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Review

Production of feed grade L-lysine using solid state fermentation for the Nigerian market

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L-Lysine, an essential amino acid crucial for both human and animal nutrition, serves as a valuable medicament and additive in animal feed. Nigeria annually imports significant quantities of L-lysine to support its animal feed industry. In developing countries like Nigeria, a viable biotechnological approach for L-lysine production involves solid-state fermentation. This method not only offers environmental advantages but also facilitates the simultaneous production of beneficial feed enzymes.

Key words: L-Lysine, solid-state fermentation, Nigerian market, *Corynebacterium glutamicum.*

INTRODUCTION

Plant proteins typically lack at least one essential amino acid, with cereal grains deficient in lysine, and legume grains lacking methionine and cysteine, both sulfur-containing amino acids (Eruvbetine, 2009). L-lysine, an indispensable amino acid crucial for both animal and human nutrition, is often supplemented in feed to compensate for these deficiencies, especially in the food and animal feed sectors. In 2021, approximately 2.2 million tonnes of L-lysine were produced.

Lysine, an essential amino acid that cannot be synthesized by the body, is crucial for maintaining health. It provides various benefits, including preventing symptoms of lysine deficiency, lowering blood pressure, and alleviating cold sores. Furthermore, lysine supplements contribute to enhanced calcium absorption. According to projections from Fior Markets (2021), the global lysine market is anticipated to expand from USD 7.32 billion in 2020 to achieve USD 13.05 billion by 2028.

L-Lysine holds particular significance for monogastric species, serving as the first limiting amino acid for pigs and the second for poultry. Major lysine manufacturers are predominantly located in Korea, China, the USA, and Germany. China, leading in both production and consumption, held a 62% market share in 2016. Noteworthy producers include CheilJedang Corp (South Korea), Ajinomoto (Japan), and Archer Daniels Midland (US). The global L-Lysine market, valued at 285.89 million USD in 2021, is projected to grow at a rate of 3.26% from 2021 to 2027 (Marketwatch, 2022).

In Nigeria, biotechnological advancements, particularly

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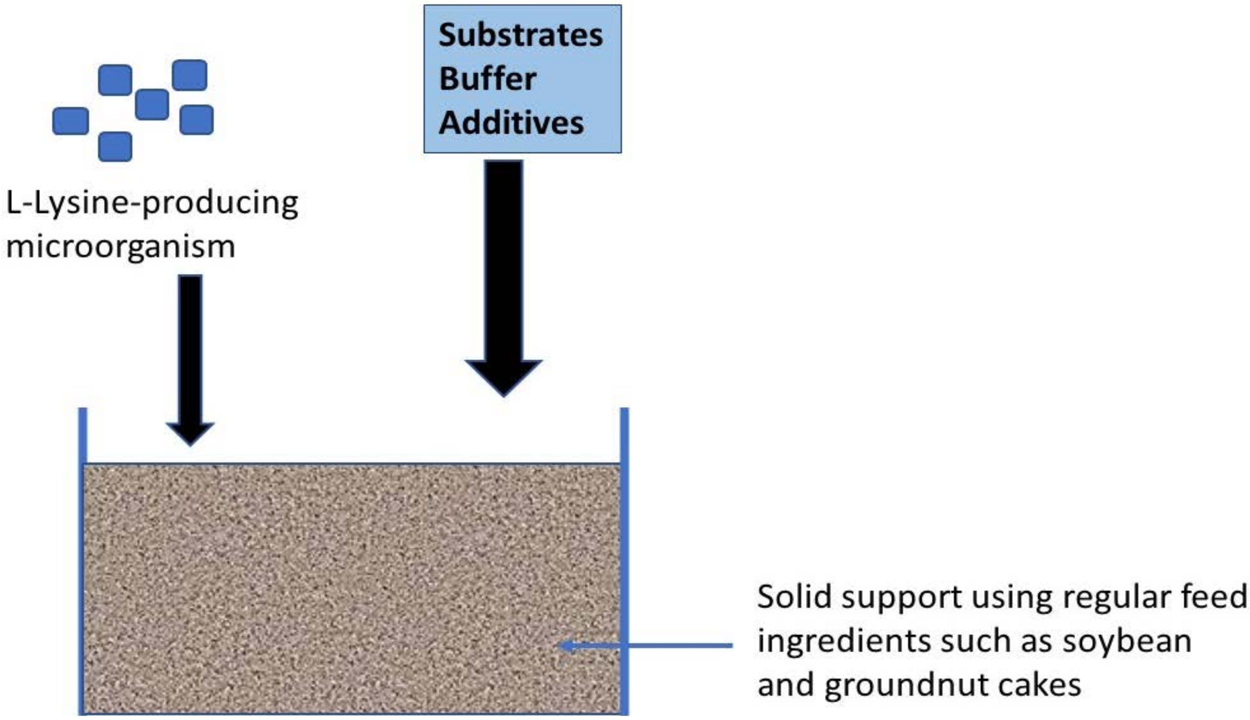

