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Biotechnological valorization of almond leaf litter: Effect of solid-state fermentation with baker's yeast, palm wine yeast, and *Rhizopus oligosporus* on nutrient and anti-nutrient

Solomon M. Utiome[1], Egoamaka O. Egbune[1]*, Oghenehosero Magbegor[2], Fidelis I. Achuba[1]

[1]Department of Biochemistry, Faculty of Science, Delta State University, P.M.B. 1, Abraka, Nigeria.
[2]Department of Microbiology, Faculty of Science, Delta State University, P.M.B. 1, Abraka, Nigeria.

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This study investigates the enhancement of almond (*Terminalia catappa*) leaf litter's nutritional quality through solid-state fermentation (SSF) using Baker's yeast (BKYe), palm wine yeast (PWYe), and *Rhizopus oligosporus* (RO). Each inoculant (1 g BKYe, PWYe, and RO at 1.4 × 10² CFU) was homogenized in 10 mL phosphate buffer (pH 6) and applied to 10 g of almond leaf litter, fermented for 72 h at room temperature. Among treatments, PWYe showed the greatest improvement. Soluble protein increased by 53%, from 3.2 mg/g in unfermented almond leaf litter (UALL) to 4.9 ± 0.05 mg/g. Glucose concentration rose by 29.6%, from 2.7 ± 0.15 mg/g (UALL) to 3.5 ± 0.13 mg/g. PWYe also elevated phenolic (5.7 ± 0.1 mg GAE/g) and flavonoid (2.3 ± 0.01 mg QE/g) contents, while achieving the highest DPPH inhibition (9.7 ± 0.4%). Enzyme activities were significantly enhanced, with amylase (4.9 ± 0.11 U/g) and protease (3.8 ± 0.15 U/g) markedly higher in PWYe treatment. Anti-nutritional compounds (tannins, saponins, oxalates) were substantially reduced, particularly under PWYe fermentation. Overall, PWYe proved most effective in enhancing proteins, sugars, antioxidants, and enzymes while lowering anti-nutrients, establishing SSF with palm wine yeast as a promising approach to upgrade almond leaf litter as a sustainable feed resource.

Key words: Solid-state fermentation (SSF), tropical almond leaf litter, palm wine yeast (PWYe), Baker's yeast (BKYe), *Rhizopus oligosporus* (RO).

INTRODUCTION

Solid-state fermentation (SSF) is an increasingly popular biotechnological process that utilizes solid substrates for the cultivation of microorganisms. In SSF, microorganisms such as fungi, yeast, and bacteria grow on moist solid materials, resembling their natural growth conditions. This method is widely used to produce a variety of bioactive compounds, enzymes, and microbial biomass, offering significant advantages over submerged fermentation. It is particularly attractive for the fermentation of agricultural residues and waste materials, providing a cost-effective and environmentally sustainable approach for waste valorization (Blasi et al., 2023; Ufitikirezi et al., 2024). Among the many agricultural by-products that can be utilized in SSF, plant leaf litter is an often-overlooked substrate. Specifically, almond (*Terminalia catappa*) leaf litter, a by-product of almond cultivation, has considerable

*Corresponding author. E-mail: egbuneegoamaka@gmail.com.

potential for biotechnological applications. Almond trees, primarily grown for their edible seeds, generate large amounts of leaf litter that are typically discarded or burned. However, almond leaves are rich in various bioactive compounds, including phenolics, flavonoids, tannins, and saponins, which are known for their antioxidant, antimicrobial, and anti-inflammatory properties (Barral-Martinez et al., 2021; Özcan, 2023). This dual nature, nutrient-rich yet containing antimicrobial metabolites makes them an interesting substrate for SSF, provided suitable microorganisms are selected or pretreatments are applied. The management of this waste is a growing concern, as improper disposal methods can lead to environmental pollution. Therefore, transforming almond leaf litter into valuable products through SSF could not only help reduce waste but also provide an opportunity to enhance the nutritional and functional properties of the substrate.

The use of microbial cultures in SSF plays a crucial role in determining the quality of the final product. Microorganisms such as Baker's yeast (*Saccharomyces cerevisiae*), Palm wine yeast (*Saccharomyces* spp.), and the fungus *Rhizopus oligosporus* are commonly employed in SSF due to their ability to degrade complex organic matter and produce valuable metabolic by-products. These microorganisms can break down the cellulose and lignin present in plant materials, releasing soluble proteins, carbohydrates, and bioactive compounds such as phenolics and flavonoids. The fermentation process can also help degrade antinutritional factors such as tannins and saponins, which are commonly present in plant materials and can limit their nutritional value (Jeyakumar and Lawrence, 2022; Arbab Sakandar et al., 2023; Egbune et al., 2025a).

In addition to improving the nutritional profile, the fermentation of plant materials with these microbes has been shown to enhance their antioxidant properties. The increase in antioxidant activity during SSF is primarily attributed to the production of bioactive compounds such as polyphenols and flavonoids, which have been linked to various health benefits, including the protection against oxidative stress and inflammation (Abdel-Aty et al., 2022; De Villa et al., 2023; Tonukari et al., 2023). The enzymatic activities of amylases and proteases, which are produced during fermentation, further enhance the digestibility and bioavailability of the nutrients, making the final fermented product more suitable for use in animal feeds or as functional ingredients in food (Adebo et al., 2022; Egbune et al., 2025b).

