscopic view of *Aspergillus niger*

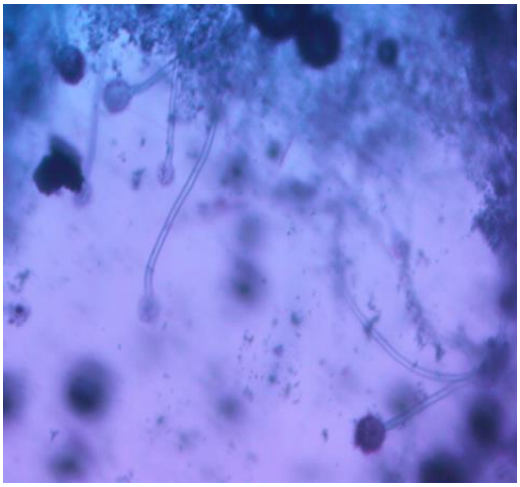

Plate 2: Microscopic view of *Aspergillus niger*

Plate 3: Macroscopic view of *Saccharromyces sp*

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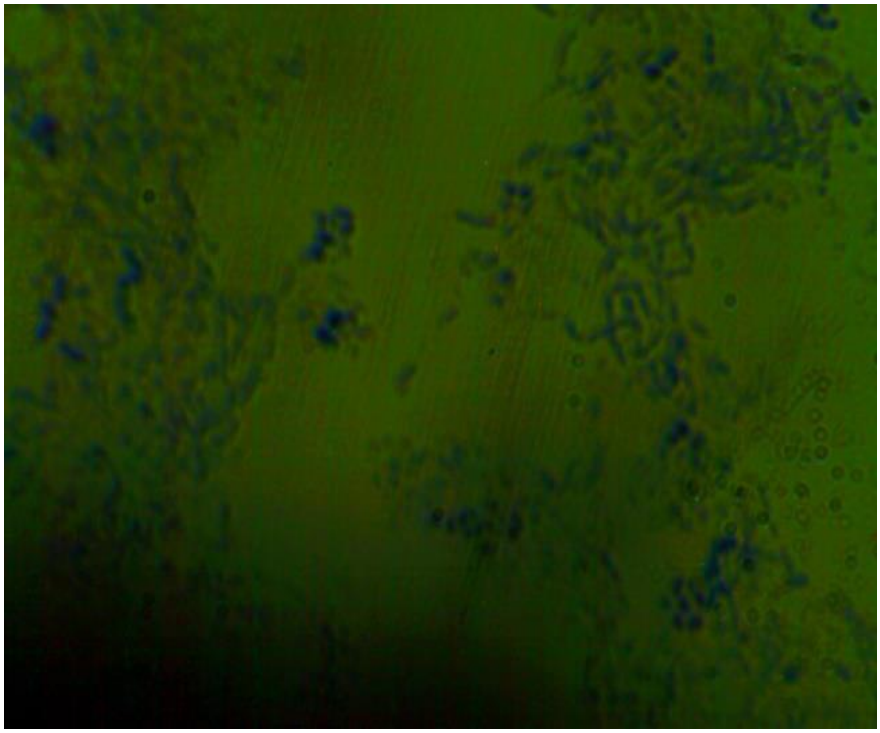

Plate 4: Microscopic view of *Saccharromyces sp*

Table 1: Morphological characteristics of lipase producing fungal isolates

	Pigmentation	**Elevation**	**Form**	**Margin**	**Colour**
SNC/C1	Black	Raised	Mold	Irregular	Yellow
SNC/D2	Creamy	Smooth	Yeast	Irregular	NA

NOTE: NA – Not Applicable

Table 2: Microscopic characteristics of isolated lipase producing fungi

Sample code	**Microscopic characteristics**	**Organism name**
SNC/C1	septate hyphae with conidiophores bearing black, spherical conidia (spores) in chains.	*Aspergillus niger*
SNC/D2	oval-shaped cells under the microscope, showing budding daughter cells attached.	*Saccharomyces* sp.

