ORIGINAL ARTICLE

Impact of Microbial Fermentation on the Nutritional Composition of Sorghum-Based Traditional Fermented Food *Nucchu ambli*

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Abstract

Aims: This study explores the biochemical and nutritional transformations occurring in *Nucchu ambli*, a traditional sorghum-based food, during microbial fermentation. **Materials and Methods:** The investigation focused on key fermentation parameters pH, titratable acidity (TTA), reducing sugars, total sugars, moisture content, and protein levels monitored over a 24 h period. **Results and Discussion:** Results revealed a marked decline in pH from 5.2 to 3.01 and a corresponding increase in TTA from 1.271% to 1.725%, reflecting heightened microbial activity. Reducing sugars decreased from 9.408 µg/mL to 7.051 µg/mL, while total sugars dropped from 4.132 mg/L to 3.126 mg/L, indicating active microbial metabolism. Protein content reduced from 0.618 mg/mL to 0.511 mg/mL, and moisture content gradually decreased due to evaporation. **Conclusion:** These findings underscore the significant impact of fermentation on the physicochemical properties of *Nucchu ambli*, enhancing its potential for improved digestibility and nutritional benefits.

Key words: Microbial fermentation, *Nucchu ambli,* nutritional composition, nutritional enhancement, sorghum, traditional fermented food

INTRODUCTION

Sorghum (*Sorghum bicolor* L. moench) was an essential staple food in many regions, particularly in Africa and Asia, where it provided significant amounts of carbohydrates and proteins. However, its nutritional value was limited due to low lysine content and the presence of anti-nutritional compounds such as tannins and phytate.[1,2] These compounds reduced protein digestibility and restricted the absorption of essential minerals, leading to deficiencies that contributed to conditions like anemia among populations dependent on sorghum-based diets. In addition, sorghum proteins had lower digestibility compared to those found in other cereals.

To improve its nutritional profile, traditional processing techniques like fermentation were utilized. Fermentation was shown to enhance protein digestibility and boost mineral absorption by breaking down tannins and phytate. The microbial activity involved in fermentation facilitated the decomposition of complex compounds, increasing amino acid content and vitamin availability.[3,4] One such example was *Nucchu ambli,* a fermented sorghum-based dish from Karnataka, India. This process not only enriched sorghum's nutritional composition but also introduced beneficial probiotics that supported gut health.

Similarly, millets played a crucial role in traditional diets and were widely cultivated in regions including China,

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India, Greece, Egypt, and parts of Africa. Varieties such as finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), little millet (*Panicum sumatrense*), and proso millet (*Panicum miliaceum*) were valued for their rich nutrient profile. These grains contributed to managing health conditions like diabetes and hyperlipidemia. In addition, other millets such as pearl millet (*Pennisetum glaucum*) and buckwheat (*Fagopyrum esculentum*) were recognized for their health benefits, reinforcing their importance in traditional diets.[5,6]

Nutritional composition and health benefits of millets

Foxtail millet (S. italica)

This grain was a rich source of complex carbohydrates that contributed to stabilizing blood glucose levels by promoting a slow and sustained release of sugar into the bloodstream. Its high iron and calcium content played a crucial role in improving immune function and maintaining bone density. Studies indicated that regular consumption of foxtail millet positively influenced lipid metabolism, notably increasing high-density lipoprotein cholesterol while reducing low-density lipoprotein (LDL) cholesterol, thereby supporting cardiovascular health.[7]

Finger millet (E. coracana)

As a naturally gluten-free cereal, finger millet served as an excellent alternative for individuals with gluten intolerance or celiac disease. It contained a high protein concentration and a well-balanced amino acid composition, making it a valuable dietary component. This millet was particularly abundant in calcium, which was essential for skeletal development, bone mineralization, and the prevention of osteoporosis. In addition, bioactive compounds such as polyphenols and flavonoids found in finger millet exhibited antioxidant and anti-inflammatory properties, contributing to metabolic health.[8]

Pearl millet (P. glaucum)

Recognized for its dense nutrient composition, pearl millet provided essential minerals such as calcium and magnesium, alongside significant amounts of protein, fiber, and iron. These nutrients collectively supported cardiovascular function, facilitated glucose metabolism, and improved insulin sensitivity. Research highlighted that its high fiber content contributed to lower glycemic responses and prolonged satiety, making it beneficial for weight management and diabetes control.