Despite the known benefits of SSF, the use of almond leaf litter as a substrate for microbial fermentation remains underexplored. Previous studies have demonstrated the potential of SSF for enhancing the nutritional and functional properties of various plant residues, including cereal grains, legumes, and other agro-industrial by-products (Aganbi et al., 2023; Egbune et al., 2024 a,b; Egbune et al., 2025c). However, limited research has been conducted on the solid-state fermentation of almond leaf litter, and the effects of different microbial cultures on its biochemical composition, enzymatic activity, and antioxidant properties have not been fully investigated.

The present study aims to explore the potential of almond (*Terminalia catappa*) leaf litter as a substrate for SSF using three microbial cultures: Baker's yeast (Saccharomyces cerevisiae), Palm wine yeast (*Saccharomyces* spp.), and *Rhizopus oligosporus*. The objectives of this research are to evaluate the biochemical properties of the fermented almond leaf litter, including soluble protein content, glucose levels, reducing sugars, and total phenolic and flavonoid content. Furthermore, the study aims to assess the antioxidant activity of the fermented substrate using the DPPH radical scavenging assay, as well as to determine the enzymatic activities of amylase and protease. Additionally, the levels of antinutritional factors such as tannins, saponins, and oxalates will be measured to evaluate the safety and nutritional suitability of the fermented product.

This study is expected to contribute valuable insights into the potential of SSF as a method for enhancing the nutritional and functional properties of almond leaf litter. By exploring the impact of different microbial cultures on the fermentation process, the research aims to provide a sustainable approach for the utilization of almond leaf litter, offering a potential solution for waste management while also creating valuable products for animal feed, food industries, and other biotechnological applications.

MATERIALS AND METHODS

Material and starter culture

The almond leaf litter used in this study was collected from Site II, Delta State University, Abraka, Delta State, Nigeria. The almond leaf litter was identified and authenticated at the Department of Botany, Delta State University, Abraka, and assigned a voucher number (DELSUH016). Prior to fermentation, almond leaves were washed with distilled water, air-dried at 45 ± 2°C, and milled to reduce the concentration of antimicrobial compounds that could inhibit microbial growth. After milling, the leaf litter was stored at room temperature until it was required for further analysis. The strains of Baker's yeast, *Saccharomyces cerevisiae* (BKYe) and *Rhizopus oligosporus* (RO) utilized in this research were provided by PT Aneka Fermentasi Industri, Bandung, Indonesia, and were obtained through Tonukari Biotechnology Company. Palm wine yeast, *Saccharomyces spp.* (PWYe) was isolated from fresh palm wine collected in Edo State, Nigeria. The cultures were maintained on Actinomycetes agar media slants at 4°C until further use. Prior to experimentation, all microbial strains were revived by culturing in a sterile nutrient-rich medium and incubating at 30°C for 24 h.

Preparation and inoculation of microbial cultures for fermentation

To prepare the inoculum, small samples of each microbial strain were obtained from stored cultures and introduced into a sterile inoculum medium. This medium was formulated with the following components: 100 mL of distilled water, 0.02 g of glucose, 0.25 g of yeast extract, 0.5 g of tryptone, 0.5 g of NaCl, and 2 g of agar, thoroughly mixed to ensure uniformity. These ingredients provided

essential nutrients to support the growth and proliferation of yeast strains and *R. oligosporus*. The resulting microbial solutions were subsequently utilized to initiate the fermentation process effectively. This approach ensured a robust and consistent inoculum, promoting optimal microbial activity during fermentation.

Preparation of substrates for solid-state fermentation

One gram of each microbial inoculant (BKYe, PWYe, and RO, each at 1.4 × 10^2 CFU) was homogenized in 10 mL of phosphate buffer (pH 6). To this, 10 g of finely ground almond leaf litter was added, and the mixture was thoroughly homogenized. The biofermenters were then covered and left to ferment for a period of 72 h at room temperature. A set of control samples, which included dried and ground almond leaf litter without any microbial inoculum, and phosphate buffer without the presence of microorganisms, was prepared concurrently with the test samples for comparison.

Following the fermentation period, 6 g aliquots of the fermented cell-litter mixture were prepared for subsequent analysis. These samples were homogenized in 60 mL of distilled water using a mortar and pestle. A 10 mL portion of the resulting homogenate was transferred into test tubes and centrifuged at 10,000 rpm for 10 minutes. The supernatant obtained was designated as the crude extract and was carefully collected and stored for further analysis. Duplicate samples of the crude extracts were prepared for subsequent testing.

Biochemical parameters

Determination of bioactive compounds

The soluble protein content was quantified using the biuret method described by Gornall et al. (1949), which measures peptide bonds through the formation of a violet-colored complex. Reducing sugars in the samples were determined using the 3,5-dinitrosalicylic acid (DNS) colorimetric assay of Miller (1959), based on the reduction of DNS to a colored product under alkaline conditions. Glucose levels were measured enzymatically using the Randox glucose kit (Randox Laboratories Ltd, UK), which employs glucose oxidase and peroxidase reactions to produce a measurable color change.