Effect of Optimization Factors

Comparative Evaluation of Lipase Production Potential of Microbial Isolates (Figures 1–6)

The enzymatic potential of *Aspergillus niger* (C1) and *Saccharomyces* sp. (D2) was assessed by subjecting both isolates to identical but varying environmental and nutritional conditions to determine differences in their lipase activity, measured in units per gram of dry substrate (U/gds). *Aspergillus niger* (C1) demonstrated superior lipase production, reaching a peak yield of 9.22 U/gds on the fourth day of fermentation at 40°C when yeast extract was used as a nitrogen supplement (C1/4D/40/Y). In comparison, *Saccharomyces* sp. (D2) attained its maximum lipase yield of 7.88 U/gds on the fifth day at 30°C in the presence of glucose as a carbon source (D2/5D/30/G). These results highlight the differential enzymatic responses of the two isolates to fermentation parameters, suggesting species-specific optimization requirements for maximal lipase biosynthesis.

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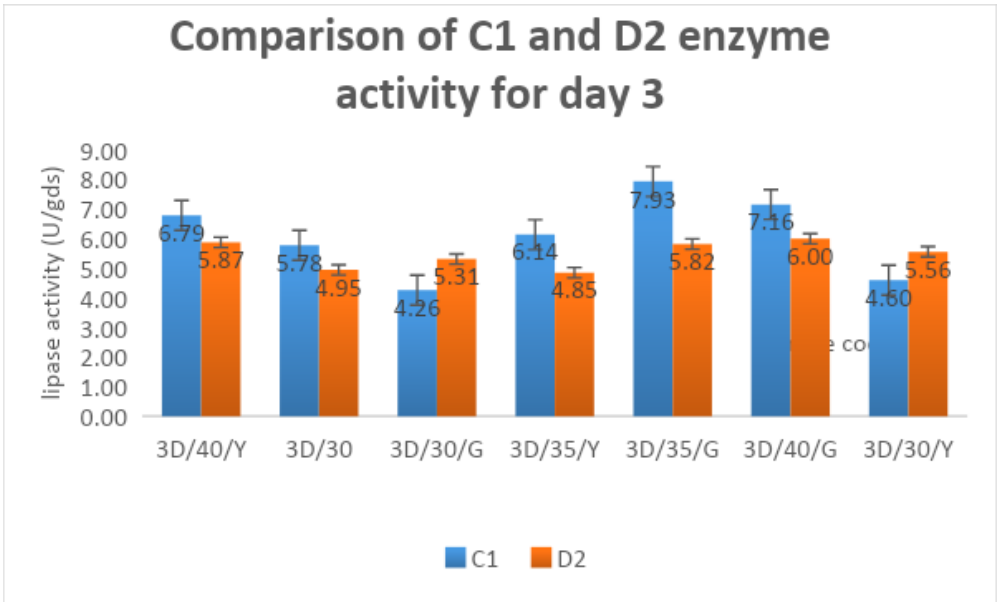

