



Research article

Nutritional and phytochemical enrichment of pineapple peel using *Aspergillus niger* through solid-state fermentation

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Abstract

Importance of the work: *Aspergillus niger* fermentation improves the nutritional and phytochemical value of underutilized pineapple peel (PP), highlighting its potential as an animal feed or additive.

Objectives: To evaluate the nutritional and phytochemical composition of PP fermented by *A. niger* using solid-state fermentation (SSF).

Materials and Methods: The PP was dried, supplemented with ammonium sulfate, sterilized, inoculated with *A. niger* strain ANL-AN01 and fermented for 120 hr. After fermentation, samples were analyzed for proximate composition, amino acid profile, total phenolic content and total antioxidant capacity.

Results: SSF of the PP using *A. niger* significantly ($p < 0.01$) reduced dry matter by 29.74% and nitrogen-free extract by 25.12%, while crude protein increased by 263.12%, crude fiber by 37.93% and ash by 38.73%, whereas crude fat remained unaffected ($p \geq 0.05$). The total phenolic content decreased by 19.47% ($p < 0.01$), whereas the total antioxidant capacity improved markedly by 119.44% ($p < 0.01$). Amino acid profiles were significantly ($p < 0.01$) enhanced, except for lysine and methionine ($p < 0.05$). Total amino acids, total essential amino acids and total non-essential amino acids increased by 102.85%, 68.69% and 124.88%, respectively. Glutamic acid, arginine and aspartic acid had the highest improvements among the individual amino acids.

Main finding: *A. niger* fermentation significantly enhanced the contents of protein and amino acids, as well as the antioxidant capacity of PP, demonstrating its novel potential as a nutrient-enriched animal feed and sustainable utilization strategy for agro-industrial byproducts.

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Introduction

Globally, the annual production of agricultural waste is predicted to reach 5 billion t from various sources, including fruit waste (Bharathiraja et al., 2017). The large number of agricultural byproducts that contribute to the issue of food waste raises serious concerns, as it may also result in numerous environmental and economic problems. The pineapple is considered by some as the queen of fruits, being the world's third most economically important tropical fruit (Chaudhary et al., 2019). Due to the increasing demand for pineapple products, production has increased, resulting in a great amount of waste (Roda and Lambri, 2019) that may lead to numerous environmental consequences, such as the generation of methane and nutrient leaching, that may reach bodies of water causing eutrophication, which is harmful to aquatic life (Sial et al., 2024).

About 60% of the weight of the pineapple fruit is considered residues and is rich in nutrients, bioactive compounds and polysaccharides, which makes it an ideal feedstuff for livestock and poultry (Roda and Lambri, 2019; Campos et al., 2020). Additionally, since pineapple waste is abundant, its utilization for feed development is highly favourable; thus, its usage has the potential to reduce expenditure on animal feed, which typically represents 65–75% of the total costs of production (Kirkpinar and Açıkgöz, 2018). Practical applications have already demonstrated that pineapple waste can successfully replace up to 50% of forage in ruminant diets (Idayanti et al., 2022) and serve as a viable feed ingredient for poultry and fish without compromising growth performance (Sukri et al., 2023). Regardless of its rich nutritional composition and economic advantage, pineapple waste is relatively low in protein, fat and mineral contents; therefore, it is imperative to enhance its nutritional value (Aruna, 2019).

A bioprocess called solid-state fermentation (SSF), based on fungal biotechnology, has provided promising results in nutrient enrichment of agro-industrial residues, including pineapple waste, making it a suitable technology for waste valorization which conforms with the concept of circular bioeconomy and the United Nations sustainable development goals (Ryan et al., 2019; Aslan and Sargin, 2023). Various studies have utilized filamentous fungi to convert agricultural waste into animal feed, with promising results (Ramli et al., 2005; Misra et al., 2006). Such is also the case for pineapple waste, where fermentation has been shown to significantly improve the nutrient content and bioactive profiles (Aruna,

2019; Paz-Arteaga et al., 2023). Among the various fungi used for such bioconversion, *Aspergillus niger* is an ideal candidate. It is a prolific producer of hydrolytic enzymes essential for degrading fibrous plant structure and is designated as a generally recognized as safe microorganism, thus ensuring its safety for animal feed applications (Schuster et al., 2002). While other studies have explored pineapple peel fermentation (Aruna, 2019; Omwango et al., 2013), there is limited information regarding the integrated analysis of amino acid profiling and phytochemical changes in pineapple peel supplemented with inorganic nitrogen during SSF. Hence, the present study aimed to evaluate the effects of *A. niger* SSF on the nutritional and phytochemical composition of pineapple peel (PP).

