

Valorisation of Fermented Millet Beverage Lees: A Viable Supplementation Alternative for Animal Feed Formulation

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Research Article

Keywords: Millet lees, Animal feed, physicochemical characteristics, FTIR spectroscopy, sustainability, circular economy

Posted Date: April 23rd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6263796/v1>

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Version of Record: A version of this preprint was published at Waste and Biomass Valorization on August 7th, 2025. See the published version at <https://doi.org/10.1007/s12649-025-03223-1>.

Abstract

The alcoholic beverage industry has endorsed significant progress with a retail valuation surpassing \$1.5 trillion in 2023. This study aims to evaluate the potential of valorising millet lees, a by-product of millet-based fermented beverages, as a sustainable ingredient for animal feed. Pearl millet (*Pennisetum glaucum*) and finger millet (*Eleusine coracana*) were preferred. Comprehensive proximate study was attended to determine the moisture, fat, ash, fibre, protein, and carbohydrate content in both fermented and unfermented millet lees. Functional characteristics were determined to evaluate the practical application of millet lees in feed formulations. Additionally, physicochemical qualities were analysed to understand the storage and handling requirements of the feed. Fourier Transform Infrared spectroscopy was employed to investigate the molecular arrangement and structural changes occurring in the millet lees all along fermentation. The verdicts plan that incorporating millet lees into animal feed formulations, also aligns with the principles of a circular economy. This study highlights the feasibility of millet fermented beverage lees as a sustainable and healthy feed ingredient, providing to the advancement of global sustainability efforts in the food and beverage industry.

1. Introduction

The global market for alcoholic beverages has grown rapidly in recent decades, with predicted worth of the industry surpassing \$1.5 trillion by 2023 (The Business Research Company, 2024). Various products, including beer, wine and spirits (and other types of fermented liquor), are produced in this sector, each with its own unique characteristics that have various cultural significances in different parts of the world. Microorganisms, such as yeast and bacteria, are an important component of fermented alcoholic drinks as they help break down fermentable substrates like grains and fruits. The process produces lees, that are dead yeast cells, leftover matters, and coarse matter that remain after fermentation. Despite being traditionally considered as waste, lees are now acknowledged for their potential as a healthy piece in animal feed (Martn and others, 2022). This is unexpected. The current focus on millets, a group of tough small-seeded grains, is on account of their ability to handle in harsh atmospheres and their extreme nutritional importance. There are two types of millets, the pearl millet (*Pennisetum glaucum*) and finger miller (*Eleusine coracana*). Pearl millet is an important crop in drought-diverse regions due to its high protein content and ability to thrive in these regions. It is also highly valued for its irrigation capabilities. In cultivating nations, food security is considerably impacted by the high calcium content and storage ability of finger millet (Patni e al, 2017). Fermented millet-based drinks are a common food item in many cultures, acknowledged for their unique flavors and health benefits. A nutrient-rich alternative, millet lees, is produced during the production of these beverages. With their high levels of protein, fibres, vitamins and mineral making them a good choice for many animal feed formulations. By combining millet leas into animal feed, we can not only promote sustainable waste management but also support actions like the circular economy and zero-waste projects. The nutritional and functional features of millet lees must be sufficiently implicit to efficiently utilize them for this purpose. To meet the nutritional needs of livestock, it is important to examine the proximate composition, such as protein, fat, fibers, moisture, and ash (Nani and others, 2024).

This research aims to thoroughly assess the proximate composition, functional properties, physicochemical characteristics, and FTIR profiles of millet lees. By doing so, the study seeks to establish the feasibility of using millet fermented beverage lees as a sustainable, nutritious ingredient in animal feed. This approach supports circular economy principles and zero-waste practices, contributing to waste reduction while creating commercial opportunities in sustainable feed production. By transforming waste into valuable resources, this research could bring significant environmental and economic benefits, advancing global sustainability efforts.

