

Nutritional Value of *Sauropus androgynus* Leaves Through Tempe-Style Fermentation

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ABSTRACT

This study evaluated the effects of ragi tempe-style fermentation on the nutritional quality, antinutritional factors, amino acid composition, and fatty acid profile of *Sauropus androgynus* (*S. androgynus*) leaves, to determine an optimal starter dose for feed application. Leaf powder was fermented for 48 h at $30 \pm 2^\circ\text{C}$ using five ragi tempe doses: 0%, 0.5%, 1.0%, 1.5%, and 2.0% (w/w). Fermentation significantly increased crude protein and lipid contents while reducing crude fiber levels, indicating improved substrate digestibility. Substantial reductions in phytic acid, tannins, oxalates, and saponins were observed with increasing doses of ragi. Essential amino acids, particularly lysine, leucine, valine, and isoleucine, were significantly enhanced following fermentation, reflecting improved protein quality. In addition, the fatty acid profile shifted toward a higher proportion of unsaturated fatty acids, including linoleic and α -linolenic acids. Among the treatments, the 1.5% ragi tempe dose consistently produced optimal nutritional improvements, with no significant additional benefits at higher doses. These findings demonstrate that

tempe-style fermentation effectively upgrades *Saurus androgynus* leaves into a nutritionally enhanced and functionally valuable ingredient, highlighting its strong potential for use in sustainable formulations for mammalian and fish feed.

Keywords-anti-nutritional factors; aquaculture nutrition; fermentation; functional feed; life below water; *Sauropus androgynus*; solid-state fermentation

I. INTRODUCTION

The global livestock and aquaculture sectors are currently facing challenges regarding the increasing demand for feed that has made traditional staples unsustainable due to volatile prices and heavy ecological footprints [1, 2]. Solid-State Fermentation (SSF), particularly tempe-style processing using specific microbial inocula, constitutes a promising approach to mitigating these limitations [3]. SSF is not just an old food preservation technique; it is a biological "upgrade" system. By using microbial powerhouses, such as *Rhizopus* or *Aspergillus*, these tough plant materials can be pre-digested [4, 5]. SSF can break down complex fibers and neutralize the chemical defenses of plants, effectively unlocking nutrients that were previously "bolted" inside the cell walls [6, 7]. In aquaculture nutrition, this reduction is especially valuable because many cultured fish species lack endogenous enzymes to break down anti-nutrients and non-starch polysaccharides, which otherwise reduce feed intake and nutrient assimilation [8].

Despite these outcomes, most research has focused on legumes, cereal by-products, and conventional plant protein sources [9]. Thus, there is a research gap regarding the application of fermentation to leafy plants, particularly those similar to *S. androgynus*, which are locally abundant in tropical regions and hold potential as low-cost alternative protein sources. Leaf meals often possess desirable nutrient profiles but are constrained by high levels of fiber and anti-nutrients. When properly processed through fermentation, these materials can yield increased protein content, liberate amino acids, and reduce anti-nutritional components, enabling their incorporation at higher inclusion levels in animal diets without compromising performance or health.

S. androgynus is of particular interest due to its high baseline nutrient density, which includes considerable crude protein and micronutrient content when compared with many forages and leaf meals. However, as with other leaf meals, direct feeding without preprocessing results in limited digestibility and potential constraints on nutrient utilization [10]. Fermentation with *Rhizopus*-based inocula (commonly sourced from tempe starter cultures such as ragi tempe) has the potential to enhance nutrient bioavailability through microbial proteolytic activity and carbohydrate degradation. This microbial bioconversion could improve the Essential Amino Acid (EAA) composition and functional lipid profiles, thereby broadening the applicability of this material in animal and aquafeed formulations while addressing key industry priorities, including feed sustainability and nutritional adequacy.

One of the key novelties of this study is the systematic evaluation of fermentation dose–response in a leaf-based substrate, an area that remains relatively underexplored. Identifying the optimal ragi tempe inoculum level provides practical insights for process optimization, scalability, and feed

ingredient production. The integration of proximate, anti-nutritional, amino acid, and fatty acid analyses enables an assessment of nutrient transformation and functional feed quality.

The need for sustainable feed alternatives is driven by economic and environmental constraints associated with conventional ingredients such as soybean meal and fishmeal. In this context, fermentation of locally available biomass such as *S. androgynus* offers a viable strategy to enhance resource efficiency and support circular bioeconomy approaches. Overall, this study contributes to the development of fermentation-based technologies to improve plant feedstuffs and advance sustainable feed formulation.