Buckwheat (F. esculentum)

Despite being classified as a pseudo-cereal, buckwheat was widely valued for its nutraceutical properties. It contained bioactive compounds like rutin, a flavonoid with strong antioxidant effects that enhanced vascular function and reduced the risk of hypertension. Studies demonstrated that buckwheat consumption was linked to improved lipid profiles, reduced inflammation, and better regulation of blood pressure. Due to its high fiber and resistant starch content, it also played a role in improving gut microbiota composition and metabolic health.[9]

Little millet (P. sumatrense)

This millet was characterized by its high dietary fiber content and significant levels of micronutrients such as potassium, zinc, iron, and calcium. It also provided essential B-complex vitamins, which were crucial for enzymatic reactions and energy metabolism. Scientific evidence suggested that little millet exhibited antioxidant properties due to the presence of phenolic compounds, which contributed to cellular protection against oxidative stress. Regular intake of little millet was associated with better glycemic control, reduced cardiovascular risk, lower incidence of asthma, and enhanced digestive health through improved gut motility.[10]

Nutritional limitations and processing interventions

Sorghum (S. bicolor L. moench)

Cultivated extensively across Africa and Asia, sorghum served as a fundamental source of carbohydrates and proteins for millions of people. However, its nutritional profile was constrained by a low lysine content and the presence of anti-nutritional factors such as tannins and phytate, which inhibited the absorption of essential micronutrients such as iron, zinc, and calcium. Traditional processing methods such as malting, soaking, and fermentation were employed to enhance its bioavailability. Fermentation, in particular, was found to significantly decrease phytate and tannin concentrations, thereby improving protein digestibility and mineral absorption. In addition, microbial fermentation contributed to the synthesis of essential amino acids and vitamins, further enriching the nutritional value of sorghum-based foods.[11]

Health benefits of millets

Millets have long been recognized for their potential to prevent and manage metabolic disorders. Their high dietary fiber content contributed to improved glycemic control by delaying glucose absorption and enhancing insulin sensitivity. Studies indicated that millet-based diets were associated with a reduced risk of type 2 diabetes and cardiovascular diseases due to their ability to regulate lipid metabolism and reduce oxidative stress. Furthermore, the presence of bioactive compounds such as polyphenols and flavonoids in millets conferred anti-inflammatory and immunomodulatory benefits, reinforcing their role in disease prevention. Regular consumption of millets was linked to enhanced gut microbiota diversity, improved digestion, and a

lower incidence of lifestyle-related disorders such as obesity and hypertension.[12]

MATERIALS AND METHODS

Sample preparation

Sorghum grains were obtained from a local supplier and underwent a thorough cleaning process to eliminate impurities. The grains were then washed with distilled water and dried under controlled conditions to prevent contamination and moisture retention. Once completely dried, they were stored in airtight containers to preserve their compositional integrity and prevent oxidative degradation. The experimental design consisted of two distinct groups: A control group comprising unfermented *Nucchu* and a treatment group consisting of fermented *Nucchu ambli.* All chemicals and reagents employed were of analytical grade.

Extraction of soluble sugars from *Nucchu* samples

Soluble sugars were extracted following a standardized protocol to ensure reproducibility. A precisely measured 5-g portion of the *Nucchu* sample was mixed with 50 mL of 80% ethanol in a conical flask. This suspension was subjected to continuous stirring at room temperature for 30 min using a magnetic stirrer to facilitate the dissolution of soluble sugars. After thorough agitation, the mixture was transferred to centrifuge tubes and centrifuged at 8,000 rpm for 10 min at ambient temperature. The resulting supernatant, containing the extracted soluble sugars, was carefully collected and stored under refrigeration for further biochemical analysis.