2. Materials and Methods

2.1. Beverage Preparation

Finger millet (*Eleusine coracana*), pearl millet (*Pennisetum glaucum*), and sugar and Brewer's yeast were purchased from the local market in Thanjavur (10.7870° N, 79.1378° E). The grains were thoroughly cleaned and stored in a refrigerator at 4 °C or further analysis. 150 g of each millet were separately cleaned, washed, and soaked in water for 12 hours. 300 g of sugar was dissolved in 1.5 L of water and boiled for 30 minutes. The boiled solution was cooled to 25°C, and 5 g of baker's yeast was added. The finger millet and pearl millet grains were separately added to the sugar solution and mixed well. The mixtures were transferred to sterile fermentation vessels and incubated at 25°C for 14 days. After fermentation, the beverages were filtered, transferred to bottles, and matured for an additional 14 days at 25°C. The separated lees were collected and spread evenly on stainless steel trays. The pearl and finger millet lees were dehydrated in a hot air stove at 50°C for 12 hours. The dried lees were pulverized into a fine powder utilizing a laboratory mixer. The pulverized lees powder was stored in airtight containers at 4°C for further analysis.

2.2. Proximate Analysis

Based on the AOAC (2005) methods, the experiment was conducted with three replications to measure moisture content, protein, fat, and ash content, while carbohydrates were calculated using the difference method. Moisture content was determined using the hot air oven method, place 5 grams of the sample were dried at 130°C for 2 hours. Ash content was analysed using the muffle furnace method, with 1 gram of the sample being incinerated at 600°C for a specific duration to ensure the complete decomposition of organic matter. The fat content of the samples was determined using the Soxhlet extraction method. The crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25.

2.3. Bulk density

For bulk density, the procedure was adapted from Feng and others. (2018). In triplicate, dry and free-flowing powdered samples were poured into a 100 ml graduated plastic cylinder without compacting. The weight of the sample was measured, and The BD were determined as per weight of the sample per unit volume of sample.

2.4. Tapped density

The tapped density procedure followed the guidelines interpreted by Smith and others. (2020). In triplicate, the dry and free-flowing powdered samples were transferred into a 100 ml graduated plastic cylinder and tapped 100 times using a tapping device or manually, guaranteeing consistent force and height. The volume of the sample after tapping was calculated. After tapping the change in volume of the powdered samples were measured and determined using the volume of powdered samples after tapping and weight.

2.5. Carr Index and Hausner Ratio

It is a mechanism that occurs due to vibration, it can be calculated from bulk and tapped density using the formula.

$$CI = 100 \times ((\rho_t - \rho_b) / \rho_t) \quad (1)$$

$$HR = \frac{\rho_t}{\rho_b} \quad (2)$$

Where ρ_t is a tapped density and ρ_b is a bulk density.

2.2. Colour measurement

The colour values, L, a, and b of the powdered samples were determined using Hunter color lab, where L* is lightness, a* is the degree of redness or greenness, and b* is yellowness or blueness.

2.3. Functional properties

The water holding capacity (WHC) and oil holding capacity (OHC) of the powdered samples were determined using the method described by Beuchat (1977). For this, 1g of the samples were weighed and added to a 10 ml centrifuge tube containing either water or refined oil. The samples were then centrifuged at 2000 rpm for 30 minutes. After centrifugation, the supernatant was removed, and the tube containing the pellets was weighed. The WHC and OHC were calculated using the formula:

$$WHC/OHC = ((Wt. \text{ of bottle after decanting} - wt. \text{ of dry bottle}) - \text{total flour}) / \text{Total flour wt. (g)}$$

The solubility and swelling power of the samples were determined following the procedure outlined by Leach et al. (1959). For solubility, 0.5g of flour was mixed with 15 ml of distilled water and heated in a water bath at 90°C for 30 minutes. The mixture was then cooled to ambient temperature and centrifuged at 4000 rpm for 15 minutes. The supernatant was decanted into a pre-weighed Petri dish and dried at 100°C for 20 minutes. The weight of the dried supernatant was measured, and the solubility (%) was calculated using the formula:

$$\text{Solubility(\%)} = \frac{\text{Dry supernatant weight}}{\text{weight of dry sample}} * 100 = (3)$$

For swelling power, the weight of the sediment was also noted. The swelling power (g/g) was calculated using the formula:

$$\text{Swelling power (g/g)} = \frac{\text{Sediment wt} * 100}{\text{dry sample} * (100 - S\%)} \quad (4)$$

2.4. Extraction method

1g of powdered samples were added to 4 ml of methanol and ultrasound for 10 mins at a power of 40W. Keep the powdered sample for 1 hr in the water bath at 30 °C. Precisely preweight the Eppendorf tube and transfer it to the cold-water bath which can be used for further analysis.