Fig.1: Comparison of C1 and D2 enzyme activity for day 3

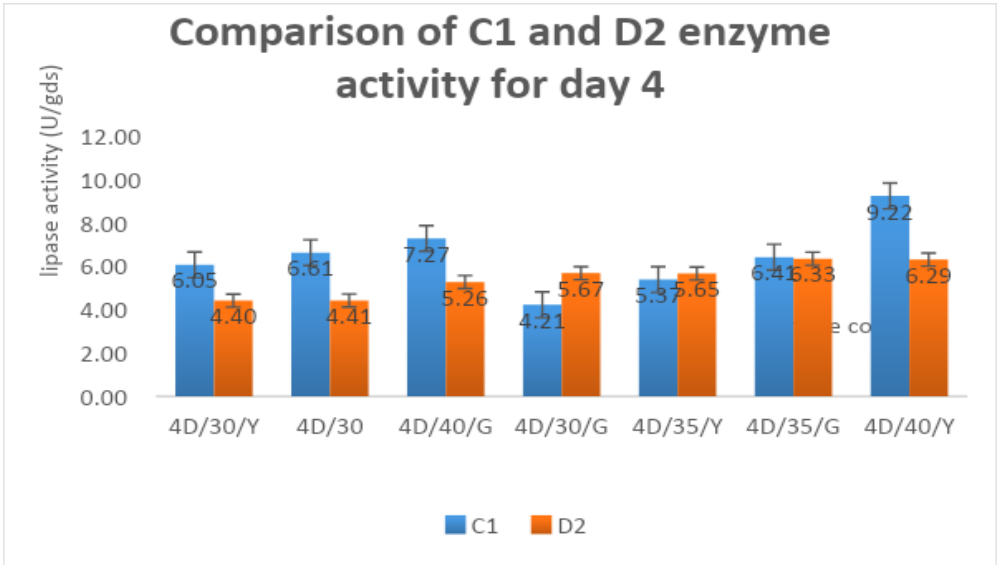

Fig.2: Comparison of C1 and D2 enzyme activity for day 4

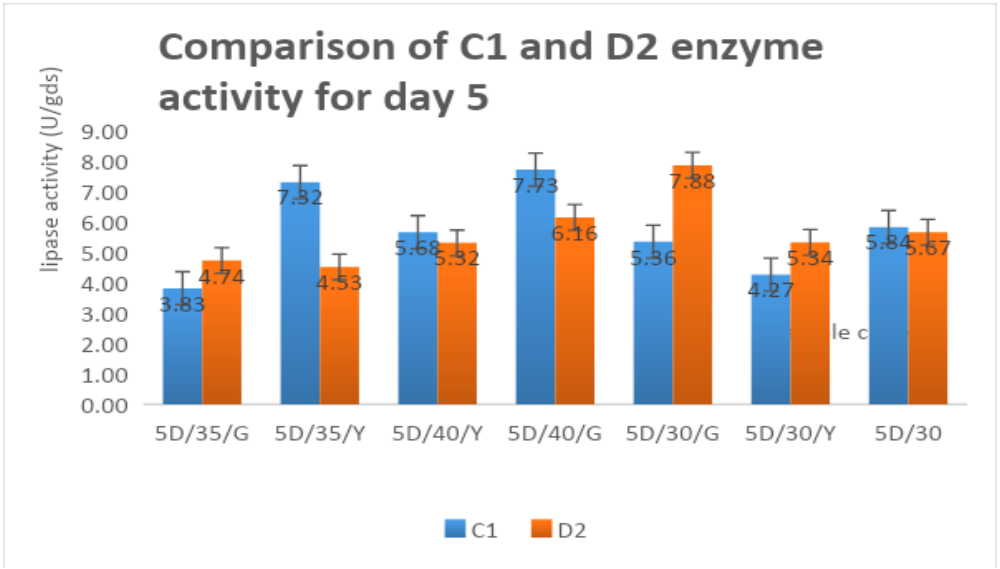

Fig.3: Comparison of C1 and D2 enzyme activity for day 5

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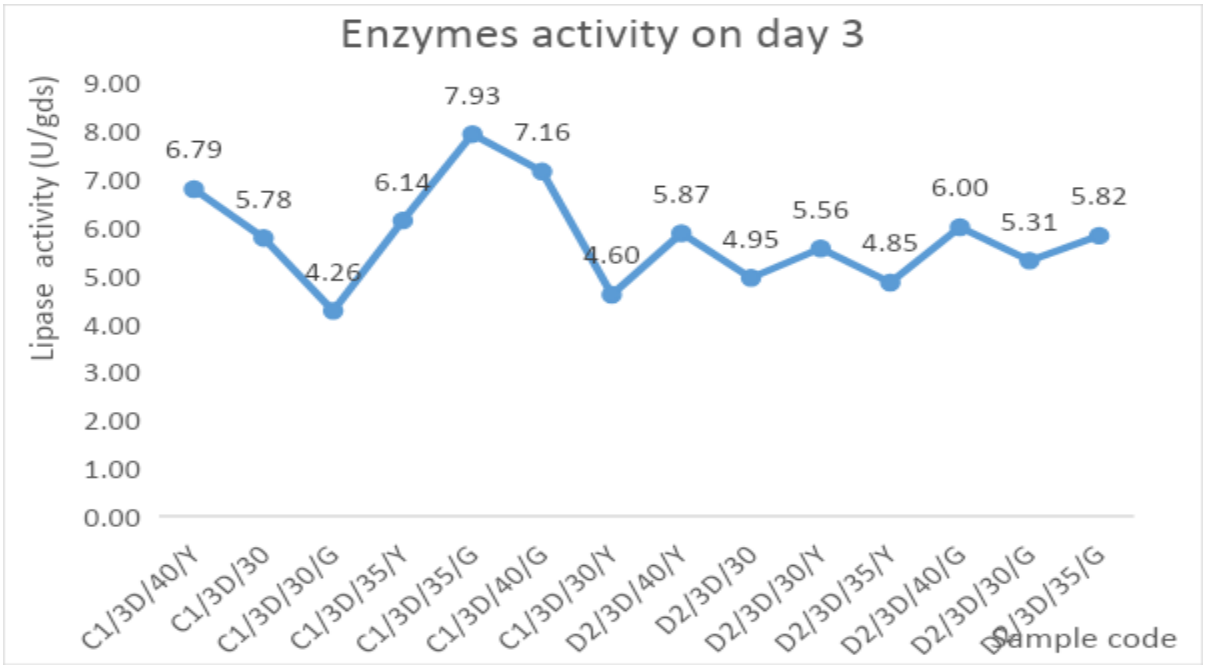