Antioxidant activity was assessed by the DPPH radical scavenging assay, following the protocol of Hatano et al. (1988), where the reduction of the purple DPPH radical to yellow indicates antioxidant capacity. The total phenolic content was determined using the Folin–Ciocalteu method described by Singleton and Rossi (1965), which relies on the reduction of the Folin reagent by phenolics. The total flavonoid content was quantified using the aluminum chloride colorimetric method developed by Jia et al. (1999), where flavonoids form a stable complex with $AlCl_3$ detectable spectrophotometrically.

Tannins were estimated using the vanillin–HCl method of Deshpande et al. (1986), which detects condensed tannins via color formation. Saponins were analyzed by the method of Makkar et al. (2007), based on their ability to form froth and react with specific reagents. Oxalates were determined following the procedure by Libert and Franceschi (1987), which quantifies oxalate through precipitation and titration.

Enzyme extraction and assay

Crude enzyme extracts were obtained from the fermented samples by mixing each sample with distilled water at a ratio of 5 mL per gram of material. The mixture was agitated at 35 °C and 200 rpm for 20 min, followed by filtration through a double layer of gauze. The filtrate was then centrifuged at 3,000 rpm for 5 min, and the resulting supernatant was collected as the crude enzymatic extract.

These extracts were stored at 4 °C and used within 12 h for enzymatic activity assays to ensure stability. α-Amylase activity was measured using the method of Nouadri et al. (2010), which is based on the hydrolysis of starch and subsequent quantification of the released reducing sugars. Protease activity was determined following the procedure of Kunitz (1947), where casein serves as the substrate and the liberated amino acids are quantified spectrophotometrically.

Statistical analysis

All data obtained were subjected to statistical analysis. Values were reported as Mean and Standard deviation (Mean ± S.D) while one-way ANOVA was used to test for differences between treatment groups. The results were considered significant at p-values of less than 0.05, that is, at 95% confidence level ($p<0.05$). Turkey post hoc tool was used as basis for comparison for level of significance.

RESULTS

The solid-state fermentation of almond (*Terminalia catappa*) leaf litter using *Saccharomyces cerevisiae* (BKYe), palm wine yeast (PWYe), and *Rhizopus oligosporus* (RO) enhanced its biochemical profile. The unfermented control (UALL) had the lowest soluble protein content at 3.2 mg/g, which increased to 4.7 ± 0.05, 4.9 ± 0.05, and 4.3 ± 0.05 mg/g with BKYe, PWYe, and RO, respectively. The highest protein yield was recorded with PWYe, representing a 53% increase over UALL ($p < 0.05$) (Figure 1A).

Glucose concentration also rose from 2.7 ± 0.15 mg/g in UALL to 3.1 ± 0.12, 3.5 ± 0.13, and 2.8 ± 0.12 mg/g following fermentation with BKYe, PWYe, and RO, respectively. The highest increase (29.6%) was observed in the PWYe-treated sample (Figure 1B). In contrast, reducing sugar levels declined after fermentation, dropping from 1.8 ± 0.05 mg/g in UALL to 1.3 ± 0.02, 1.1 ± 0.03, and 1.7 ± 0.02 mg/g with BKYe, PWYe, and RO, respectively. This reduction, particularly in the PWYe treatment, likely reflects microbial utilization during growth (Figure 1C).The total phenolic content (TPC) of the unfermented almond leaf litter (UALL) was 4.3 ± 0.2 mg GAE/g. Following fermentation, TPC increased across all treatments. Samples fermented with *S. cerevisiae* (ALL+BKYe), palm wine yeast (ALL+PWYe), and *R. oligosporus* (ALL+RO) recorded TPC values of 5.6 ± 0.2, 5.7 ± 0.1, and 6.5 ± 0.3 mg GAE/g, respectively (Figure 2A). Total flavonoid content (TFC) also increased after fermentation. UALL had a flavonoid level of 1.2 ± 0.02 mg QE/g, while ALL+BKYe, ALL+PWYe, and ALL+RO recorded 1.9 ± 0.03, 2.3 ± 0.01, and 2.2 ± 0.03 mg QE/g, respectively (Figure 2B). The inhibition of DPPH free radicals increased with fermentation. UALL exhibited 6.7 ± 0.2% inhibition, while ALL+BKYe, ALL+PWYe, and ALL+RO showed 9.4 ± 0.3%, 9.7 ± 0.4%, and 10.6 ± 0.5% inhibition, respectively (Figure 2C). The amylase activity of the unfermented almond leaf litter (UALL) was 3.6 ± 0.12