From an engineering and applied science perspective, tempe-style fermentation can be framed as a controllable solid-state bioprocess in which key operational variables, such as inoculum dose, temperature, moisture content, and fermentation time, govern substrate transformation efficiency. Optimizing these parameters is important not only for maximizing nutritional enhancement but also to ensure process reproducibility, scalability, and compatibility with industrial feed manufacturing systems. Identifying an optimal inoculum level represents a crucial process control variable that directly influences microbial kinetics, substrate utilization, and metabolic output. Therefore, this study approaches fermentation not solely as a nutritional modification strategy but as a bioprocess optimization problem with practical implications for scale-up and integration into feed production pipelines.

II. MATERIALS AND METHODS

A. Raw Materials and Starter Culture

Fresh leaves of *S. androgynus* were obtained from a local agricultural area at Kediri, East Java, Indonesia, and were transported to the laboratory under ambient conditions. The leaves were washed thoroughly with running water to remove adhering soil and debris, then drained and oven-dried at 50 °C until a constant weight was reached. Dried leaves were milled using a laboratory grinder and sieved to obtain a uniform powder (mesh size 60). Commercial ragi tempe containing a mixed culture dominated by *Rhizopus* spp. was used as the fermentation starter. All chemicals and reagents employed for proximate, anti-nutritional, amino acid, and fatty acid analyses were of analytical grade.

B. Experimental Design and Fermentation Procedure

The experiment was arranged in a completely randomized design with five treatments corresponding to different ragi tempe doses: 0% (T0, unfermented control), 0.5% (T1), 1.0% (T2), 1.5% (T3), and 2.0% (T4) (w/w, based on dry substrate). Each treatment was conducted in triplicate. For fermentation, *S. androgynus* leaf powder was adjusted to approximately 60%

moisture content using sterile distilled water, thoroughly mixed, and steamed for 20 min to reduce native microbial load. After cooling to room temperature, the substrate was inoculated with ragi tempe according to the designated treatment levels, combined uniformly, and packed in perforated polyethylene bags to allow aeration.

Fermentation was carried out at $30 \pm 2^\circ\text{C}$ for 48 h. The selection of fermentation conditions was based on established parameters for *Rhizopus*-dominated tempe fermentation and SSF systems. This specific temperature was chosen as it represents the optimal growth range for *Rhizopus* spp., ensuring efficient enzymatic activity and substrate colonization. A 48 h fermentation period was selected to allow sufficient microbial proliferation and enzymatic hydrolysis while avoiding over-fermentation, which may lead to nutrient degradation or undesirable metabolite accumulation. The inoculum range (0–2.0%, w/w) was designed to capture the dose-response relationship while avoiding excessive microbial loading that could result in substrate competition, oxygen limitation, or heat accumulation. The upper limit of 2.0% was therefore selected based on practical and biological constraints commonly reported in SSF systems. The potential existence of a dose-response plateau was not assumed a priori but was empirically evaluated through the experimental design.

C. Proximate Composition Analysis

Proximate composition, including crude protein, crude fat, crude fiber, ash, and moisture content, was determined following standard Association of Official Analytical Collaboration (AOAC) procedures. Crude protein was analysed using the Kjeldahl method with a nitrogen conversion factor of 6.25. Crude fat content was measured by Soxhlet extraction using petroleum ether as solvent, while crude fiber was determined by sequential acid-alkali digestion. Ash content was obtained by incineration in a muffle furnace at 550°C . Carbohydrate content was calculated by difference, and all analyses were performed in triplicate and expressed on a dry weight basis.

D. Determination of Anti-Nutritional Factors

Anti-nutritional compounds were quantified using established spectrophotometric and titrimetric methods. The phytic acid content was determined using the Wade reagent method, with the results expressed as milligrams per 100 grams of dry sample. Tannin content was measured by deploying the Folin-Ciocalteu method and expressed as mg tannic acid equivalents per gram. Oxalate content was assessed by acid extraction followed by titration with standardized potassium permanganate, while saponin content was determined using a gravimetric method after solvent extraction. These analyses were conducted in triplicate for each treatment.

E. Amino Acid Profile Analysis

Amino acid composition was analysed following acid hydrolysis of samples with 6 N HCl at 110°C for 24 h under vacuum conditions. After hydrolysis, the samples were neutralized, filtered, and evaluated employing High-Performance Liquid Chromatography (HPLC) equipped with a fluorescence or UV detector, following pre-column

derivatization. EAAs were quantified using external standards and expressed as g/100 g protein [11].