Fig.4: Enzymes activity on day 3

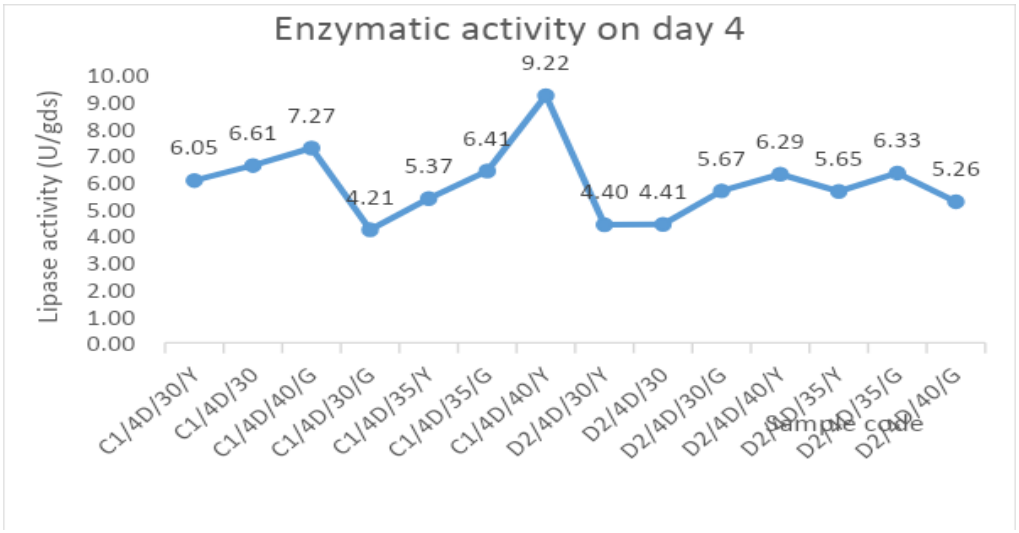

Fig.5: Enzymes activity on day 4

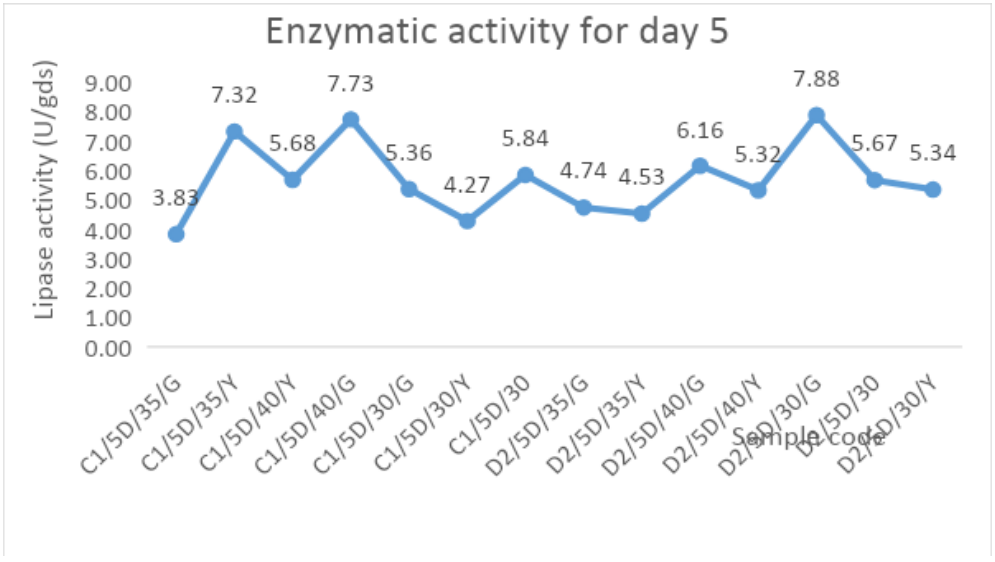