Figure 1. Production of L-lysine via solid state fermentation using regular animal feed components, such as soybean as support, substrates and microorganisms with appropriate buffers and additives, including minerals and vitamins at specific temperature and pH.

in solid-state fermentation (Figure 1), present an opportunity to competitively produce lysine locally, eliminating the need for extensive fermenters and costly downstream processing (Tonukari et al., 2010).

ANIMAL FEED PRODUCTION IN NIGERIA

Based on estimates from the Federal Ministry of Agriculture and Rural Development, the Nigerian poultry industry makes a substantial contribution of 6.0 to 8.0% to the country's GDP. In 2019, the net worth of this industry was reported to be N1.6 trillion, constituting 25% of the agricultural gross domestic product, according to the Central Bank of Nigeria (Punch, 2022). Since its humble beginnings with only two feed mills in 1957, Nigeria has now grown to accommodate over 1,000 feed mills, positioning itself as the 40th leading country in global livestock feed production. The industry directly or indirectly engages more than 5 million Nigerians, including technical or skilled personnel, distributors, tool and machinery fabricators, input suppliers, and others (Vanguard, 2021).

Considerable progress has been made in localizing inputs for poultry production in Nigeria; especially regarding bulk feed ingredients such as maize and soybeans. While most feed mills utilize locally produced bulk raw materials, certain specialized ingredients like lysine, vitamins, toxin binders, and other additives are still imported (Netherlands Enterprise Agency, 2020). The Nigerian feed industry reportedly generated 5.5 million metric tonnes of animal feed in 2016 (Vanguard, 2021). Consequently, the cost of a 25 kg bag of poultry feed, primarily composed of maize, millet, soybean, and wheat, surged from N3600 in 2019 to a range between N8500 and N10,000 in July 2022 (Premium Times, 2022).

Lysine plays a vital role as a feed additive in the animal feed industry, particularly for monogastric animals such as broilers, poultry, and swine. It is incorporated into common livestock feed, like cereals, which lack sufficient levels of L-lysine to meet the animals' nutritional requirements. Lysine enhances feed quality by facilitating the absorption of other amino acids in the digestive systems of the animals (Anastassiadis, 2007). To achieve its annual target of 50 million tonnes of animal feed production, Nigeria is estimated to require approximately 100,000 tonnes of lysine.

PRODUCTION OF L-LYSINE BY SOLID-STATE FERMENTATION

The significance of L-lysine research and development has rapidly grown since the discovery of fermentative amino acid production in the 1950s (Kinoshita et al., 1958). This progress has introduced innovative fermentation processes, replacing more costly classical manufacturing methods like acid hydrolysis. Lysine biosynthesis primarily involves two pathways, α-aminoadipate and diaminopimelate, each utilizing distinct substrates and enzymes present in various organisms. A typical culture medium for L-lysine production includes inorganic salts (potassium monohydrogen phosphate, potassium dihydrogen phosphate, magnesium sulfate, sodium chloride, magnesium chloride, ferrous chloride, ferrous sulfate, and manganese sulfate), organic compounds (yeast extract, peptone, meat extract, corn steep liquor, soybean cake), as well as monosaccharide starting materials, minerals, and vitamins (Anastassiadis, 2007).

The predominant method for lysine production involves fermentation using strains of *corynebacteria*, notably *Corynebacterium glutamicum*. This process comprises multiple steps, including fermentation, cell separation through centrifugation or ultrafiltration, product separation and purification, evaporation, and drying (Anastassiadis, 2007). Limited information exists regarding lysine production through solid-state fermentation. Giannoutsou et al. (2012) reported lysine production by a *Paecilomyces variotii* strain using solid-state fermentation with two-phase olive mill waste. Anusree et al. (2015) employed *C. glutamicum* DM 1729 and starch hydrolysate of jackfruit seed powder as a substrate, with sugarcane bagasse acting as the inert solid support matrix in solid-state fermentation for microbial growth and lysine production. Mageshwaran and Parvez (2016) observed lysine enrichment during gossypol detoxification in cottonseed cake through solid-state fermentation.