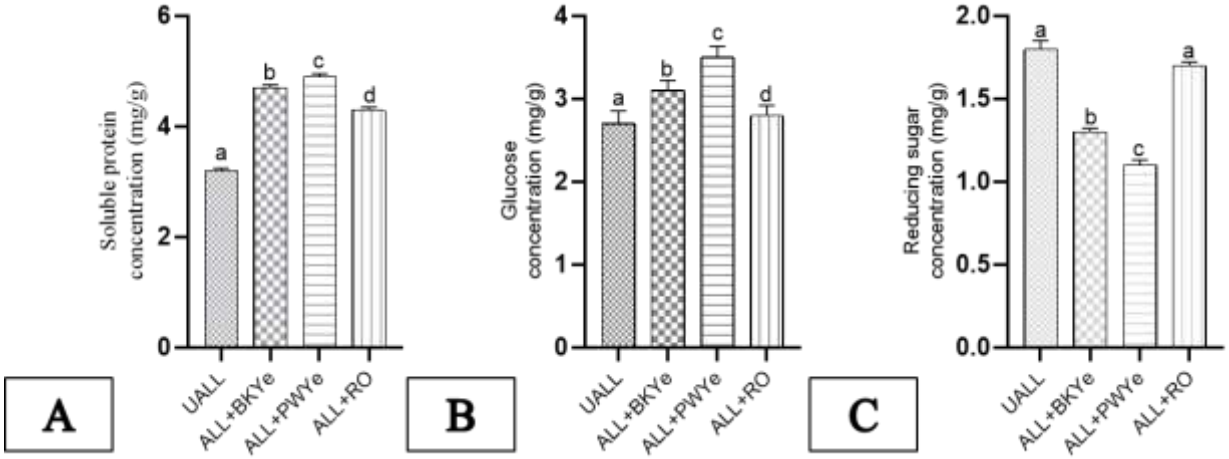

Figure 1. (A) Soluble protein content, (B) Glucose level, and (C) Reducing sugar level of state-state fermentation of almond leaf litter with Baker's yeast (BKYe), Palm wine yeast (PWYe), and *Rhizopus oligosporus* (RO). UALL = Unfermented almond leaf litter (control), ALL = Almond leaf litter. The bars displayed represent the average values ($\bar{x}$) along with the standard deviation (SD) derived from three separate measurements (n=3) of the parameter in question, presented as $\bar{x}\pm SD$. It's important to note that any differences indicated by different lowercase letters are statistically significant at $p<0.05$.

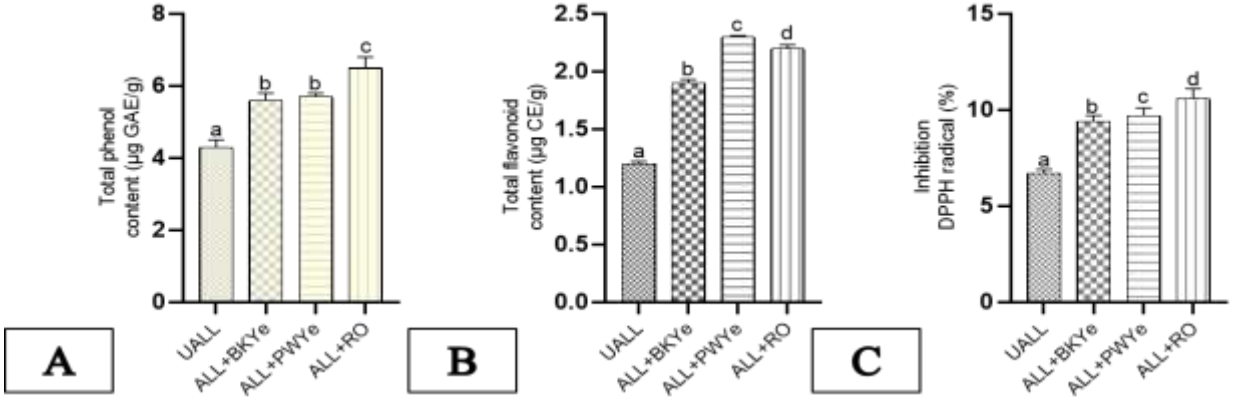

Figure 2. A) Total phenol content (TPC), (B) Total flavonoids content (TFC), and (C) Inhibition of DPPH Free Radicals of state-state fermentation of almond leaf litter with Baker's yeast (BKYe), Palm wine yeast (PWYe), and *Rhizopus oligosporus* (RO). UALL = Unfermented almond leaf litter (control), ALL = Almond leaf litter. The bars displayed represent the average values ($\bar{x}$) along with the standard deviation (SD) derived from three separate measurements (n=3) of the parameter in question, presented as $\bar{x}\pm SD$. It's important to note that any differences indicated by different lowercase letters are statistically significant at $p<0.05$.

U/g. Following fermentation, there was a significant increase in amylase activity across all treatments. The *S. cerevisiae* (ALL+BKYe) treatment increased amylase activity to 4.4 ± 0.13 U/g, while *R. oligosporus* (ALL+RO) and palm wine yeast (ALL+PWYe) treatments showed amylase activities of 4.5 ± 0.13 U/g and 4.9 ± 0.11 U/g, respectively. These increases were statistically significant compared to the unfermented control ($p < 0.05$) (Figure 3A).

The protease activity of UALL was 2.7 ± 0.05 U/g. Fermentation resulted in a significant increase in protease activity in all treatments. The *S. cerevisiae* (ALL+BKYe) treated samples exhibited a protease activity of 3.2 ± 0.11 U/g, while palm wine yeast (ALL+PWYe) and *R. oligosporus* (ALL+RO) showed protease activities of 3.8 ± 0.15 U/g and 3.7 ± 0.15 U/g, respectively. These values were significantly higher than the unfermented almond leaf litter (UALL) ($p < 0.05$) (Figure 3B).