Materials and Methods

Cultivation medium and inoculum

MD-2 variety pineapple waste consisting of the fruit peel was collected from Mount Kitanglad Agri-Development Corporation, Valencia City, Bukidnon, Philippines. The collected PP sample was washed and cleaned with distilled water before being subjected to oven-drying using a natural circulation oven following Association of Official Analytical Chemists International (2006). After the moisture had been removed, the peel was subjected to milling using a Cyclotec™ 293 laboratory mill (FOSS; Hillerød, Denmark) to reduce particle size.

The microorganism used was a local isolate of *A. niger* (AN) obtained from a rotting onion bulb (*Allium cepa* L.) collected in Bukidnon, the Philippines. As a non-commercial strain, it has been designated with the laboratory accession number ANL-AN01. The isolate was identified based on its macroscopic and microscopic morphological characteristics according to the taxonomic keys of Klich (2002). Macroscopically, colonies on potato dextrose agar (PDA) exhibited rapid growth, initially presenting a white basal mycelium that transitioned to a dense layer of black conidial heads (carbon black) after 72 hr of incubation at 30°C. Microscopically, the isolate displayed smooth, thick-walled conidiophores terminating in globose vesicles, with biserial phialides covering the entire vesicle surface—characteristic of the AN group.

The isolate was cultured on PDA and incubated statically at 30°C for 72 hr. After incubation, pure cultures of AN were maintained in PDA slants at 4°C until inoculum preparation. Thereafter, the fungus was subcultured in PDA slants for 168 hr

before substrate cultivation. Tween 80 at 0.01% volume per volume and sterile distilled water were used to prepare spore suspension and were shaken using a vortex mixer. A hemocytometer was used to adjust the count of spores to approximately 1×10^6 spores/mL. Then, the prepared PP substrate was distributed among the assigned experimental groups: 1) negative control group (unfermented PP powder without ammonium sulfate (AS) addition); 2) positive control group (unfermented PP powder with AS addition); and 3) fermented PP with AS addition.

Solid-state fermentation of pineapple peel

All fermentation experiments were performed in triplicate ($n = 3$) for each treatment group. For the fermented and positive control groups, oven-dried PP in 50 g portions was weighed into separate 500 mL Erlenmeyer flasks to allow sufficient headspace for aeration. The moisture content and pH were adjusted using a standardized protocol to ensure reproducibility.

The appropriate amount of AS (3% weight per weight) was dissolved in distilled water. A strict 60% (volume per weight, v/w) moisture content was maintained based on calculating the volume of water added by subtracting the displacement volume of the ammonium sulfate. Due to the strong buffering capacity of PP and the difficulty of measuring the pH in a solid-state matrix, the required volume of 0.1 N NaOH to achieve a final pH of 4.5 was determined in a preliminary trial. In this trial, the substrate was mixed with the ammonium sulfate solution, homogenized and allowed to equilibrate for 1 hr before the pH measurement. The optimized volume of NaOH established in this preliminary step was then consistently applied to all treatment flasks.

The negative control group consisted of 100 g portions of oven-dried PP stored under identical conditions. The flasks were plugged with cotton wool and wrapped with aluminum foil for autoclaving at 121°C at approximately 103.4 kPa for 20 min. After cooling the medium at ambient temperature ($24 \pm 3^\circ\text{C}$), it was inoculated separately with 10% v/w spore suspension (1×10^6 spores/mL) of AN and incubated statically at 30°C for 120 h.