2.5. Total phenolics content

The total phenolic content of the extracts was determined using the Folin-Ciocalteu method as detailed by Murugan et al. (2018). In this method, 50 µL of the extract in triplicate was mixed with 450 µL of distilled water in a test tube. A Folin-Ciocalteu (FC) reagent was prepared by diluting it 1:1 with distilled water. To the test tube, 0.5 mL of FC reagent was added, followed by 2.5 mL of a 20% sodium carbonate solution. The test tubes were then kept in the dark for 40 minutes. After incubation, the absorbance of the mixture was determined at 725 nm against a blank reagent to determine the total phenolic content.

2.6. Flavonoids content

The flavonoid content in the samples was determined using the colorimetric method described by Zhang et al. (2013). In this process, 100 µL of the extract was mixed with 100 µL of distilled water, followed by the addition of 150 µL of sodium nitrite solution. The mixture was incubated at room temperature for 6 minutes. Subsequently, 150 µL of aluminium chloride solution was added, and the combination was further incubated for 6 minutes. After incubation, 2 mL of sodium hydroxide solution was added, and the solution was vortexed thoroughly. The combination was allowed to stand at room temperature for 15 minutes, and the flavonoid content was determined by measuring the absorbance at 510 nm against a blank reagent.

2.7. Anti-oxidant assay

2.7.1. DPPH- Scavenging assay

The DPPH radical scavenging activity of the extracts was determined using the method described by Blois (1958). In this process, the extracts were prepared at various concentrations (5 µL, 10 µL, 15 µL, 20 µL, 25 µL). A DPPH solution was prepared by dissolving 4 mg of DPPH powder in 100 mL of 99% methanol. To each test tube, 3 mL of the DPPH solution was added, and the tubes were observed in the dark for 30 minutes. The inhibition of the DPPH radical was measured by determining the absorbance at 517 nm against a blank reagent. The percentage of DPPH radical inhibition was calculated using the formula

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance A} - \text{Absorbance Sample})}{\text{Absorbance A}} * 100 \quad (5)$$

2.8. Fourier Transform Infrared (FTIR) spectroscopy

The spectral measurements were conducted using a Shimadzu IR Tracer-100 FTIR spectrophotometer. Samples were located on a diamond crystal surface and secured with a sample clamp. The spectral measurements were recorded over a range of 4000 to 400 cm⁻¹, targeting the identification of functional groups based on their characteristic vibration frequencies. For each sample, 45 scans were acquired to generate interferograms, ensuring a high signal-to-noise ratio. Background scans were recorded and deducted from the sample scans to eliminate any impedance from environmental or instrumental noise. The spectral features acquired were analysed to guarantee they were consistent with the chemical nature of the samples.

2.9. Statistical Analysis

The data on proximate and physicochemical properties of pearl millet lees and finger millet lees were analysed using SPSS software. One-Way ANOVA: Conducted to compare means between the two types of millet lees. The null hypothesis (H₀) posited no significant differences, while the alternative hypothesis (H₁) suggested at least one difference. Significance was determined at a 5% level ($p \leq 0.05$). Post-Hoc Tests: Applied if significant differences were found ($p \leq 0.05$) to identify specific differences between groups. Descriptive statistics, including means and standard deviations, were calculated and reported for each parameter to support the analysis.