F. Fatty Acid Composition Analysis

Total lipids were extracted utilizing a chloroform-methanol mixture following a modified Folch method. Extracted lipids were converted to Fatty Acid Methyl Esters (FAMES) by transesterification using methanolic KOH. FAMES were examined employing gas chromatography equipped with a flame ionization detector and a capillary column suitable for fatty acid separation. Individual fatty acids were identified by comparing their retention times with those of certified standards, and expressed as a percentage of the total fatty acids.

G. Statistical Analysis

All data were expressed as mean \pm Standard Deviation (SD), with $n = 3$ independent biological replicates per treatment. Before analysis, data were assessed for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively. As assumptions were satisfied, statistical differences among treatments were evaluated using One-Way Analysis of Variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons when significant differences were detected ($p < 0.05$). Statistical analyses were performed utilizing the Statistical Package for the Social Sciences (SPSS) software. In addition to significance testing, percentage changes relative to the control were calculated to support the interpretation of practical relevance.

III. RESULTS

A. Proximate Composition Enhancements

The fermentation process significantly increased crude protein and crude fat contents in treated leaves compared to the unfermented control, as shown in Table I. The highest protein enrichment was observed in the 1.5% ragi tempe treatment, reaching a mean of $29.72 \pm 1.47\%$ (dry basis), which was significantly greater than the unfermented control. Increasing the dose to 2.0% did not result in additional protein, suggesting a dose-response plateau. This overall increasing trend aligns with findings in other plant fermentations, where microbial biomass contributes protein, and cellulolytic activity liberates protein previously bound within plant matrices. For instance, fermented tropical leaf meals have demonstrated higher crude protein and lower fiber, attributed to the enzymatic breakdown of the cell wall.

In parallel, crude fat content reached its peak in treatments T3 and T4, showing a significant improvement over the control. The reduction in crude fiber reflects enzymatic degradation of structural carbohydrates by the starter culture. Similar observations have been made in the fermentation of legumes and leaf meals, where cellulase and hemicellulase activities from fungal cultures reduce insoluble fiber and increase digestibility metrics. Consistent with these changes, the ash content remained relatively stable across all treatments ($p > 0.05$), while carbohydrate content, calculated by difference, decreased as protein and fat levels rose.

TABLE I. PROXIMATE COMPOSITION OF FERMENTED *S. ANDROGYNUS* LEAVES (% DRY MATTER BASIS). (N = 3, BIOLOGICAL REPLICATES)

Treatment	Crude protein	Crude fat	Crude fiber	Ash	Carbohydrate*
T0	21.35 ± 0.82 ^d	3.12 ± 0.41 ^c	14.88 ± 0.76 ^a	9.42 ± 0.18	51.23
T1	23.12 ± 1.15 ^c	3.48 ± 0.35 ^{bc}	13.56 ± 0.92 ^b	9.51 ± 0.32	50.33
T2	26.44 ± 0.98 ^b	4.15 ± 0.52 ^b	11.24 ± 0.65 ^c	9.68 ± 0.21	48.49
T3	29.72 ± 1.47 ^a	4.46 ± 0.62 ^a	10.32 ± 1.18 ^d	9.83 ± 0.44	45.67
T4	28.95 ± 1.25 ^a	4.58 ± 0.56 ^a	10.71 ± 0.84 ^d	9.91 ± 0.22	45.85

Values are expressed as mean ± SD. Different superscripts within the same column indicate significant differences among treatments ($p < 0.05$), with "a" representing the highest mean value. *Carbohydrate content was calculated by difference. Values without superscripts indicate no significant differences among treatments ($p > 0.05$).

B. Reduction of Anti-Nutritional Factors

A key functional outcome of fermentation was the significant reduction in anti-nutritional factors, including phytic acid, tannins, oxalates, and saponins. As presented in Table II, phytic acid decreased to 246.9 ± 18.8 mg/100 g in T3. This reduction of more than 50% aligns with the known role of microbial phytases in degrading phytic acid complexes in plant materials. Phytate reduction is important because high phytate levels are associated with impaired mineral uptake and reduced protein digestibility in both fish and terrestrial animals.