Proximate nutrient composition

Proximate analysis was performed to determine the dry matter (DM), crude protein (CP), crude fat, crude fiber (CF), ash and nitrogen-free extract (NFE) contents of the

samples following standard international protocols. First, the moisture content was determined according to method 930.15 (Association of Official Analytical Chemists International, 2006) by drying the samples in a natural circulation oven (UNB 308; Memmert; Schwabach, Germany) until a constant weight was reached. To ensure homogeneity and reduce particle size, the oven-dried samples were milled using a Cyclotec™ 293 Laboratory Mill (FOSS; Hillerød, Denmark). Then, representative samples were collected from each treatment for subsequent chemical analysis.

The crude fat content was determined using the Soxhlet extraction method according to standard 6492 (International Organization for Standardization, 1999). Crude protein was evaluated via the Kjeldahl method (International Organization for Standardization, standard 5983-2:2009 (International Organization for Standardization, 2009) using a block digester (Labtec™ DT208; FOSS Analytical A/S; Hillerød, Denmark) and steam distiller (Kjeltec™ 8100; FOSS Analytical A/S; Hillerød, Denmark). CF analysis followed the Weende method based on method 978.10 (Association of Official Analytical Chemists International, 2023), involving sequential cold and hot extraction steps. The ash content was determined based on incineration in a muffle furnace (Thermolyne™ F6010; Thermo Fisher Scientific; Waltham, MA, USA) according to method 942.05 (Association of Official Analytical Chemists International, 2006). Finally, following the methods of Rivera et al. (2022), the nitrogen-free extract (NFE) was calculated using the Equation 1:

$$\text{NFE} = 100 - (\text{Crude protein} + \text{Ash} + \text{Crude fat} + \text{Crude fiber}) \quad (1)$$

All nutrient compositions were calculated and reported on a DM basis.

Phytochemical analysis

The total phenolic content (TPC) of the samples was analyzed using Folin-Ciocalteu colorimetric assay (Ainsworth and Gillespie, 2007). The total antioxidant capacity (TAC) was determined using phosphomolybdenum colorimetric assay (Prieto et al., 1999). All the samples were pipetted to 96-well plates and their absorbance was measured using an ultraviolet-visible light microplate spectrophotometer (Multiskan™ SkyHigh; Thermo Fisher Scientific; Waltham, MA, USA).

Amino acid profiling

The samples underwent hydrolysis with 6N HCl at 120°C for 24 h, followed by filtration through a 0.45 µm filtration assembly. Then, they were analyzed after pre-column derivatization of amino acids using a high-performance liquid chromatography system equipped with an integrated autosampler (Prominence-i Plus; Shimadzu Corporation; Kyoto, Japan). O-phthalaldehyde was used to derivatize the primary amino acids, while fluorenylmethyl chloroformate was used for the secondary amino acids before injection. The chromatograms of the samples were compared to those of the standard amino acid mixture to match their retention time and quantification was achieved based on mathematical methods (Banerjee and Ghosh, 2016).

Statistical analysis

Data were analyzed using one-way Analysis of Variance appropriate for a completely randomized design. The results were expressed as mean ± SD of triplicate determinations ($n = 3$). Tukey's honestly significant difference test was used to compare significant differences among treatment means and a probability value of $p < 0.05$ was considered statistically significant and $p < 0.01$ was highly significant. All statistical analyses were performed using the IBM SPSS Statistics for Windows software (version 24.0; IBM Corp.; Armonk, NY, USA).

Results

Proximate nutrient composition of pineapple peel

Macroscopic observation confirmed extensive fungal colonization of the substrate. As shown in Fig. 1, the negative control (A) retained its original fibrous texture and light color. The positive control (B) had a golden-brown discoloration (likely indicative of a Maillard reaction induced by the autoclaving process), yet it retained its fibrous structure and showed no visible signs of microbial growth. In contrast, the AN-fermented substrate (C) had a distinct darker pigmentation and altered physical structure characteristic of extensive fungal biomass accumulation and sporulation following oven drying. All the parameters obtained in the proximate analysis are summarized in Table 1. Overall, AN highly significantly affected the PP's DM, CP, CF, Ash and NFE contents, but not the crude fat content ($p \geq 0.05$). The DM contents were decreased ($p < 0.01$) by AN by 29.74%. In contrast, the CP was increased markedly by 263.12% ($p < 0.01$) relative to unfermented PP. Similarly, the CF and ash contents increased by 37.93% and 38.73% respectively ($p < 0.01$), whereas the NFE declined by 25.12%. There was a slight but not significant ($p \geq 0.05$) decrease in the crude fat content.