3. Result and discussion

3.1 Proximate Analysis

Table 1
The proximate analyses of fermented and unfermented millets

Parameters	UPM	FPML	UFM	FFML
Moisture Content (%)	8.16 ± 0.28 ^c	8.56 ± 0.05 ^b	8.46 ± 0.05 ^b	9.33 ± 0.08 ^a
Fat (%)	4.33 ± 0.28 ^a	2.14 ± 0.12 ^b	1.33 ± 0.28 ^c	1.35 ± 0.10 ^c
Ash (%)	1.33 ± 0.28 ^c	2.40 ± 0.08 ^b	2.54 ± 0.04 ^b	3.12 ± 0.09 ^a
Crude Fiber (%)	2.52 ± 0.06 ^d	12.33 ± 0.29 ^b	3.48 ± 0.02 ^c	13.43 ± 0.01 ^a
Protein (%)	11.21 ± 0.02 ^b	13.39 ± 0.04 ^a	7.51 ± 0.23 ^d	7.83 ± 0.03 ^c
Carbohydrates (g)	72.31 ± 0.03 ^b	60.96 ± 0.16 ^d	76.44 ± 0.05 ^a	64.84 ± 0.03 ^c

The proximate composition of fermented and unfermented millet samples is demonstrated in Table 1. Moisture content increased a little after fermentation, aligning with prior studies that indicate fermentation retains more moisture (Gopalan et al., 2021; Sharma et al., 2019). Fat content declined in both millets post-fermentation, consistent with reports of fat decline during fermentation (Mukherjee et al., 2022). Ash content rose following fermentation, likely on account of enhanced mineral availability

(Reddy et al., 2020). Crude fiber presented a solid increase, supporting findings by Singh et al. (2021), who noted similar trends in fermented cereals. Protein content also increased, which could be from enhanced digestibility all along fermentation (Sharma et al., 2018). Carbohydrate levels decreased post-fermentation, consistent with reports of carbohydrate decline on account of fermentation (Chauhan and others., 2020). Significant differences ($p < 0.05$) were seen across all measured parameters (moisture, fat, ash, fiber, protein, and carbohydrates), highlighting the influence of formulation and prepare in determining product attributes. Fermented pearl millet (FPM) and finger millet (FFM) lees show promise as animal feed on account of their high moisture, improved mineral content, and improved protein levels. However, proper drying is needed to prevent spoilage. While the high crude fiber benefits ruminants, adaptations can be required for non-ruminants to secure balanced nutrition.

Table 2
Functional Properties of fermented and unfermented millets

Parameters	UPM	FPML	UFM	FFML
Water Holding Capacity (d.b)	1.56 ± 0.06 ^b	2.15 ± 0.25 ^a	1.78 ± 0.02 ^b	1.81 ± 0.24 ^b
Oil Holding Capacity	1.34 ± 0.05 ^b	0.94 ± 0.02 ^d	1.48 ± 0.02 ^a	1.11 ± 0.02 ^c
Solubility (%)	14.33 ± 0.28 ^c	17.46 ± 0.02 ^a	11.21 ± 0.02 ^d	16.91 ± 0.05 ^b
Swelling power	8.16 ± 0.28 ^a	8.12 ± 0.01 ^a	7.48 ± 0.02 ^b	6.35 ± 0.05 ^c

Table 2 summarizes the functional properties of fermented and unfermented millet lees. Fermented pearl millet lees (FPML) presented the highest water holding capacity (WHC), consistent with studies signifying enhanced WHC improves the functionality of flour products (Singh et al., 2011). In contrast, unfermented millets (UPM and UFM) had lower WHC, with fermented finger millet lees (FFML) presenting the lowest capacity, reflect findings by Chandra et al. (2015). For oil holding capacity (OHC), unfermented finger millet (UFM) had the highest value, while FPML demonstrated a lower OHC of 0.92 ± 0.04 , similar to results from Shobana and Malleshi (2007). Regarding solubility, FPML had the highest solubility, followed by FFML, with UFM show the least, a pattern supported by Chandra et al. (2015). Higher solubility displays better functionality and nutrient assimilation. FPML also had the highest swelling capacity, exceeding UPM, while FFML shown the lowest swelling capacity, in line with Singh et al. (2011). These functional properties—WHC, OHC, solubility, and swelling capacity—are key to developing productive animal feed. High WHC helps retain moisture, developing feed hydration and digestibility, while elevated OHC improves energy quantity by retaining fats. High solubility supports nutrient assimilation, and greater swelling capacity enhances texture, making feed more palatable for animals.