A significant reduction in all measured anti-nutritional factors was observed as the ragi dose increased up to 1.5%. Fermentation also effectively reduced tannins and saponins, which otherwise interfere with nutrient absorption and protein utilization. The levels of these anti-nutrients remained statistically similar between the 1.5% and 2.0% doses, indicating that the enzymatic degradation had reached a saturation point at the T3 level. The effectiveness of *Rhizopus* fermentation in reducing anti-nutrients has been documented in tempeh production, where proteolytic activity, microbial hydrolysis, and co-metabolism contribute to the degradation of compounds that negatively affect digestibility. It should be noted that in Tables II-IV, the values are expressed as mean ± SD. Different superscripts within the same column indicate significant differences among treatments ($p < 0.05$), with "a" representing the highest mean value. Values without superscripts indicate no significant differences among treatments ($p > 0.05$).

C. Essential Amino Acid Improvements

Fermentation significantly enhanced the concentration of EAAs across all treatments, with T3 displaying optimal increases, particularly for limiting amino acids such as lysine, leucine, and valine, as shown in Table III. Compared to the control, the concentrations of these EAAs increased by 20-40% in the medium-to-high dose treatments. For instance, lysine content rose from 4.12 ± 0.28 g/100 g in the control to a peak of 5.91 ± 0.52 g/100 g in T3. This result parallels research on fermented legumes, where microbial proteases break down

complex proteins into free amino acids, increasing both the quantity and bioavailability of EAAs.

Enhanced EAA profiles are especially beneficial in aquaculture and livestock feeding, as a balanced amino acid supply directly influences growth rates, muscle accretion, and immune function. In aquafeeds, the fermentation of plant proteins has been linked to improved growth performance, enhanced nutrient digestibility, and positive modulation of the gut microbiota in species such as Nile tilapia.

TABLE II. ANTI-NUTRITIONAL FACTORS OF FERMENTED *S. ANDROGYNUS* LEAVES. (N = 3, BIOLOGICAL REPLICATES)

Treatment	Phytic Acid (mg/100 g)	Tannins (mg TAE/g)	Oxalate (mg/100 g)	Saponin (mg/100 g)
T0	512.4 ± 12.6 ^a	7.92 ± 0.74 ^a	386.2 ± 18.8 ^a	214.5 ± 10.7 ^a
T1	445.2 ± 15.4 ^b	6.15 ± 0.32 ^b	341.6 ± 14.1 ^b	188.6 ± 9.4 ^b
T2	332.8 ± 22.4 ^c	5.32 ± 0.58 ^c	248.3 ± 11.5 ^c	135.8 ± 12.9 ^c
T3	246.9 ± 18.8 ^d	3.81 ± 0.45 ^d	198.6 ± 12.7 ^d	102.7 ± 8.8 ^d
T4	241.4 ± 14.1 ^d	3.85 ± 0.21 ^d	196.2 ± 10.1 ^d	104.3 ± 7.2 ^d

TABLE III. EAA PROFILE OF FERMENTED *S. ANDROGYNUS* LEAVES (G/100 G PROTEIN). (N = 3, BIOLOGICAL REPLICATES)

Amino acid	Treatment				
	T0	T1	T2	T3	T4
Lysine	4.12 ± 0.28 ^c	4.86 ± 0.34 ^b	5.42 ± 0.41 ^a	5.91 ± 0.52 ^a	5.82 ± 0.38 ^a
Leucine	6.34 ± 0.45 ^b	6.51 ± 0.52 ^b	7.12 ± 0.48 ^{ab}	7.96 ± 0.61 ^a	7.88 ± 0.55 ^a
Isoleucine	3.11 ± 0.19 ^b	3.42 ± 0.24 ^{ab}	3.79 ± 0.31 ^a	4.08 ± 0.29 ^a	4.15 ± 0.33 ^a
Valine	3.92 ± 0.22 ^c	4.51 ± 0.31 ^b	4.98 ± 0.35 ^{ab}	5.12 ± 0.42 ^a	5.17 ± 0.39 ^a
Methionine	1.42 ± 0.12 ^b	1.55 ± 0.15 ^{ab}	1.79 ± 0.18 ^a	1.94 ± 0.21 ^a	1.92 ± 0.17 ^a

D. Modulation of Fatty Acid Profiles

Fatty acid composition shifted toward a more favorable profile for aquaculture and livestock nutrition, characterized by a higher proportion of unsaturated fatty acids, particularly oleic (C18:1n9c) and linoleic (C18:2n6c) acids, alongside a significant decrease in saturated palmitic acid (C16:0) content (Table IV). For instance, palmitic acid decreased from $18.42 \pm 0.65\%$ in the control to $15.92 \pm 0.74\%$ in the 1.5% ragi tempe treatment (T3). The substitution of Saturated Fatty Acids (SFAs) by unsaturated types reflects the lipolytic activity of fungal enzymes and the preferential utilization of SFAs as energy sources during microbial metabolism.