Fermentation process

The fermentation process was conducted to assess its impact on the biochemical composition of *Nucchu*. For this, 30 g of fine sorghum particles were cooked in 300 mL of water for 20 min to gelatinize the starch, making it more accessible for microbial metabolism. After cooling to room temperature, 40 g of curd, serving as an inoculum, was added to introduce a microbial culture rich in lactic acid bacteria. The mixture was then left to ferment at ambient temperature for 24 h under controlled conditions. Samples were systematically collected at specific time intervals (0, 6, 12, 18, and 24 h) to monitor biochemical transformations occurring during the fermentation process.[13]

Biochemical and analytical methods

A comprehensive set of biochemical assays was performed to evaluate changes in the nutritional composition during fermentation. Carbohydrate analysis was conducted using Molisch's, Anthrone, and Fehling's tests to detect the presence and quantify carbohydrate content. The Ninhydrin and Nitrous Acid tests were employed for amino acid profiling to assess protein hydrolysis and amino acid release. Lipid content was determined using the saponification test, which provided insights into changes in fatty acid composition. The pH of the samples was continuously monitored using a calibrated pH meter to observe acidification trends, while titratable acidity (TTA) was quantified through titration with NaOH using phenolphthalein as an indicator. Moisture content was measured using the oven-drying method to assess water retention during fermentation. Protein concentration was estimated using the Lowry method, enabling a precise evaluation of protein degradation and synthesis during microbial activity. These analyses provided a detailed understanding of the biochemical transformations induced by fermentation and their potential implications for enhancing the nutritional profile of *Nucchu ambli.*[14]

RESULTS AND DISCUSSION

Qualitative test for fat by saponification test

Saponification test was performed to identify presence of fat content for both control and treated, formation of foam indicates the presence of lipid content, if lipid was present in sample then soap or foam will form and float on water layer of NaOH solution, compared to treated there was more foam present in control sample because of more content of lipid present in sample as shown in Figures 1 and 2.

Analysis of the pH versus fermentation time graph

The analysis of effect of pH on fermentation time was resulted in pH variations during fermentation, the control sample maintained a stable pH of around 7 for the first 20 h, after which it exhibited a slight decline but remained above 6 as shown in Figure 3. This was the indication of minimal

Figure 1: Saponification test (control)

acid production. In contrast, the treated sample initially had a pH of approximately 5.2, which was continuously dropped to around 3.01 by the 25th h. This significant decrease suggested an increased rate of microbial activity and organic acid production, contributing to enhanced acidogenesis. Previous research confirmed that lower initial pH levels accelerated acid production during fermentation, whereas higher pH conditions limited acidogenesis due to reduced microbial activity. The substantial decline in pH observed in the treated sample aligned with findings that linked pH reduction to an increase in acidic metabolite synthesis. These results highlighted the crucial role of pH regulation in optimizing fermentation efficiency and microbial metabolic pathways.[15,16]

The role of pH in the fermentation of *Nucchu ambli* was crucial. Changes in pH were monitored at 5-h intervals throughout the fermentation process. The pH profile of the treated sample declined from an initial value of 5 over the course of 25 h, showing a continuous decrease.[17] This reduction in pH correlated with an increase in amino acid production, driven by the growth of acid-producing bacteria, such as lactic acid bacteria, which were particularly active during the early stages of fermentation. In addition, Figure 3 illustrates the TTA measured over a 24-h fermentation period for both control and treated *Nucchu ambli* samples.