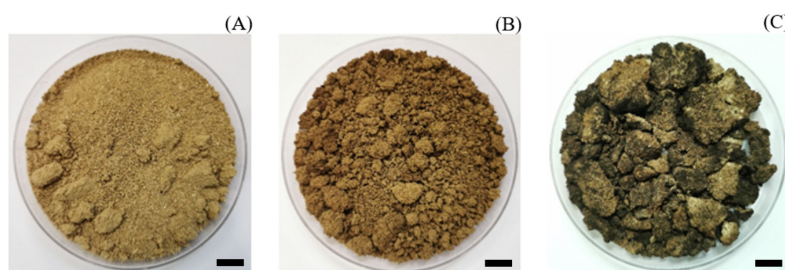


Fig. 1 Macroscopic appearance of pineapple peel substrates: (A) negative control without nutrient solution or inoculation; (B) positive control with nutrient solution, showing heat-induced golden-brown discoloration without fungal colonization; (C) AN-fermented substrate showing extensive mycelial growth and sporulation, where scale bar = 1 cm.

Table 1 Effect of *Aspergillus niger* solid-state fermentation on proximate composition (g/100g) of pineapple peel (PP)

Parameter	Treatment			<i>p</i> values
	NC	PC	AN	
Dry matter	94.28±0.53 ^a	90.44±0.17 ^b	66.24±1.62 ^c	< 0.001
Crude protein	4.23±0.30 ^c	11.80±0.17 ^b	15.36±0.71 ^a	< 0.001
Crude fat	0.88±0.07	0.97±0.04	0.80±0.09	0.065
Crude fiber	14.71±0.08 ^b	14.04±0.07 ^c	20.29±0.31 ^a	< 0.001
Ash	5.50±0.27 ^b	5.86±0.36 ^b	7.63±0.59 ^a	0.002
Nitrogen-free extracts	74.68±0.27 ^a	67.33±0.44 ^b	55.92±1.13 ^c	< 0.001

NC = negative control (unfermented PP); PC = positive control (unfermented PP with nutrient solution); AN = *Aspergillus niger* fermented PP. Values (mean ± SD, $n = 3$) within the same row with different lowercase superscripts are highly significantly different ($p < 0.01$).

Bioactive components of pineapple peel

Both the TPC and TAC (Table 2) were highly significantly influenced by AN fermentation. Specifically, the TPC decreased by 19.47% compared to the unfermented PP, whereas the TAC showed a marked increase of 119.44% ($p < 0.01$) relative to the negative control.

Amino acid profile of pineapple peel

The influence of AN on the PP's amino acid profile is presented in Table 3. Compared to the unfermented control

Table 2 Effect of *Aspergillus niger* solid-state fermentation on total phenolic content and total antioxidant capacity of pineapple peel (PP)

Parameter	Treatment			<i>p</i> values
	NC	PC	AN	
TPC (mg GAE/g)	1.13±0.06 ^a	1.21±0.04 ^a	0.91±0.04 ^b	< 0.001
TAC (mg AAE/g)	0.36±0.09 ^c	1.39±0.06 ^a	0.79±0.11 ^b	< 0.001

NC = negative control (unfermented PP); PC = positive control (unfermented PP with nutrient solution); AN = *Aspergillus niger* fermented PP.

Values (mean ± SD, $n = 3$) within the same row with different lowercase superscripts are highly significantly different ($p < 0.01$).

Table 3 Effect of *Aspergillus niger* solid-state fermentation on amino acid profile (g/100g) of pineapple peel (PP).