The tannin content of the unfermented almond leaf litter (UALL) was 3.4 ± 0.05 mg TAE/g. After fermentation, tannin levels decreased across all treatments. The *S. cerevisiae* (ALL+BKYe) treatment resulted in a reduction

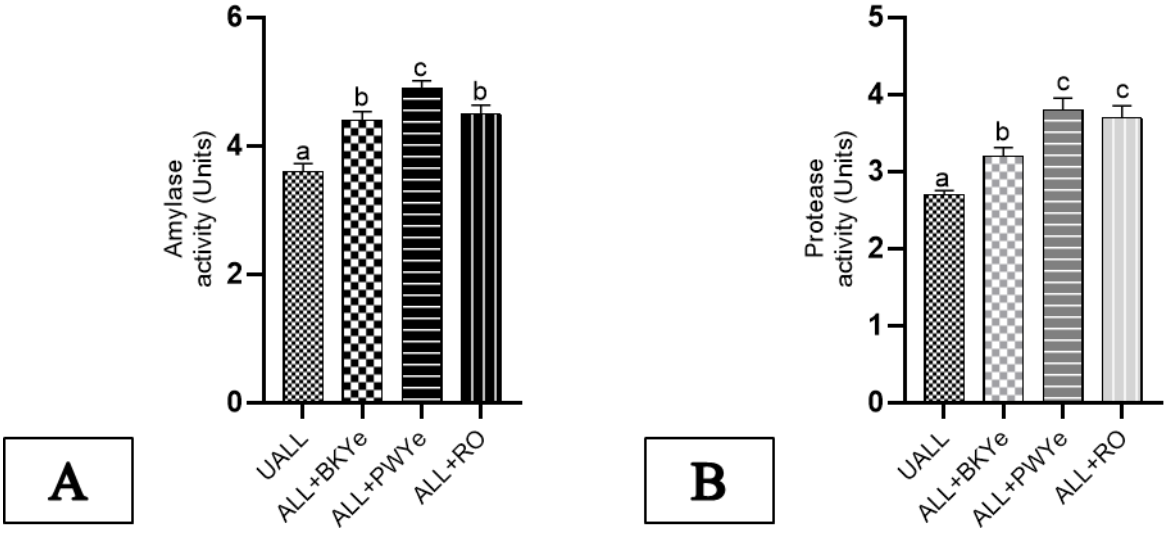

Figure 3. A) Amylase activity and (B) protease activity of state-state fermentation of almond leaf litter with Baker's yeast (BKYe), Palm wine yeast (PWYe), and *Rhizopus oligosporus* (RO). UALL = Unfermented almond leaf litter (control), ALL = Almond leaf litter. The bars displayed represent the average values ($\bar{x}$) along with the standard deviation (SD) derived from three separate measurements (n=3) of the parameter in question, presented as $\bar{x}\pm SD$. It's important to note that any differences indicated by different lowercase letters are statistically significant at $p<0.05$.

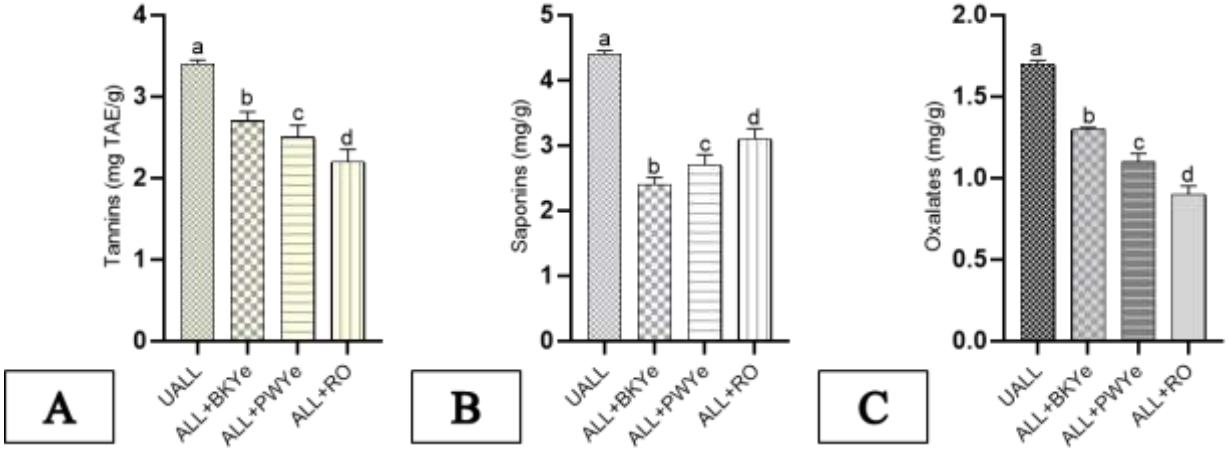

Figure 4. A) Tannins, (B) Saponins and (C) Oxalate level of state-state fermentation of almond leaf litter with Baker's yeast (BKYe), Palm wine yeast (PWYe), and *Rhizopus oligosporus* (RO). UALL = Unfermented almond leaf litter (control), ALL = Almond leaf litter. The bars displayed represent the average values ($\bar{x}$) along with the standard deviation (SD) derived from three separate measurements (n=3) of the parameter in question, presented as $\bar{x}\pm SD$. It's important to note that any differences indicated by different lowercase letters are statistically significant at $p<0.05$.