Fig.6: Enzymes activity on day 5

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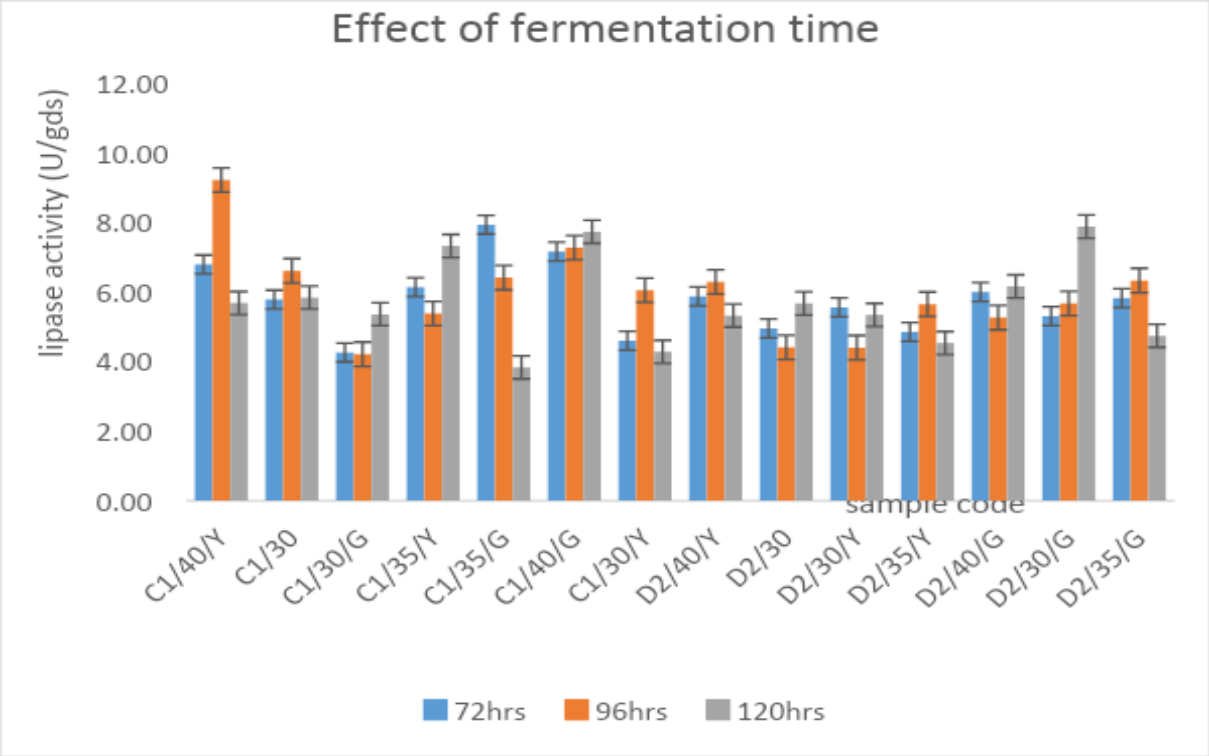