Parameter	Treatment			<i>p</i> values
	NC	PC	AN	
Methionine	0.26±0.02 ^{ab}	0.23±0.01 ^b	0.30±0.01 ^a	0.003
Lysine	1.25±0.08 ^b	1.67±0.04 ^a	1.09±0.16 ^b	0.001
Threonine	0.55±0.06 ^b	0.49±0.02 ^b	1.23±0.01 ^a	< 0.001
Histidine	-	0.19±0.01 ^b	0.32±0.01 ^a	< 0.001
Phenylalanine	0.49±0.03 ^c	0.59±0.03 ^b	1.04±0.01 ^a	< 0.001
Leucine	0.63±0.02 ^b	0.59±0.02 ^b	1.26±0.02 ^a	< 0.001
Isoleucine	0.42±0.02 ^b	0.38±0.02 ^c	0.77±0.01 ^a	< 0.001
Valine	0.52±0.03 ^b	0.50±0.02 ^b	0.94±0.00 ^a	< 0.001
Cysteine	0.24±0.03 ^b	0.24±0.02 ^b	0.49±0.01 ^a	< 0.001
Arginine	0.47±0.01 ^c	1.25±0.05 ^b	2.43±0.04 ^a	< 0.001
Tyrosine	0.33±0.02 ^b	0.32±0.02 ^b	0.74±0.01 ^a	< 0.001
Alanine	0.77±0.07 ^b	0.69±0.03 ^b	1.34±0.01 ^a	< 0.001
Proline	0.89±0.07 ^b	1.24±0.01 ^a	1.46±0.01 ^a	< 0.001
Glycine	0.70±0.09 ^b	0.75±0.02 ^b	1.47±0.11 ^a	< 0.001
Serine	0.85±0.12 ^b	0.85±0.05 ^b	1.82±0.07 ^a	< 0.001
Aspartic Acid	1.05±0.10 ^b	1.02±0.06 ^b	2.14±0.08 ^a	< 0.001
Glutamic Acid	1.07±0.08 ^b	1.02±0.06 ^b	2.50±0.02 ^a	< 0.001
TNEAA	6.39±0.54 ^b	7.39±0.24 ^b	14.37±0.18 ^a	< 0.001
TEAA	4.12±0.19 ^b	4.55±0.28 ^b	6.95±0.20 ^a	< 0.001
TAA	10.51±0.72 ^c	11.94±0.52 ^b	21.32±0.13 ^a	< 0.001

NC = negative control (unfermented PP); PC = positive control (unfermented PP with nutrient solution); AN = *Aspergillus niger* fermented PP; TNEAA = total non-essential amino acids; TEAA = total essential amino acids; TAA = total amino acids;

Values (mean ± SD, $n = 3$) within the same row with different lowercase superscripts are highly significantly different ($p < 0.01$).

group, there was a highly significant enhancement in almost all amino acids profiles for the PP fermented with AN, with moderate improvement for methionine ($p < 0.05$), while lysine was comparable to the control group. The TAA, TEAA and TNEAA increased by 102.85%, 68.69% and 124.88% respectively ($p < 0.01$), following fermentation. Specifically, individual amino acids of PP were affected by AN. Notably, glutamic acid, arginine and aspartic acid were the most enhanced amino acids followed by serine, glycine, proline, alanine, leucine, threonine, lysine, phenylalanine, valine, isoleucine, tyrosine while cysteine, histidine and methionine were the lowest in improvement.

Discussion

Proximate nutrient composition of pineapple peel

The observed DM contents loss aligned with other reports on fungal SSF. For example, AN reduced the DM content by 4–15% in various plant substrates after 3 d of fermentation (Jannathulla et al., 2017; Rajesh et al., 2010). Similarly, tea dregs fermented with AN and *Trichoderma koningii* showed DM content reductions of 24% and 12%, respectively (Cui et al., 2021). Longer fermentation durations, as in Wang et al. (2023), resulted in further DM content loss, consistent with the present study's results. This loss (approximately 30% in the current study), could be attributed primarily to fungal metabolic processes, particularly the breakdown of carbohydrates into CO₂, alcohols and volatile organic acids (Cui et al., 2021).