3.1. Density Studies of Fermented and Unfermented Millets Lees

Table 3
The Functional Properties of fermented and unfermented millets

Parameters	UPM	FPML	UFM	FFML
Bulk density (g/cm²)	0.76 ± 0.01 ^a	0.64 ± 0.05 ^b	0.67 ± 0.01 ^b	0.75 ± 0.05 ^a
Tapped density (g/cm²)	0.87 ± 0.02 ^b	0.84 ± 0.05 ^b	0.87 ± 0.05 ^b	0.91 ± 0.02 ^a
Carr's index (%)	13.47 ± 0.01 ^d	24.00 ± 0.58 ^a	22.73 ± 0.01 ^b	15.33 ± 0.01 ^c
Hausner ratio	1.14 ± 0.05 ^c	1.31 ± 0.01 ^a	1.30 ± 0.01 ^a	1.17 ± 0.01 ^b

Table 3 highlights the density-related properties of fermented and unfermented millet lees. Fermented pearl millet lees (FPML) demonstrated the lowest bulk density, making it the least dense, while fermented finger millet lees (FFML) had the highest bulk density, indicating it is the most compact. Similarly, FFML led in tapped density, closely followed by unfermented gem millet (UPM), both indicating better packing adaptability. FPML had the lowest tapped density, suggesting it packs less capably. For Carr's index, FPML scored highest, indicating poorer flowability and greater compressibility, while FFML had the lowest index, indicating better flowability and less compressibility. The Hausner ratio, another measure of flowability, was also highest for FPML, indicating lower ease of flow. UPM, accompanying a lower Hausner ratio, shown better flowability. These findings are consistent with previous research on grain lees, with determinants like particle size, shape, and moisture content influencing the distinctnesses in bulk and tapped densities (Hoseney, 1994). Such physical qualities are essential for animal feed formulation, as factors like FFML, accompanying larger densities, determine better packing and storage effectiveness. Lower Carr's index and Hausner percentage principles signify better flowability, that is important for efficient feed mixing and processing.

Colour Analysis of Fermented and Unfermented Millets Lees

Table 4
Colour Analysis of fermented and unfermented millets lees

Parameters	UPM	FPML	UFM	FFML
L	59.23 ± 0.25 ^a	57.08 ± 0.01 ^b	37.16 ± 0.28 ^d	39.32 ± 0.25 ^c
L*	60.50 ± 0.50 ^b	64.13 ± 0.01 ^a	45.16 ± 0.28 ^d	45.80 ± 0.05 ^c
a	4.16 ± 0.28 ^c	5.26 ± 0.01 ^a	4.66 ± 0.28 ^b	5.50 ± 0.01 ^a
a*	7.16 ± 0.28 ^b	6.19 ± 0.05 ^d	7.46 ± 0.02 ^a	7.14 ± 0.05 ^b
b	18.15 ± 0.25 ^a	17.76 ± 0.01 ^b	8.10 ± 0.17 ^c	7.36 ± 0.01 ^d
b*	25.10 ± 0.17 ^b	26.66 ± 0.14 ^a	10.16 ± 0.28 ^d	11.17 ± 0.02 ^c
ΔE	22.77 ± 0.06 ^a	11.46 ± 0.03 ^b	8.61 ± 0.01 ^c	7.93 ± 0.01 ^d