to 2.7 ± 0.11 mg TAE/g, while palm wine yeast (ALL+PWYe) and *R. oligosporus* (ALL+RO) treatments showed further decreases to 2.5 ± 0.15 mg TAE/g and 2.2 ± 0.15 mg TAE/g, respectively. These reductions were statistically significant compared to the unfermented control (UALL) ($p < 0.05$) (Figure 4A). The saponin content of UALL was 4.4 ± 0.05 mg/g. Following fermentation, there was a significant decrease in saponin levels in all treatments. The *S. cerevisiae* (ALL+BKYe) treated samples showed a decrease to 2.4 ± 0.11 mg/g, while palm wine yeast (ALL+PWYe) and *R. oligosporus* (ALL+RO) exhibited saponin contents of 2.7 ± 0.15 mg/g and 3.1 ± 0.15 mg/g, respectively. The decrease was statistically significant compared to UALL ($p < 0.05$) (Figure 4B). The

Table 1. Effects of solid-state fermentation of almond (Terminalia catappa) leaf litter with Baker's yeast (BKYe), Palm wine yeast (PWYe), and Rhizopus oligosporus (RO) on biochemical composition and enzymatic activities.

Parameter	UALL (Control)	BKYe (*S. cerevisiae*)	PWYe (*Saccharomyces* spp.)	RO (R. *oligosporus*)	Notable Outcome
Soluble protein (mg/g)	3.2 ± 0.05[a]	4.7 ± 0.05[b]	4.9 ± 0.05[c]	4.3 ± 0.05[d]	PWYe gave highest increase (↑53%)
Glucose (mg/g)	2.7 ± 0.15[a]	3.1 ± 0.12[b]	3.5 ± 0.13[c]	2.8 ± 0.12[a]	PWYe highest rise (↑29.6%)
Reducing sugar (mg/g)	1.8 ± 0.05[a]	1.3 ± 0.02[c]	1.1 ± 0.03[d]	1.7 ± 0.02[b]	Decline due to microbial utilization
Phenolics (mg GAE/g)	4.3 ± 0.2[a]	5.6 ± 0.2[b]	5.7 ± 0.1[b]	6.5 ± 0.3[c]	RO highest
Flavonoids (mg QE/g)	1.2 ± 0.02[a]	1.9 ± 0.03[b]	2.3 ± 0.01[c]	2.2 ± 0.03[c]	PWYe highest
DPPH inhibition (%)	6.7 ± 0.2[a]	9.4 ± 0.3[b]	9.7 ± 0.4[b]	10.6 ± 0.5[c]	RO strongest antioxidant activity
Amylase (U/g)	3.6 ± 0.12[a]	4.4 ± 0.13[b]	4.9 ± 0.11[c]	4.5 ± 0.13[b]	PWYe highest
Protease (U/g)	2.7 ± 0.05[a]	3.2 ± 0.11[b]	3.8 ± 0.15[c]	3.7 ± 0.15[c]	PWYe slightly higher
Tannins (mg TAE/g)	3.4 ± 0.05[a]	2.7 ± 0.11[b]	2.5 ± 0.15[c]	2.2 ± 0.15[d]	RO lowest
Saponins (mg/g)	4.4 ± 0.05[a]	2.4 ± 0.11[d]	2.7 ± 0.15[c]	3.1 ± 0.15[b]	BKYe lowest
Oxalates (mg/g)	1.7 ± 0.02[a]	1.3 ± 0.01[b]	1.1 ± 0.05[c]	0.9 ± 0.05[d]	RO lowest

Values are mean ± SD (n = 3). Different superscripts within a row ([a–d]) indicate statistically significant differences at $p < 0.05$. UALL = Unfermented almond leaf litter (control); BKYe = Baker's yeast (*Saccharomyces cerevisiae*); PWYe = Palm wine yeast (*Saccharomyces* spp.); RO = *Rhizopus oligosporus*.

oxalate content in UALL was 1.7 ± 0.02 mg/g. Fermentation resulted in a significant reduction of oxalate content across all treatments. The *S. cerevisiae* (ALL+BKYe) treatment resulted in a reduction to 1.3 ± 0.01 mg/g, palm wine yeast (ALL+PWYe) showed 1.1 ± 0.05 mg/g, and *R. oligosporus* (ALL+RO) exhibited the lowest oxalate level of 0.9 ± 0.05 mg/g. All decreases were statistically significant when compared to UALL ($p < 0.05$) (Figure 4C).

To provide a clearer overview of the comparative effects of the different microbial strains, the key biochemical and enzymatic changes observed during fermentation are summarized in Table 1. The results highlight distinct microbial contributions: PWYe was most effective in enhancing soluble proteins, glucose, amylase, protease, and flavonoids, while RO yielded the highest increases in total phenolics and antioxidant activity (DPPH), as well as the greatest reductions in tannins and oxalates. BKYe showed moderate improvements overall but was particularly effective in lowering saponin levels. This comparative summary emphasizes that the choice of microorganism can tailor the biochemical outcomes of almond leaf litter fermentation toward specific nutritional or functional targets.