Fig.7: Effect of fermentation time

Keys

C1 – *Aspergillus niger* D2 – *Saccharomyces* sp. 3D – Fermentation period of 3 days (72 hrs); 4D – Fermentation period of 4 days (96 hrs); 5D – Fermentation period of 5 days (120 hrs); 30 – Fermentation Temperature at 30°C; 35 – Fermentation Temperature at 35°C 40

Fermentation was carried out at a controlled temperature of 40 °C, utilizing yeast extract (denoted as Y) and glucose (denoted as G) as nutrient supplements, as illustrated in Figure 7. Sample codes were systematically designated to reflect key experimental parameters, including the test organism, duration of incubation, incubation temperature, and type of nutrient supplement. For instance, the code C1/5D/35/G refers to a fermentation setup involving *Aspergillus niger* (C1), incubated for 5 days (120 hours) at 35 °C with glucose as the carbon source. Similarly, D2/4D/30/Y indicates a fermentation trial using *Saccharomyces* sp. (D2), incubated for 4 days (96 hours) at 30 °C with yeast extract supplementation. All other sample identifiers were formulated using this consistent nomenclature.

DISCUSSION

Fungi are widely acknowledged as prolific microbial producers of lipase due to their capacity for extracellular enzyme secretion, which simplifies downstream recovery from fermentation media. Among fungi, filamentous forms and yeasts are particularly advantageous for commercial enzyme production owing to their robust metabolic capabilities and ease of enzyme extraction (Cesário *et al.,* 2021). Moreover, the generally recognized as safe (GRAS) status of several lipolytic fungi further enhances their industrial applicability, though certain exceptions do exist (Olalla *et al.,* 2015).

The present study supports these observations by establishing the lipase-producing potential of indigenous fungal isolates *Aspergillus niger* and *Saccharomyces sp*. when cultivated on shea nut cake (SNC) under solid-state fermentation (SSF) conditions. SNC, a lignocellulosic by-product from shea butter processing, was evaluated as a low-cost, nutrient-rich substrate for microbial lipase biosynthesis. Abdul-Mumeen *et al.* (2013) previously reported SNC's suitability for microbial growth due to its high protein and lipid content, which supports the proliferation of lipolytic fungi. Our findings corroborate this, as isolates from SNC, particularly *A. niger* (C1) and *Saccharomyces sp.* (D2), demonstrated substantial lipolytic activity.

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The microbial communities isolated align with prior studies, such as Ibrahim *et al.* (2020), who identified similar fungal species including *Aspergillus* and *Saccharomyces* in SNC and comparable agro-industrial residues. Among the optimized parameters, temperature was a key determinant of enzyme activity. *A. niger* displayed peak lipase production at 40 °C, while *Saccharomyces sp.* exhibited maximal output at 30 °C, consistent with findings by Hidayat *et al*. (2017) and Nema *et al.* (2019), who reported optimal lipase production within the mesophilic range of 30–40 °C. Enzyme activity declined beyond 50 °C, likely due to thermal denaturation a known limitation of mesophilic enzyme systems (Böhme *et al.,* 2019).

Incubation time also critically influenced enzyme yield. A general trend of increasing activity up to 96 hours followed by a decline at 120 hours was observed, mirroring Basak *et al.* (2020), who attributed such declines to nutrient depletion and accumulation of metabolic inhibitors. Furthermore, the presence of glucose resulted in reduced lipase activity during early fermentation (72–96 hours), likely due to carbon catabolite repression. However, by 120 hours, glucose-enhanced enzyme activity was observed, suggesting delayed repression or microbial adaptation again consistent with Basak *et al.* (2020).