Colour Analysis of fermented and unfermented millets lees is listed in Table 4. The color analysis of the samples reveals distinct distinctnesses in their color attributes. UPM and FPML exhibit lighter colours compared to UFM and FFML, with UPM being the lightest and FPML slightly lighter than UFM and FFML. Specifically, FPML has the highest lightness value (L*), indicating it is the brightest among the samples, while UFM and FFML show lower lightness values, indicating darker hues. In agreements of the red-green value (a and a*), FPML and FFML show more distinct red hues distinguished to UPM and UFM, with FFML show the highest red hue. UPM and UFM have comparable a* values, displaying a strong red hue, but FPML has the lowest a* value, suggesting a less intense red coloration. The yellow-blue value (b and b*) is higher in UPM and FPML, indicating a more intense yellow color. Conversely, UFM and FFML exhibit lower b and b* values, that correspond to a more blueish or less yellow appearance. UPM and FPML are thus more yellow in color distinguished to UFM and FFML. Finally, the total color difference (ΔE) shows that UPM has the largest color departure from the reference, indicating the most significant color difference. In contrast, FPML, UFM, and FFML have lower ΔE values, with FFML being the closest in color to the reference. This suggests that FFML is the most similar in color to the standard, while UPM exhibits the greatest variation. Overall, UPM and FPML are characterized by lighter and more intense yellow-red hues, whereas UFM and FFML are darker and less yellow. These variations in color attributes highlight the diverse appearance of the samples. When comparing these results to other grain lees, analogous trends maybe noticed. Significant differences in all parameters between the groups imply that formulation, ingredient selection, and processing conditions substantially impact product traits. Understanding these distinctnesses is critical for optimizing condition, consistency, and consumer satisfaction. For instance, rice lees generally exhibit higher L* values, indicating better lightness, that joins with the higher L* values seen in pearl millet lees (Zhu et al., 2023). Wheat lees, on the other hand, likely to have lower a* and b* values, indicating a more neutral colour profile compared to the distinct yellow hue of millet lees (Hoseney, 1994).

3.2. Total Phenolics and Flavonoids Content

Table 5
Total Phenolics and Flavonoids Content fermented and unfermented millets lees

Parameters	UPM	FPML	UFM	FFML
Phenolics (mgGAE/g)	3.33 ± 0.28 ^d	23.85 ± 0.01 ^a	4.33 ± 0.28 ^c	23.05 ± 0.02 ^b
Flavonoids (mgRE/g)	1.45 ± 0.05 ^d	32.04 ± 0.12 ^a	2.36 ± 0.32 ^c	29.32 ± 0.01 ^b

Table 5 shows the phenolic and flavonoid content of fermented and unfermented millet lees. Fermented pearl millet (FPML) and fermented finger millet (FFML) have significantly higher phenolic and flavonoid levels compared to their unfermented counterparts. FPML has the highest phenolic content, followed by FFML, indicating their superior antioxidant potential. In contrast, unfermented pearl millet (UPM) has the lowest phenolic level at 3.00 mgGAE/g, reflecting lower antioxidant capacity.

Similarly, FPML leads in flavonoid content, followed closely by FFML, while UPM and UFM lag behind. This suggests that fermentation enhances the health benefits and antioxidant properties of millet lees, as higher levels of these compounds are linked to better antioxidant activity.

Using FPML and FFML in animal feed provides numerous benefits due to their higher phenolic and flavonoid content. These compounds offer antioxidant protection, enhance immune function, and improve feed quality by extending shelf life. They also contribute to better feed palatability and support animal health by reducing oxidative stress and inflammation.

3.3. Antioxidant Assay

3.3.1. DPPH Radical Scavenging Assay

Table 6
DPPH Radical Scavenging Assay of Fermented and Unfermented Millets Lees

Parameters	UPM	FPML	UFM	FFML
5µl	14.28 ± 0.05 ^c	27.03 ± 0.02 ^b	7.14 ± 0.05 ^d	32.66 ± 0.01 ^a
10µl	28.55 ± 0.01 ^c	35.73 ± 0.03 ^b	21.43 ± 0.05 ^d	37.42 ± 0.02 ^a
15µl	42.86 ± 0.05 ^b	45.83 ± 0.02 ^a	35.71 ± 0.05 ^c	42.83 ± 0.02 ^b
20µl	57.14 ± 0.05 ^a	55.81 ± 0.01 ^b	50.01 ± 0.02 ^c	47.22 ± 0.01 ^d
25µl	71.41 ± 0.01 ^a	65.31 ± 0.01 ^b	64.25 ± 0.01 ^c	52.05 ± 0.05 ^d