Our study (Egbune et al., 2023a, 2024; Eedom et al., 2023) introduces a feasible method for L-lysine production using agricultural by-products such as palm kernel cake, soybean cake, groundnut cake, and wheat offal. Solid-state fermentation with *C. glutamicum* ATCC 13032 resulted in the production of L-lysine from these agricultural by-products (Figure 2). Additionally, the addition of elephant grass extract was found to enhance L-lysine production. Furthermore, soluble proteins, glucose, amylase, and protease activities significantly increased alongside L-lysine production in the fermented agricultural by-products. Lysine produced through solid-state fermentation has been incorporated into broiler and pig feed formulations. In contrast to commercial lysine precipitated from submerged fermentation, solid-state produced lysine lacks hydrochloric acid (HCl) or sulfate. It remains in its natural state, is environmentally friendly, and proves more cost-effective than imported L-lysine monohydrochloride and L-lysine sulfate.

SOLID-STATE FERMENTATION PROCESS USED FOR L-LYSINE PRODUCTION IN NIGERIA

In the solid-state fermentation process for L-lysine production in Nigeria, agricultural residues like rice bran or wheat bran are carefully selected substrates, sterilized to create a conducive environment for the growth of L-lysine-producing microorganisms, typically strains of Corynebacterium or Brevibacterium. Maintaining controlled environmental conditions, including temperature and humidity, is crucial for optimizing microbial growth and L-lysine synthesis. The process emphasizes the strategic use of local agricultural by-products, contributing to efficiency and sustainability (Egbune et al., 2023a, Ezedom et al., 2023; Egbune et al., 2024). Downstream processing involves extracting and purifying lysine from the fermented solid substrate using solvents and various techniques, such as ion exchange chromatography, highlighting the importance of a well-defined production pathway for economic viability and sustainability (Sinha et al., 2022; Ewing et al., 2022). While solid-state fermentation (SSF) for L-lysine production offers several advantages, such as the utilization of agricultural residues and the potential for a sustainable production process, it is important to consider potential challenges and drawbacks:

Uniformity and control

Achieving uniformity in substrate composition and maintaining precise control over environmental factors in solid-state fermentation can be challenging. Variability in raw material composition and uneven microbial distribution within the solid substrate may lead to fluctuations in lysine production (Zhao et al., 2023).

Scaling-up difficulties

Transitioning from laboratory-scale SSF to larger industrial scales can pose challenges. Maintaining consistent conditions and ensuring even microbial distribution becomes more complex in larger fermentation vessels, potentially impacting the reproducibility of lysine production (Ganeshan et al., 2021).

Heat transfer limitations

Solid-state fermentation generates heat as a byproduct, and poor heat transfer within the solid substrate may result in temperature gradients. This can lead to localized areas of higher or lower temperatures, affecting microbial activity and lysine production. Effective heat removal becomes crucial for maintaining optimal fermentation conditions (Vauris et al., 2022; Anigboro et al., 2022).

Substrate utilization efficiency

The efficiency of substrate utilization in SSF may vary, as microorganisms may not utilize all components of the