Figure 2: Saponification test (treated)

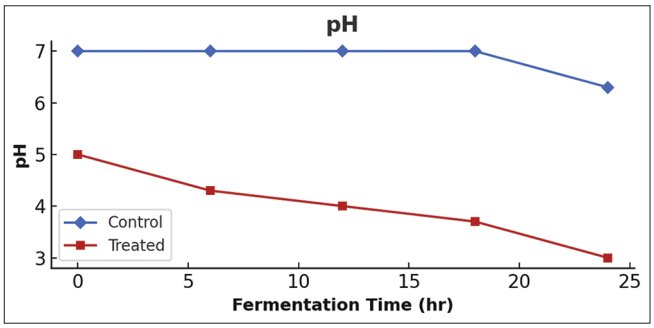

Figure 3: Influence of pH on fermentation time

The treated samples demonstrated a significant rise in TTA compared to the control, suggesting notable biochemical transformations during the fermentation process.[18,19]

TTA increased from 1.271 to 1.725 (Treated) and there was no much increase in TTA (control) as the pH decreased the TTA increased, as the fermentation time increased was due to the effect of bacterial population increases, the TTA value increases drastically.

Figures 4 and 5 illustrate the changes in % TTA over a 24-h fermentation period for both control (unfermented) and treated (fermented) *Nucchu ambli* samples. TTA was a measure of the total acidity present in a sample, reflecting the concentration of acidic compounds produced during fermentation. The % TTA values for the control samples remain relatively stable throughout the 24-h period, fluctuating slightly between 0.211 and 0.299. This stability indicates minimal biochemical activity in the absence of fermentation. In contrast, the treated samples exhibit a significant increase in % TTA over time. Starting at approximately 1.271, the acidity rises steadily, reaching around 1.725 by the 24-h mark. This progressive increase suggests active fermentation,

Figure 4: *Nucchu ambli* sample

Figure 5: Titratable acidity of *Nucchu ambli*

leading to the production of organic acids. The rising TTA in the treated samples was indicative of microbial fermentation, during which microorganisms metabolize substrates to produce organic acids such as lactic acid. This acidification process not only enhances the flavor and preservation of fermented products but also improves their nutritional profile by increasing the bioavailability of certain nutrients.[20,21]

The total sugar content increased until the 12th h of fermentation, after which it began to decline as shown in Figure 6. In the treated sample, the sugar concentration peaked at 12 h, reaching 4.132 mg/L, before decreasing to 3.126 mg/L by the 24th h (Figure 7). In comparison, the control sample showed a slight rise, beginning at 0.638 mg/L by the 6th h, maintaining a relatively stable level, and eventually reducing to 0.432 mg/L at 24 h. Similarly, protein estimation was performed by DNS method and protein concentration was almost the same for control sample whereas treated sample has shown maximum protein concentration between 4 and 6 h of fermentation as shown in Figure 8.[22,23]