The results from the DPPH radical scavenging assay, presented in Table 6, demonstrate clear differences in antioxidant ability between the fermented and unfermented millet lees. Fermented pearl millet lees (FPML) usually show the highest antioxidant activity across all tested concentrations. This strong antioxidant response is closely connected to its elevated phenolic and flavonoid content, both of that are known to enhance antioxidant properties. As noted by Dykes and Rooney (2006), phenolic compounds play a crucial part in neutralizing free radicals, that helps in preventing oxidative stress. These verdicts imply that FPML not only has potential nutritional benefits but can also significantly help improving animal health by protecting against oxidative damage. This makes FPML an excellent candidate for inclusion in animal feed formulations. Fermented finger millet lees (FFML) also exhibit robust antioxidant project, specifically at higher concentrations. This joins with previous studies, like those by Devi et al. (2014) and Chandrasekara and Shahidi (2010), that highlight the health benefits of finger millet, attributing its antioxidant potential to its rich composition of bioactive compounds. FFML's ability to neutralize free radicals, particularly at bigger dosages, further supports its role as a valuable feed ingredient that can help lower oxidative stress and promote overall animal well-being. On the other hand, unfermented pearl millet (UPM) and finger millet lees (UFM) display much lower antioxidant activity, particularly at lower concentrations. This diminished capacity to neutralize free radicals is likely due to the lower phenolic and flavonoid content in these unfermented samples. As a result, UPM and UFM may not offer the same level of protection against oxidative stress as their fermented counterparts. This reduced activity suggests that these unfermented samples might not be as effective in improving the health and resilience of animals through antioxidant mechanisms.

3.4. FTIR

The FTIR interpretation of pearl millet (PMF1) and finger millet (FMF1) lees has provided valuable insights into their biochemical profiles, revealing a diverse range of functional groups that contribute to their nutritional potential. In the case of pearl millet lees, the FTIR spectrum highlights several significant peaks, including a prominent O-H stretching peak at 3275.19 cm^{-1} , indicating the presence of hydroxyl groups. Other key peaks at 2931.85 cm^{-1} and 2894.24 cm^{-1} correspond to C-H stretching, while a peak at 1719.57 cm^{-1} suggests the presence of carbonyl groups ($\text{C}=\text{O}$). Additionally, peaks at 1643.38 cm^{-1} and 1236.39 cm^{-1} show C = C and C-O elongated, individually. Notably, the range likewise discloses a phosphate group peak at 1147.67 cm^{-1} , stressing the food-rich type of these remains (Kumari et al., 2023). For finger millet lees, the FTIR spectrum presents similar features, accompanying O-H stretching observed at 3299.30 cm^{-1} and C-H elongated at 2935.71 cm^{-1} . The presence of C = C elongated at 1645.31 cm^{-1} and C-N extended at 1342.48 cm^{-1} signifies a rich biochemical arrangement. Similar to pearl millet, finger millet also shows a phosphate group peak at 1147.67 cm^{-1} , reinforcing the nutritional significance of these lees (Kumari et al., 2023). When comparing the FTIR spectra of millet remains to those of fermented wine lees, we observe important correspondences. The common spectral features, such as O-H elongated about $3200\text{--}3600\text{ cm}^{-1}$ and C = O extended near 1700 cm^{-1} , imply that both types of lees share fermentation-derived organic compounds. However, slight differences in peak intensity and wavenumber, like the higher O-H stretching wavenumber in finger millet lees, indicate unique biochemical characteristics resulting from different raw materials and fermentation processes (El-Massry et al.,