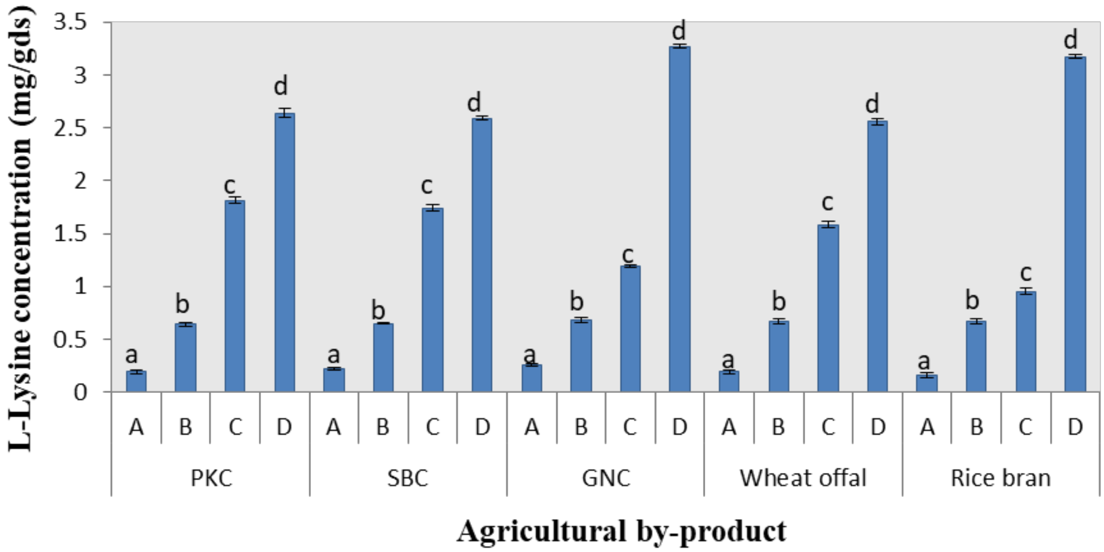

Figure 2. Differences in L-Lysine concentrations were observed in solid-state fermented agricultural by-products utilizing *C. glutamicum*. Bars marked with distinct superscripts indicate significant variations at p<0.05. The abbreviations used are PKC for Palm Kernel Cake, SBC for Soybean Cake, and GNC for Groundnut Cake. The experimental conditions are denoted as follows: A represents 500 ml water (control), B represents 500 ml water with the addition of *C. glutamicum*, C represents 50 ml elephant grass extract with 450 ml water and *C. glutamicum*, and D represents 500 ml elephant grass extract with *C. glutamicum.*
Source: Egbune et al. (2024).

solid substrate equally. This inefficiency can affect overall lysine yield and may require additional optimization efforts in substrate selection and microbial strain engineering (Catalán and Sánchez, 2020).

Downstream processing challenges

Extracting and purifying lysine from the solid substrate can be more complex in SSF compared to liquid fermentation. Solid residues, microbial biomass, and impurities present in the fermented mass may complicate downstream processing, requiring additional steps for effective lysine recovery (Kumar et al., 2021).

Longer fermentation times

SSF often requires longer fermentation times compared to liquid fermentation methods. This prolonged process may increase the risk of contamination and requires careful monitoring and control to prevent unwanted microbial growth and maintain the desired lysine production (Rudakiya, 2019). Despite these challenges, ongoing research and technological advancements aim to address these drawbacks, making solid-state fermentation a promising avenue for sustainable L-lysine production. Optimization of process parameters, strain improvement, and innovative engineering solutions can help overcome these challenges and enhance the overall efficiency of lysine production through SSF.

Comparison or analysis of the solid-state fermentation method with other conventional methods of L-lysine production, such as fermentation using strains of corynebacteria

The comparison between solid-state fermentation (SSF) and other conventional methods of L-lysine production, particularly fermentation using strains of corynebacteria in submerged liquid cultures, involves considering various factors such as efficiency, scalability, operational costs, and environmental impact (Table 1).

PERSPECTIVE

The availability of L-Lysine is a crucial determinant for achieving high-quality animal feed production and ensuring a sustainable global protein economy (Leinonen et al., 2019). While the majority of commercially available L-lysine comes in the form of L-lysine monohydrochloride and L-lysine sulfate, solid-state produced L-lysine retains its natural state. The use of solid-state fermentation for lysine production offers a significant advantage due to the concurrent generation of beneficial enzymes, such as protease and amylase, which prove highly beneficial for monogastric animals (Egbune et al., 2022, 2023b). Nigeria has the potential to fulfill its L-lysine requirements

Table 1. Key factors involved in the comparison between solid-state fermentation and liquid fermentation using strains of Corynebacteria for L-lysine production.

Factor	Solid-state fermentation (SSF)	Liquid fermentation (corynebacteria)
Substrate utilization	Variable efficiency; may not fully utilize all components of solid substrate (Rudakiya, 2019).	Controlled composition for efficient substrate utilization (Halle et al., 2023)
Microbial growth and productivity	Uneven growth; variations in productivity (Londoño-Hernandez et al., 2020).	Uniform growth; potentially higher productivity (Park et al., 2018)
Scaling-up and industrialization	Challenges in maintaining uniform conditions; potential environmental benefits (Niakousari et al., 2021).	Easily scalable with established technologies; may have resource and environmental concerns (Igbokwe et al., 2022).
Downstream processing	Complex due to solid residues and impurities (Mehta, 2019).	Generally straightforward in liquid medium (Gabelman, 2022).
Environmental impact	More environmentally friendly; utilizes agricultural residues (Wang et al., 2019).	Water and energy-intensive; liquid waste disposal concerns (Kumar et al., 2014).
Cost considerations	Potential cost advantages; reduced energy requirements; challenges in downstream processing (Costa et al., 2018).	Higher operational costs; benefits from well-established technologies (D'Este et al., 2018).

for animal feed by embracing solid-state fermentation. Additionally, the agricultural by-products utilized in L-lysine production are abundant in most regions of the country.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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