DISCUSSION

In recent years, solid-state fermentation (SSF) has emerged as a promising biotechnological approach to enhance the nutritional and biochemical properties of agro-industrial residues. This method utilizes microorganisms to break down complex plant materials, facilitating the release of bioactive compounds and improving the digestibility of the substrate. The almond (*T. catappa*) leaf litter, often regarded as an underutilized agricultural waste, contains various anti-nutritional factors such as tannins, saponins, and oxalates that hinder its use as a potential feed ingredient. However, microbial fermentation has been shown to reduce these compounds, making the substrate more suitable for animal consumption. This study aimed to evaluate the impact of different microbial inoculants—*S. cerevisiae* (baker's yeast, BKYe), palm wine yeast (PWYe), and *R. oligosporus* (RO)—on the biochemical composition of almond leaf litter. The results provide insight into how fermentation can improve the soluble protein content, sugar profiles, phenolic and flavonoid contents, and enzymatic activities, while also mitigating the levels of anti-nutritional factors.

The present study demonstrates the potential of solid-state fermentation (SSF) to enhance the biochemical properties of almond (*T. catappa*) leaf litter through microbial action. Fermentation using *S. cerevisiae* (baker's yeast, BKYe), palm wine yeast (PWYe), and *R. oligosporus* (RO) resulted in notable changes in soluble protein content, glucose concentration, and reducing sugar levels when compared to the unfermented control (UALL).

A significant increase in soluble protein content was observed across all treatments, with the PWYe-inoculated sample (ALL+PWYe) exhibiting the highest level (4.9 ± 0.05 mg/g), representing a 53% increase over the control (3.2 mg/g) (Figure 1A). This enhancement can be attributed to microbial biomass accumulation and the secretion of extracellular enzymes such as proteases, which may have facilitated protein liberation from the plant matrix. Yeast fermentation, particularly with PWYe, likely induced metabolic activity conducive to protein synthesis or mobilization, consistent with findings from previous

studies where fermentation improved protein content in agro-wastes (Anigboro et al., 2022; Bibi et al., 2023; Ughe et al., 2025).

Glucose concentration also increased following fermentation, with ALL+PWYe showing the highest glucose yield (3.5 ± 0.13 mg/g), a 29.6% increase over UALL (2.7 ± 0.15 mg/g) (Figure 1B). This rise suggests effective enzymatic hydrolysis of complex carbohydrates into fermentable sugars. Palm wine yeast, being a diverse consortium of *Saccharomyces* and non-*Saccharomyces* species, may possess broader enzymatic capabilities, enhancing cellulose and hemicellulose breakdown (Sumerta et al., 2024). The moderate increases observed with BKYe and RO further support the capacity of these microbes to hydrolyze polysaccharides, though to a lesser extent than PWYe (Lee et al., 2024).

Conversely, a general decline in reducing sugar levels was recorded after fermentation (Figure 1C). UALL had the highest reducing sugar concentration (1.8 ± 0.05 mg/g), while ALL+PWYe had the lowest (1.1 ± 0.03 mg/g). The reduction may be due to the consumption of reducing sugars by fermenting microbes as carbon sources to support growth and metabolism (Zhao et al., 2023). The relatively lower decline observed in the RO-treated sample (1.7 ± 0.02 mg/g) could indicate a slower uptake rate or less aggressive fermentation process compared to yeast-based treatments (Seo et al., 2020).

The results indicate a significant increase in the total phenolic content (TPC) of almond leaf litter after fermentation with different microorganisms. The TPC values of the fermented samples (5.6–6.5 mg GAE/g) were higher than that of the unfermented sample (UALL: 4.3 mg GAE/g), suggesting that fermentation enhanced the extraction or synthesis of phenolic compounds. This finding aligns with previous studies that have shown the positive impact of fermentation on phenolic content in plant materials, such as studies on fermented rice husks and other agricultural residues (Egbune et al., 2022, 2023). Similarly, the increase in TPC in the present study could be attributed to the activity of microorganisms, which might hydrolyze the cell wall components, releasing more phenolic compounds from the plant matrix (Wang et al., 2022). Among the fermentation treatments, *R. oligosporus* (ALL+RO) resulted in the highest TPC, which is consistent with the findings of Šelo et al. (2023), who demonstrated that Rhizopus species could enhance the phenolic content of fermented substrates more effectively than other microorganisms.

For total flavonoid content (TFC), a marked increase was observed across all fermented treatments compared to UALL. The TFC values of 1.9–2.3 mg QE/g in the fermented samples suggest that fermentation promoted the synthesis or release of flavonoids. Previous studies, such as those by Zhao et al. (2021), have reported similar increases in flavonoid content upon fermentation of plant-based materials. This increase could be linked to the activity of enzymes like glycosidases, which hydrolyze flavonoid glycosides into their aglycone forms, thus enhancing the bioavailability of flavonoids (Chen et al., 2022). The highest flavonoid content was observed in the palm wine yeast (ALL+PWYe) treatment, which may indicate a more efficient conversion of flavonoid precursors compared to other microbial strains, as noted in similar studies (Edema-Eyena et al., 2023; Egbune et al., 2024a,b).