The CP content of the PP significantly increased after solid-state fermentation, consistent with Omwango et al. (2013), who reported a 178% protein enhancement in fermented pineapple waste, while Atere et al. (2016) observed a 161% increase using AN. This improvement could be attributed to fungal biomass growth during fermentation, as fungi utilize available carbon sources and synthesize protein-rich cellular material (Aruna, 2019). While *Trichoderma* species are preferred often for fiber degradation, AN was selected for the present study based on comparative evidence showing its superior capacity for protein synthesis; notably, Cui et al. (2021) demonstrated that AN fermentation yielded significantly higher total and essential amino acid profiles compared to *Trichoderma koningii*. Notably, the DM content loss (approximately 30%) contributed to a concentration effect that naturally elevated the nutrient percentages (Iyayi and Aderolu, 2004). However, the calculations showed that mass loss alone

could not account for the threefold protein increase, confirming that true fungal synthesis was the primary driver. Additionally, supplementation with ammonium sulfate likely contributed to protein enrichment by providing not only nitrogen but also sulfur, which is essential for the synthesis of sulfur-containing amino acids such as methionine (Walker and White, 2017).

No significant ($p \geq 0.05$) change in the crude fat content was observed, aligning with Atere et al. (2016), who reported stable fat levels in PP fermented with AN. Variations in other studies, such as the increase observed by Aruna (2019) using *Trichoderma viride*, may have been due to differences in substrate composition or the use of lipid-accumulating fungi (Zhu et al., 2021).

The CF content increased after fermentation with AN, consistent with findings by Cui et al. (2021) and Ibarruri et al. (2021), who reported elevated fiber, neutral detergent fiber and acid detergent fiber levels in fermented plant substrates. This increase was attributed primarily to the structural components of the fungal cell walls, particularly glucans and chitin. In filamentous fungi, chitin can comprise 10–15% of the cell wall dry weight, contributing to tensile strength and overall biomass (Osheroov and Yarden, 2010). The observed fiber increase in the present study supported evidence of fungal biomass accumulation, aligning with simultaneous increases in the CP and ash contents and reductions in the DM and NFE contents.

The ash content in the present study increased with fermentation using AN, which aligned with Cui et al. (2021) and Rajesh et al. (2010). This increase was partly due to the DM reduction due to fungal metabolic processes and enzyme activity. Notably, phytase production by the fungus contributes to ash content increase by breaking down phytate complexes and releasing bound minerals (Troesch et al., 2013).

The decrease in the NFE content observed in the present study indicated substantial carbohydrate utilization during fungal fermentation. Similar reductions were reported by Aruna (2019), Souza et al. (2016) and Omwango et al. (2013), highlighting the fungi's reliance on available sugars for energy and biomass synthesis. As heterotrophs, fungi oxidize organic substrates, such as pineapple peel, to support mycelial growth (Wang et al., 2023). Additionally, an inverse relationship between the carbohydrate and protein contents during fermentation would explain the decline in the NFE and a rise in the CP contents.

The nutritional changes observed in the present study have distinct implications for different livestock classes. The significant increase in the crude protein and fiber contents

makes the fermented product particularly suitable for ruminants, which can efficiently utilize the fiber fraction as an energy source while benefiting from the enriched microbial protein level. Conversely, for monogastric animals, the elevated fiber content and the finding that lysine and methionine remained as limiting amino acids might limit inclusion rates due to digestibility constraints, although the enhanced protein digestibility often associated with fermented products could mitigate this effect. From an environmental perspective, this process links PP valorization to circular bioeconomy concepts. By converting a high-volume agro-industrial residue into high-value feed, the process mitigates the disposal burden of the millions of tonnes of pineapple waste generated globally, returning nutrients to the food chain (Meena et al., 2022). Furthermore, although AN is generally recognized as safe (GRAS), specific strains must be screened to ensure the absence of mycotoxins, such as ochratoxin A and fumonisins, prior to industrial scale-up (Schuster et al., 2002). In addition, *in vivo* feeding trials and digestibility studies are required to validate these findings and to determine the optimal inclusion levels for specific animal diets.