Nitrogen supplementation significantly enhanced lipase biosynthesis, with yeast extract yielding the highest enzymatic output. This is attributed to its rich composition of amino acids, peptides, and vitamins that facilitate microbial growth and enzyme induction (Ramos-Sanchez *et al.,* 2015). The observed effect underscores the role of nitrogen sources in modulating enzyme productivity.

These findings contribute to the broader understanding of using agricultural by-products for enzyme production and align with sustainable bioprocessing principles. As Sharma *et al.* (2019) emphasized, using agro-residues like SNC supports green chemistry initiatives by reducing waste and improving economic feasibility. Comparative analysis with earlier studies, such as Pandey et al. (2000), suggests SNC is competitive with traditional substrates for lipase production.

The deployment of indigenous fungal strains in SSF has practical relevance, as locally adapted microorganisms often exhibit improved substrate utilization and enzymatic efficiency under native conditions. The use of these strains thus presents an opportunity to enhance large-scale enzyme production while minimizing reliance on synthetic fermentation media.

The methodology employed for fungal isolation and screening was consistent with standard microbiological practices. Pure cultures were obtained using Potato Dextrose Agar (PDA), a medium favored for its ability to support diverse fungal taxa (Eltaweel *et al.,* 2018). Primary screening for lipase activity was conducted on lipid-enriched media such as Tween-80 or tributyrin agar, where lipolytic activity is visualized through clear hydrolysis zones—a rapid, qualitative method validated by Eltaweel *et al.* (2018).

Among the tested isolates, SNC/C1 (A. niger) and SNC/D2 (Saccharomyces sp.) demonstrated the most pronounced hydrolysis zones, indicative of high extracellular lipase production. These isolates were further selected for fermentative evaluation in SSF trials. SSF remains a preferred approach for fungal lipase production due to its cost-effectiveness and its ability to mimic natural fungal growth conditions, which enhances enzyme output (Zhao, 2024).

The study employed a structured nomenclature system to track fermentation parameters. For example, the code C1/5D/35/G denotes fermentation using *A. niger* (C1), incubated for five days at 35 °C with glucose. Likewise, D2/4D/30/Y refers to Saccharomyces sp. (D2) incubated for four days at 30 °C with yeast extract. This systematic coding is consistent with good scientific practice, enhancing reproducibility and clarity, especially in complex optimization studies (Pandey *et al.,* 2000).

Glucose and yeast extract, selected as carbon and nitrogen sources respectively, are widely documented for their roles in microbial fermentation. Glucose supports rapid microbial proliferation and secondary metabolite production (Singh *et al.,* 2016), while yeast extract is recognized for promoting enzyme synthesis due to its comprehensive nutrient profile (Balaraman & Prabhakar, 2007).

The comparative analysis of C1 and D2 under varying SSF conditions revealed species-specific enzymatic responses. *A. niger* reached a peak lipase yield of 9.22 U/gds on day 4 at 40 °C with yeast extract, while Saccharomyces sp. achieved a maximum of 7.88 U/gds on day 5 at 30 °C with glucose supplementation. These values are consistent with earlier studies by Nema *et al.* (2019) and Putri *et al*. (2020), who also noted high lipase activity in *A. niger* under SSF, particularly when supplemented with agro-industrial waste materials.

Although *Saccharomyces sp*. is not conventionally associated with high lipase productivity, findings from Tsekova and Ganeva (2009) indicate that under optimized conditions, *S. cerevisiae* can achieve appreciable enzyme yields, thereby supporting our observations.

CONCLUSION

In summary, this study validates the use of SNC as a promising substrate for SSF-based lipase production. It emphasizes the critical role of process optimization temperature, time, nutrient supplementation and highlights the potential of indigenous fungal strains for industrial enzyme production. These findings support the advancement of low-cost, eco-friendly biotechnological processes and pave the way for future research focused on scale-up and strain improvement. Further research is necessary to explore large-scale applications and genetic improvements of indigenous fungal strains for enhanced enzyme production.

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