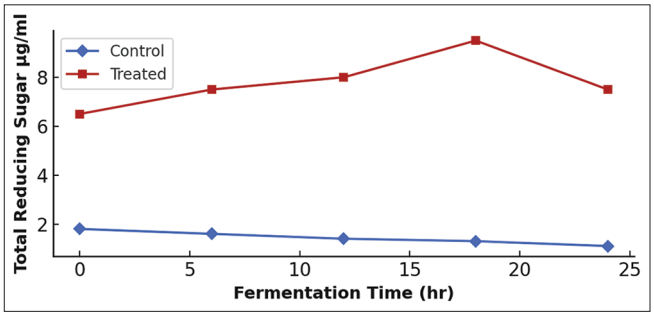

Figure 6: Total reducing sugar of *Nucchu ambli*

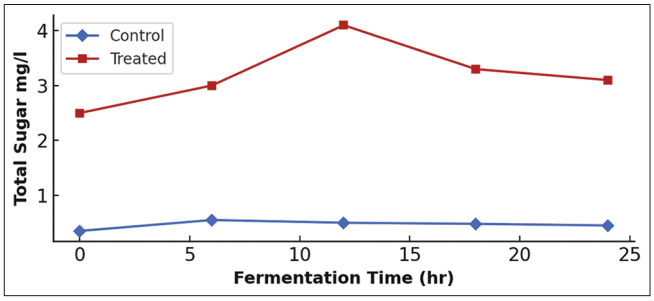

Figure 7: Total sugars present in *Nucchu ambli*

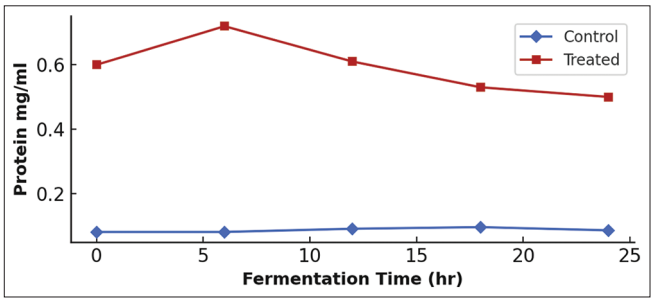

Figure 8: Estimation of protein content during fermentation process

Moisture content and drying process analysis

The experimental data represents a comprehensive comparison of weight loss, moisture content, and drying rate for both the Control (C) and Treated (T) samples over time. A gradual reduction in weight was observed across both sample groups, indicating progressive moisture loss. However, the treated samples exhibited a more substantial weight reduction compared to the control, suggesting an enhanced rate of water evaporation due to the applied treatment.

The cumulative water loss (Σm) consistently remained higher in treated samples than in control samples, highlighting the treatment's effectiveness in accelerating moisture removal. Similarly, the moisture content (XT) exhibited a decreasing trend over time, with treated samples experiencing a faster decline than the control, reinforcing the impact of the treatment in facilitating moisture loss.

The drying rate was initially high for both sample groups but progressively declined over time, following the well-documented drying kinetics observed in hygroscopic materials. The treated samples demonstrated a significantly higher drying rate than the control, indicating improved moisture removal efficiency. This behavior aligned with conventional drying mechanisms, where an initial constant rate period, governed by surface water evaporation, was followed by a falling rate period, characterized by moisture diffusion from the material's internal structure. Understanding these drying dynamics was essential for optimizing industrial drying processes, reducing energy consumption, and preserving the physicochemical integrity of materials across diverse applications, including agricultural product preservation, pharmaceutical formulation, and advanced material processing.[24]

The moisture content graph depicted a typical drying process as shown in Figure 9, with moisture levels decreasing over time. During the initial phase (0–1 h), the moisture content remained nearly constant, indicating an adaptation phase in which surface water started evaporating but had not yet reached a steady removal rate. Between 1 and 3 h, the drying rate increased significantly, representing the constant rate period, where moisture was efficiently removed due to sustained water migration from the surface. After 3 h, the rate of moisture loss declined, marking the transition into the falling rate period, during which internal diffusion became the primary limiting factor for further moisture removal. By the 4th h, the curve flattened, suggesting that the material had reached its equilibrium moisture content, beyond which additional drying would require considerably more energy. This drying behavior was essential in optimizing industrial processes, particularly in food processing, pharmaceuticals, and material science, where precise moisture control influenced product stability, microbial safety, and overall quality retention. Understanding the drying kinetics allowed

for better process efficiency, reduced energy consumption, and improved preservation of heat-sensitive materials.[25,26]

Table 1 provides data on weight loss, moisture content, and drying rate for Control (C) and Treated (T) samples over time (h). Both control and treated samples show a gradual decrease in weight over time. Treated samples exhibit higher weight loss than control samples, suggesting increased water evaporation due to treatment effects. The cumulative water evaporated (Σm) was consistently higher for treated samples than control, indicating that the treatment enhances moisture loss. The moisture content (XT) decreases over time, with treated samples losing moisture faster than control samples. The drying rate was initially higher and gradually decreases over time for both conditions. The treated sample shows a significantly higher drying rate than the control, suggesting better moisture removal efficiency. This trend was common in drying processes, where the constant rate period was followed by a falling rate period.[27,28]

The moisture content increased from 0.9914 and decreased at 0.9525 (control), as the fermentation time increased, the moisture content decreased due to the loss of water content present in the sample. The moisture content graph illustrates