Bioactive components of pineapple peel

Beyond macronutrient enhancement, the functional value of fermented feed is defined critically by its bioactive profile, which contributes to animal health and stress resilience. Phenolic compounds in plant-based feedstuffs exist in free and bound forms, with insoluble phenolics often complexed within the lignocellulosic matrix, limiting their bioavailability (Acosta-Estrada et al., 2014). SSF can enhance phenolic release through fungal enzymatic activity (Kleinert and Bart, 2008). However, in the present study, the TPC of the PP significantly decreased after SSF with AN. While some studies have reported increased phenolics after SSF, such as in pineapple peel fermented with *Rhizopus oryzae* (Rivera et al., 2022) or Sambucus berry pomace with AN (Dulf et al., 2015), others have observed notable reductions, depending on the fungal species and fermentation duration (Starzyńska-Janiszewska et al., 2022). These variations suggest that the phenolics that result during SSF are highly dependent on the species and substrate.

The reduction in the TPC may be attributed to fungal enzymes, such as peroxidases and laccases, which degrade phenolic compounds through oxidative mechanisms (Hur et al., 2014; Leite et al., 2021). These enzymes generate reactive oxygen species (ROS) that oxidize phenols and

under nutrient-depleted conditions, the free phenolics can be diminished further due to stress-induced polymerization and lignification, reducing their reactivity with the Folin-Ciocalteu reagent and thereby lowering the measurable TPC (Vattem et al., 2004; Nagarajan et al., 2020). However, the concomitant increase in the TAC (119.44%) necessitates a critical interpretation of these results. As reviewed by Amorati and Valgimigli (2015), the Folin-Ciocalteu assay is fundamentally an electron-transfer based method that measures reducing capacity rather than true radical-trapping antioxidant activity. Consequently, while polymerization reduces the availability of the specific reducing groups required for the Folin-Ciocalteu reaction, these transformed phenolic complexes may still retain potent radical-scavenging activity via hydrogen atom transfer mechanisms, which are better captured by total antioxidant capacity assays. Additionally, the fermentation process may have generated non-phenolic antioxidant compounds, such as organic acids or bioactive peptides, which contribute to the increased TAC independent of phenolic content (Hur et al., 2014).

The growing interest in natural antioxidants for animal nutrition is driven by concerns over the harmful effects of ROS on animal health and performance. PP, a fruit by-product, contains bioactive compounds with antioxidant potential (Nordin et al., 2023). SSF is a promising method to enhance this activity, as shown in the present study, where the TAC increased after fermentation with AN. Similar results were reported by Casas-Rodríguez et al. (2024) and Dulf et al. (2015) using fruit by-products fermented with AN. However, not all substrates respond similarly, with Starzyńska-Janiszewska et al. (2022) noting a decline in antioxidant activity in fermented moringa leaves, especially after extended durations, likely due to the polymerization of free phenolics and the reduced availability of active compounds (Nagarajan et al., 2020).

Amino acid profile of pineapple peel

While bioactive compounds protect health (Balasubramanian et al., 2023)), the ultimate nutritional quality of the feed depends on the specific balance of amino acids available for protein synthesis. Protein quality is determined by its amino acid composition, which is critical for evaluating feed potential. In the present study, AN fermentation improved the amino acid profile of the PP, consistent with the findings by Fan et al. (2022) and Shi et al. (2015), who reported increased essential amino acids in various fermented substrates. These enhancements reflect the ability of AN to generate single-cell proteins through mycelial biomass accumulation on

diverse substrates. However, variations in individual amino acid responses, such as the unexpected decrease in lysine in the present study, may result from protein degradation, polymerization or transformation during fermentation (Cui et al., 2021).

Conclusions

The PP was enriched based on the AN strain using SSF compared with unfermented PP. Notably, the CP, ash and CF contents increased considerably, while the DM and NFE contents decreased, driven by both true fungal biomass synthesis and a concentration effect resulting from the loss of DM. The amino acid contents improved significantly, although lysine remained a limiting factor. Notably, while the TPC decreased (possibly due to enzymatic oxidation or polymerization), the TAC increased substantially, likely due to the release of bound phenolics and fungal metabolites.

However, the present study represents a preliminary valorization step. The findings are limited by the absence of *in vivo* feeding trials to confirm digestibility, nutrient bioavailability and animal performance. Therefore, future research should focus on: 1) optimizing the fermentation conditions, including the fermentation time, moisture content and inorganic nitrogen sources; 2) measuring enzymatic parameters; and 3) conducting comprehensive animal experiments to assess safety and palatability, thereby validating the practical application of the fermented product in sustainable livestock production.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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