The inhibition of DPPH free radicals, an indicator of antioxidant activity, also increased significantly in all fermented samples. The increase in antioxidant activity, with ALL+RO showing the highest inhibition (10.6%), suggests that fermentation enhanced the bioactivity of the almond leaf litter. This is in agreement with studies that have shown that fermentation can increase the antioxidant potential of plant materials by releasing bioactive compounds or by modifying them into more potent forms (Leonard et al., 2021). The observed increase in DPPH inhibition for ALL+RO was particularly noteworthy and supports findings from previous research (Wu et al., 2022), where fermentation with Rhizopus species was shown to enhance antioxidant properties in plant residues.

The amylase activity in the fermented samples increased across all treatments, with *S. cerevisiae* (ALL+BKYe), palm wine yeast (ALL+PWYe), and *R. oligosporus* (ALL+RO) showing a 22.2, 36.1, and 25% increase, respectively, compared to the unfermented control (UALL). The highest amylase activity was recorded in the palm wine yeast-treated sample (ALL+PWYe), suggesting that this microorganism was particularly effective at enhancing the breakdown of starches in the almond leaf litter. Similar results have been reported in other studies, where fermentation with *S. cerevisiae* increased amylase activity in various lignocellulosic biomass substrates (Khanpanuek et al., 2022).

Similarly, protease activity showed an increase in all fermented samples, with palm wine yeast (ALL+PWYe) exhibiting the highest increase (41.5%) in protease activity. This suggests that Saccharomyces spp. and *R. oligosporus* were effective in secreting proteases, which facilitated the breakdown of proteins in almond leaf litter. Other studies have also highlighted the potential of *R. oligosporus* to enhance protease production during fermentation of plant materials (Egbune et al., 2023). The enhanced protease activity in the palm wine yeast fermentation could be attributed to the yeast's ability to produce extracellular proteases during fermentation, which has been reported to increase the bioavailability of protein in various substrates (Christensen et al., 2022; Egbune et al., 2024a).

The decrease in tannin content observed in all fermented samples compared to the unfermented almond leaf litter (UALL) suggests the effectiveness of fermentation in reducing the levels of these potentially toxic compounds. Tannins are known for their astringency and ability to bind proteins, which can interfere with the digestion and absorption of nutrients in animals (Besharati

et al., 2022). In this study, fermentation with *S. cerevisiae* (ALL+BKYe), palm wine yeast (ALL+PWYe), and *R. oligosporus* (ALL+RO) resulted in significant reductions in tannin content, with the lowest reduction observed in ALL+RO. This is consistent with previous studies where microbial fermentation has been shown to degrade tannins, thereby improving the digestibility and nutritional value of plant materials (Chen et al., 2021; Ke et al., 2022; Utiome and Achuba, 2025). The reduction in tannins may be attributed to the metabolic activities of the microbes involved, particularly their ability to hydrolyze tannin-polymer complexes, which further enhances the feed value of the almond leaf litter.

Similarly, the significant decrease in saponin content following fermentation aligns with previous research indicating that microbial fermentation can reduce saponin concentrations. Saponins are known to have anti-nutritional properties, including the inhibition of digestive enzymes and interference with nutrient absorption (Singh et al., 2023). The fermentation treatments, especially those involving *R. oligosporus* (ALL+RO), which exhibited the lowest saponin levels, demonstrate the potential of fungal fermentation in breaking down saponins and reducing their anti-nutritional effects. This result is in line with earlier studies on the fermentation of various plant materials, where microbial action, particularly from yeasts and fungi, has been shown to detoxify or lower the saponin content, thereby improving feed quality (Jeyakumar and Lawrence, 2022; Kholif, 2023).

The significant reduction in oxalate content across all fermentation treatments is also noteworthy. Oxalates are considered anti-nutrients because they can bind calcium and form insoluble salts, leading to reduced mineral bioavailability (Zayed et al., 2025). The decrease in oxalate levels following fermentation with *S. cerevisiae*, *R. oligosporus*, and palm wine yeast (ALL+PWYe) indicates the microbial ability to degrade or transform oxalates into less harmful compounds. Previous studies have demonstrated the potential of fermentation to decrease oxalate content in plant-based substrates, which could enhance the overall nutritional quality of almond leaf litter as a feed ingredient (Hashimoto et al., 2024; Mashiah et al., 2025; Chisoro et al., 2025).

Conclusion

This study demonstrates that solid-state fermentation (SSF) using *S. cerevisiae* (BKYe), palm wine yeast (PWYe), and *R. oligosporus* (RO) significantly enhances the biochemical properties of almond leaf litter. Fermentation improved soluble protein content, sugar profiles, phenolic and flavonoid contents, antioxidant activity, and enzymatic activities, while reducing anti-nutritional factors like tannins, saponins, and oxalates. Among the treatments, PWYe (ALL+PWYe) showed the most significant improvements in nutritional components and enzymatic activities, indicating its superior efficacy. Microbial fermentation enhances the nutritional value of almond leaf litter, making it more suitable for animal feed. These findings highlight the potential of SSF to valorize agricultural residues, contributing to sustainable feed production and waste reduction. Further optimization of fermentation conditions and exploration of other microbes could enhance these benefits.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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