While oleic and linoleic acids showed significant increases reaching their peak at T3 and T4 (Table IV), α -linolenic acid (C18:3n3) remained relatively stable across all treatments. This indicates that while fermentation effectively enhances certain monounsaturated and polyunsaturated fatty acids, some long-chain omega-3 fatty acids are preserved without significant

degradation or synthesis by the starter culture. This overall reduction in Total SFA from $24.88 \pm 1.12\%$ to $21.52 \pm 1.21\%$ demonstrates that tempe-style fermentation improves the lipid quality of *S. androgynus* leaves, making them a more suitable ingredient for high-quality animal feeds.

TABLE IV. FATTY ACID COMPOSITION OF FERMENTED *S. ANDROGYNUS* LEAVES (% OF TOTAL FATTY ACIDS). (N = 3, BIOLOGICAL REPLICATES)

Fatty acid	Treatment				
	T0	T1	T2	T3	T4
Palmitic (C16:0)	18.42± 0.65 ^a	17.15± 0.82 ^b	16.44± 0.55 ^c	15.92± 0.74 ^c	16.12 ± 0.48 ^c
Oleic (C18:1n9c)	9.15± 0.42 ^c	10.55± 0.58 ^b	11.82± 0.71 ^a	12.44± 0.62 ^a	12.31± 0.55 ^a
Linoleic (C18:2n6c)	14.22± 0.95 ^b	14.85± 1.12 ^{ab}	15.94± 0.88 ^a	16.52± 1.05 ^a	16.48± 0.92 ^a
α-Linolenic (C18:3n3)	32.15± 1.45	31.88± 1.28	32.42± 1.62	32.05± 1.35	31.94± 1.51
Total SFA	24.88± 1.12 ^a	23.15± 0.95 ^b	22.04± 1.08 ^c	21.52± 1.21 ^c	21.74± 0.88 ^c

IV. DISCUSSION

The present study demonstrates that tempe-style fermentation using ragi tempe enhances the nutritional quality of *S. androgynus* leaves, improving their suitability as an alternative feed ingredient. Significant enhancements in proximate composition, anti-nutritional factors, and nutrient profiles indicate effective bioconversion of a fiber-rich substrate into a more bioavailable material.

Fermentation increased crude protein and lipid contents while reducing crude fiber (Table I). Protein content peaked at the 1.5% inoculum level, indicating efficient microbial biomass accumulation and enzymatic degradation of structural components. The absence of further protein gain at 2.0% (T4) suggests a metabolic plateau, consistent with previous reports on fungal fermentation of leaf-based substrates [12].

The progressive reduction in crude fiber from $14.88 \pm 0.76\%$ to a minimum of $10.32 \pm 1.18\%$ reflects the effective degradation of cellulose and hemicellulose by fungal cellulolytic enzymes. This outcome is nutritionally crucial, as high fiber is a great constraint in leaf meal inclusion for monogastric animals and fish [13]. Reduced fiber enhances nutrient accessibility and feed conversion efficiency, particularly for species with limited endogenous carbohydrase activity, such as Nile tilapia [14]. The stability of ash content across treatments further confirms that the fermentation process primarily reconfigures organic macronutrients without altering the total mineral matrix. A significant functional advantage was the substantial reduction of anti-nutritional compounds (Table II). The marked decrease in phytic acid, exceeding 50% in the T3 treatment compared to the control, is attributed to microbial phytase activity associated with *Rhizopus* spp. [15].

The reduction of tannins and saponins further improves protein digestibility and nutrient absorption, as these compounds are known to interfere with digestive enzymes and form indigestible complexes with dietary proteins and minerals [16]. Such reductions are consistent with reports on fermented legumes and plant materials, where enzymatic hydrolysis and

microbial metabolism collectively diminish polyphenolic and glycosidic anti-nutrients [17]. These findings are particularly relevant for aquaculture, where anti-nutritional factors severely limit the utilization of plant-based feed ingredients due to the limited detoxification capacity of fish [18].