2023). Furthermore, the FTIR spectra of raw pearl and finger millet flour mainly exhibit peaks about 3300 cm^{-1} for O-H elongated, accompanying C-H and C = O stretching noticed at about 2900 cm^{-1} and 1700 cm^{-1} , respectively. These peaks distinct in intensity compared to those in the lees, indicating that fermentation considerably alters the biochemical arrangement. During fermentation, compounds such as alcohols and acids become more evident, enhancing the nutritional profile of the millet lees (Patil et al., 2019; Kumar et al., 2019). The analysis emphasizes that fermentation plays a crucial role in changing the biochemical composition of millet products. The resulting functional groups, that are more prominent in the lees than in the raw flour, highlight the potential health benefits of these fermented products. The similarities between millet lees and fermented wine lees suggest shared fermentation pathways, while the differences indicate the unique processes that characterize each type of feed ingredient. Overall, the FTIR results explain that millet lees carry a rich biochemical profile, making them promising competitors for sustainable and nutritious animal feed. The presence of essential functional groups such as hydroxyl, carbonyl, and phosphate displays a high fiber content, including carbohydrates, proteins, fats, and vital minerals. These nutrients are essential for animal growth and health, reinforcing the value of millet lees as a feed ingredient. Moreover, the fermentation process enhances the digestibility and bioavailability of these vitamins by break down complex carbohydrates and proteins into simpler forms. The identified unsaturated fatty acids and esters further suggest that millet lees can serve as functional additives, promoting better gut health and immune function in animals.

Incorporating millet lees into animal feed not only supports animal health and productivity but also aligns with sustainable agricultural practices. By valorizing fermentation by-products, we can reduce waste and promote resource efficiency in food production systems. Therefore, the findings from the FTIR analysis support the potential of millet lees to improve feed formulations, enhance animal well-being, and contribute to more sustainable agricultural practices.

4. Conclusion

The study extensively assessed the nutritional and functional properties of fermented and Unfermented millet lees, specifically pearl millet and finger millet. The results revealed important differences in the proximate composition, total phenolics, flavonoids content, antioxidant activity, and physicochemical properties between the samples. Fermented pearl millet lees (FPML) and fermented finger millet lees (FFML) regularly demonstrated superior nutritional characterizations and higher antioxidant capacities compared to unfermented pearl millet (UPM) and unfermented finger millet (UFM). This is attributed to the higher concentrations of phenolic and flavonoid compounds in the fermented samples, which are known for their antioxidant properties. The findings emphasize the potential of FPML and FFML as valuable components in animal feed formulations. Their greater phenolic and flavonoid contents not only boost the antioxidant activity but also offer additional health benefits, including protection against oxidative stress, improved immune function, and reduced inflammation. Moreover, the incorporation of these fermented lees supports sustainable waste management practices by repurposing by-products from the beverage manufacturing into valuable animal feed ingredients.

5. Future scope

The study suggests several future research and development instructions. Firstly, detailed nutritional analysis of millet lees, containing amino acid characterizations, vitamin content, and mineral bioavailability, can provide a more inclusive understanding of their benefits in animal nourishment. Long-term animal feeding trials are essential to figure out health impacts, growth performance, and feed efficiency, establishing optimum inclusion rates and validating antioxidant benefits. Exploring the microbial communities in the fermentation process could optimize conditions to enhance nutritional and functional properties. Additionally, expanding commercially viable animal feed products, in the way that pelletized feeds, supplements, and treats, tailored to different livestock species' dietary needs, can increase market potential. Investigating fermented millet lees' applications in human nutrition, especially as functional food ingredients, is promising, given their high antioxidant content. Conducting life cycle assessments and economic studies to assess the environmental and economic benefits, containing waste reduction, resource utilization efficiency, and cost savings for the beverage and feed industries, is crucial. Addressing these research directions can fully accomplish millet lees' potential as sustainable and nutritious feed ingredients, enhancing both animal health and environmental sustainability.

Declarations

Author's Contribution

Rithika S- Conceptualization, development or design of methodology, conducting the experiments ,acquisition of data ,analysis and interpretation of data ,writing the original draft

Dr. A. Amudhasurabi-Conceptualization, development or design of methodology,analysis and interpretation of data, writing the original draft,review and edit the draft, Supervision ,Project administration

Dr. K. Suresh Kumar-Development or design of methodology, Review and edit the draft, Supervision, Project administration

Dr. T. Suman-Development or design of methodologyReview and edit the draft Supervision

Conflict of Interest

The authors declare no conflict of interest in this research article.

Acknowledgement

The authors would like to thank NIFTEM-T for providing technical support and facilities required for this research work.

Data Availability statement

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