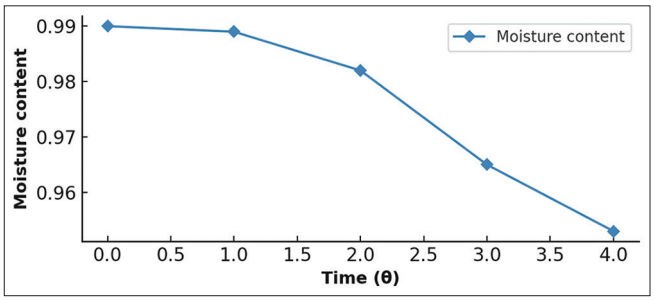

Figure 9: Graph of moisture content versus time (ɵ) at 0 h (control)

a typical drying process, where moisture decreases over time. Initially (0–1 h), the moisture content remains nearly constant, indicating an adaptation phase where surface water begins to evaporate. Between 1 and 3 h, the drying rate increases, representing the constant rate period**,** where moisture is efficiently removed. Beyond 3 h, the rate of moisture loss declines, marking the falling rate period, where internal diffusion limits further moisture removal. By the 4th h, the curve flattens, suggesting the approach of equilibrium moisture content. This trend was crucial in optimizing drying conditions in food processing, pharmaceuticals, and material science to enhance efficiency while preserving quality.[29,30]

The moisture content increased from 0.9750 and decreased to 0.940 (Treated), as the fermentation time increased, the moisture content decreased, due to the loss of water content present in the sample, as shown in Figure 10.

Both graphs, Figures 11 and 12, demonstrated a reduction in moisture content over the drying period. An initial rapid decline was observed during the 1st h, primarily attributed to the evaporation of surface moisture. This was followed by a more gradual moisture loss during the constant rate period (1–3 h), and finally, a stabilization phase from 3 to 4 h, where internal moisture diffusion became the limiting factor. The second graph displayed a smoother moisture depletion trend, indicative of a more uniform drying mechanism, possibly due to better sample uniformity or controlled environmental conditions. In contrast, the first graph exhibited minor fluctuations, which might have been caused by sample heterogeneity or external variations such as airflow or humidity. Despite these discrepancies, both datasets converged to a similar final moisture content (0.82 g/g dry basis), suggesting that equilibrium drying was achieved. These drying kinetics provided critical insights for optimizing dehydration protocols in sectors such as food

Table 1: Calculation of moisture content at 24 h Fermentation

Time in h (ɵ)	Weight (w) in g	Weight difference M=(wi−wi+1) in g	Water Evaporated Σm in g	Moisture content at any time (wi-Σm) in g	Total moisture content (XT)	Average (XTavg)	Rate of drying kg/ m^{2}s
0	96.91 (C)	-	-	-	-	-	-
	115.29 (T)	-	-	-	-	-	-
1	90.30 (C)	6.31	6.31	83.99	0.9301	0.9301	0.7011
	108.79 (T)	6.50	6.50	102.29	0.9402	0.9402	0.7222
2	82.67 (C)	7.63	13.94	68.73	0.8313	0.8807	0.7744
	101.70 (T)	7.09	13.59	88.11	0.8663	0.9032	0.7550
3	82.66 (C)	0.01	13.95	68.71	0.8312	0.8312	0.5166
	100.32 (T)	1.38	14.97	85.35	0.8507	0.8585	0.5544
4	81.68 (C)	0.98	14.93	66.75	0.8172	0.8242	0.4147
	98.89 (T)	1.43	16.40	82.49	0.8341	0.8424	0.4555
5	80.97 (C)	0.71	15.64	65.33	0.8068	0.8120	0.3475
	97.86 (T)	1.03	17.43	80.43	0.8218	0.8279	0.3873