Fermentation significantly enhanced the EAA profile of *S. androgynus* leaves, with important increases in lysine, leucine, isoleucine, valine, and methionine (Table III). These improvements are consistent with previous findings, according to which, microbial proteolysis during fermentation enhances amino acid availability and digestibility in plant-based feed ingredients [19]. The increase in lysine from 4.12 to 5.91 g/100 g protein is particularly relevant, as lysine is often the first limiting amino acid in plant-based aquafeeds, and the dietary requirement for Nile tilapia is approximately 5.0-5.5% of dietary protein [20]. This indicates that the fermented product approaches nutritionally relevant levels for practical feed formulation and could partially substitute conventional protein sources, such as soybean meal or other leaf meals, when combined with complementary ingredients to achieve amino acid balance.

Fermentation also induced a favourable shift in fatty acid composition, characterized by a reduction in saturated fatty acids, particularly palmitic acid, and a concomitant increase in unsaturated fatty acids, including oleic, linoleic, and α-linolenic acids (Table IV). These changes likely reflect selective microbial utilization of SFAs and the activity of lipolytic enzymes during fungal metabolism [21]. However, despite the improvements in fatty acid composition, the overall lipid content of leaf-based ingredients remains relatively low. Therefore, the contribution of these changes to total dietary lipid supply is likely limited, although they may enhance the ingredient's functional quality in balanced feed formulations.

The enrichment of linoleic and α-linolenic acids is nutritionally significant, as these fatty acids serve as essential precursors for long-chain polyunsaturated fatty acids and play key roles in membrane fluidity, inflammatory regulation, and metabolic processes [22]. In aquaculture nutrition, plant-derived sources rich in these fatty acids are particularly valuable for freshwater fish species with limited endogenous synthesis capacity. Consequently, the observed improvements in lipid profile contribute additional functional benefits to fermented *S. androgynus* leaves as a feed ingredient.

Across all evaluated parameters, the 1.5% ragi tempe treatment (T3) consistently achieved optimal improvements, with no statistically meaningful gains observed at the 2.0% dose. This indicates the presence of a dose-response plateau, a phenomenon widely reported in fermentation optimization studies, where excessive inoculum levels may lead to nutrient competition or metabolic inefficiencies [23]. Identifying this optimal dose is crucial for practical feed processing, as it balances nutritional enhancement with economic efficiency.

The results confirm that tempe-style fermentation effectively upgrades *S. androgynus* leaves into a nutritionally improved and functionally superior feed ingredient. These simultaneous improvements in protein quality, amino acid balance, lipid profile, and reduced anti-nutritional factors

enable partial replacement of conventional protein sources in sustainable mammalian and aquaculture feeds. These findings align with research emphasizing fermentation as a strategic tool to improve plant-based feed ingredients and support the transition toward more resilient and environmentally responsible feed systems.

From a process engineering standpoint, the results demonstrate that the fermentation system exhibits a clear dose-response behavior with an identifiable optimum at 1.5% inoculum. This has direct implications for scalability, as excessive inoculum levels do not yield proportional benefits and may increase production costs without improving output quality. The SSF approach used in this study is inherently scalable, as it can be adapted to tray, packed-bed, or bioreactor systems commonly employed in agro-industrial processing. Key parameters, such as temperature and moisture, fall within standard industrial SSF ranges, supporting process transferability. Furthermore, integration into feed manufacturing is feasible, as the fermented product can be directly incorporated after drying and milling, like conventional leaf meals. However, process optimization at a larger scale must consider heat buildup, oxygen transfer, and uniform inoculum distribution to ensure consistent product quality. These findings contribute to developing scalable fermentation-based feed ingredient production systems.

V. CONCLUSION

Tempe-style fermentation effectively transforms *S. androgynus* leaves from a fiber-heavy forage into a high-quality protein source. The obtained results show a clear "sweet spot" at a 1.5% ragi tempe dose, which maximized protein enrichment and slashed phytic acid by over 50%. Beyond this dose, the nutritional gains plateaued, meaning higher inoculum levels only add production costs without improving feed quality. This bioprocessing strategy significantly improves lysine levels and shifts the lipid profile toward beneficial unsaturated fatty acids, making the fermented meal a viable candidate for sustainable aquafeeds. The next logical step is to conduct in vivo feeding trials to determine the maximum inclusion level that can replace expensive fishmeal or soybean meal in practical diets.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request. No datasets were generated or analyzed during the current study.

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