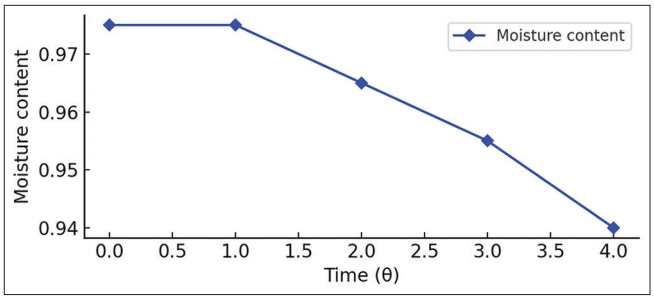

Figure 10: Moisture content versus time (ɵ) at 0 h (treated)

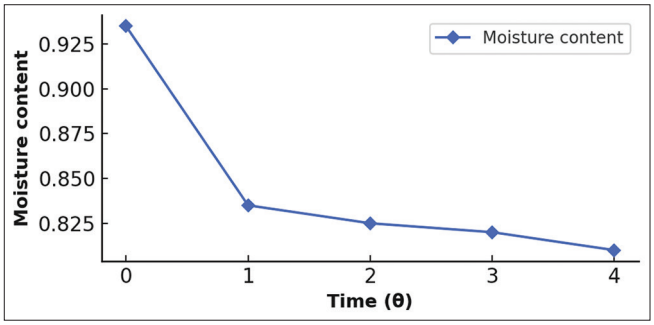

Figure 11: Moisture content versus time (ɵ) at 24 h (control)

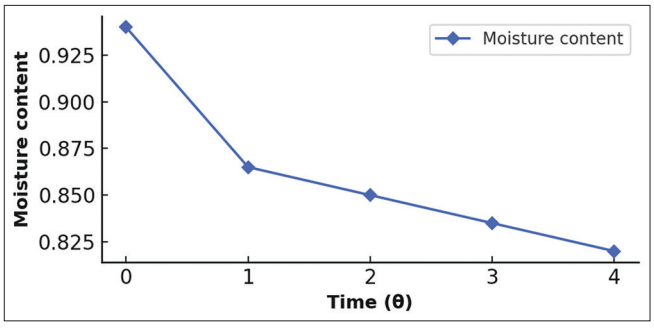

Figure 12: Moisture content versus time (ɵ) at 24 h (treated)

processing, pharmaceuticals, and materials engineering, where precise moisture control is essential for ensuring product stability, shelf life, and quality.[31,32] The findings suggest that microbial fermentation can significantly enhance the nutritional quality of sorghum-based traditional foods such as *Nucchu ambli.* This highlights its potential role in improving food security, promoting gut health, and supporting the development of sorghum as a sustainable functional food

CONCLUSION

The investigation into the primary fermentation of *Nucchu ambli* revealed the presence of diverse microbial consortia, predominantly amylolytic (starch-degrading) and lactic acid bacteria. The type of sorghum used had a notable impact on key physicochemical properties such as pH, TTA, sugar content, protein levels, and moisture. Initially, the pH rose to 5.1, likely due to deamination or buffering effects, but later dropped to 3.01 after 24 h, driven by increased amino acid synthesis and the active growth of acid-producing microorganisms like *Lactobacillus* spp. Correspondingly, TTA rose from 1.271% to 1.725%, indicating the accumulation of organic acids, particularly lactic acid, through microbial metabolism.

Total sugar concentration peaked at 4.1 mg/mL around 12 h before declining to 3.1 mg/mL, aligning with carbohydrate catabolism and microbial assimilation. Protein content exhibited an initial increase, reaching 0.71 mg/mL at 6 h, possibly due to microbial biomass synthesis and enzymatic protein release. It later declined to 0.51 mg/mL, which may have resulted from protein degradation or redistribution as the microbial community shifted. Concurrently, moisture content decreased steadily, indicating progressive water loss, likely due to microbial metabolic activity and ambient evaporation during fermentation. These findings underscore the transformative impact of fermentation on the nutritional and functional properties of *Nucchu ambli*, highlighting its potential for enhanced digestibility and health benefits.

AUTHOR CONTRIBUTIONS

All authors contributed equally.

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