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SAFETY ASSESSMENT OF FERMENTED CHEESE WHEY-BASED NUTRACEUTICAL: MICROBIAL, PHYSICOCHEMICAL AND *IN VIVO* TOXICOLOGICAL STUDIES IN ALBINO RATS

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Abstract

Cheese whey is a rich source of nutraceutical compounds, and little is known about how long-term preservation affects its microbial profile, physicochemical characteristics, and possible toxicity to important organs. Therefore, the microbial composition, pH, titratable acidity, and sub-chronic toxicity (in adult albino rats) of fermented cheese whey were investigated in this work during 28 days of storage. However, *Staphylococcus aureus*, *Lactobacillus* species, *Micrococcus* species, and *S. epidermidis* were the most common bacteria found in laboratory-fermented whey, which had low bacterial counts (1.7×10^3 CFU/ml) and no fungi. Locally-fermented whey on the other hand, had significantly higher microbial loads (2.3×10^3 CFU/ml bacteria and 9.0×10^3 CFU/ml fungi), which also included a number of known pathogens and spoilage organisms. The fermented cheese whey acidified during storage: laboratory-fermented whey changed more slowly (pH 4.0-3.52; acidity 0.28%-0.35%), whereas locally-fermented whey showed a quicker pH drop (4.11-3.17) and increase in titratable acidity (0.29%-0.39%). Nevertheless, at day 14 no effect on the liver or kidneys but day 28, showed dose-dependent toxicity, in both fermented whey. We found that long preservation of cheese whey leads to toxicity in our *in-vivo* model.

Keywords: Cheese-whey; physico-chemical; microbial; histopathology; liver; kidney.

Introduction

Cheese whey is a white to yellow or green opalescent liquid by-product of cheese production which contains varying levels of proteins, lipids, and mineral salts, as well as up to 6% lactose (Fancello *et al*., 2024). Fresh whey has traditionally been consumed by individuals together with cheese in many households in Nigeria localize and towns due

to its high nutritional content and potential health effects such as; improved digestion, hydration and significant antibacterial or antiviral properties (Gupta & Prakash, 2017). However, whey high moisture content, nutrient richness, low pH, and vulnerability to unchecked microbial fermentation during storage also makes it extremely perishable (WHO, 2004). Fermentation can alter the kind and structure of components when taken in large amounts or after long storage such as; oxidation, immunological response, and microbial growth (Zeng *et al*., 2023). Whey often has a larger bacterial content than milk since the processes used to make cheese encourage the growth of bacteria, even when pasteurized milk is used because bacterial starter cultures are added (da Silva Duarte *et al*., 2020). Also, potential renal acid load (PRAL produce and uncontrolled fermentation, which is linked to the emergence of metabolic changes such as; insulin resistance, diabetes, hypertension, liver disfunction, chronic renal disease, bone diseases, poor muscle mass, and other issues (Osuna-Padilla *et al*., 2019). Therefore, more research is required to understand how the physicochemical alterations that take place during whey fermentation and storage may affect toxicological results. Thus, the pH, acidity, microbial load, and histological effects of locally and laboratory-fermented cheese whey given to albino rats are assessed in this work.

Materials and Methods

Raw cow milk and locally produced whey were collected aseptically from Fulani settlements in Ilorin metropolis, Nigeria, transported on ice, and stored at 4°C. Laboratory-fermented whey was produced following Bos *et al*., (2000) and pasteurized milk (71.3°C) was acidified with food-grade lactic acid, coagulated with rennet, incubated for 30-40 minutes, and curd was removed by filtration to obtain whey. Serial dilutions were plated on Nutrient Agar (NA), MacConkey Agar (MCA), De Man Rogosa Sharpe (MRS), and Potato Dextrose Agar (PDA) following standard procedures.

Colony-forming units (cfu/mL) were recorded as mean ± SD of triplicate plates. pH was recorded using a calibrated digital pH meter, and total acidity was quantified titrimetrically using NaOH and phenolphthalein indicator. Measurements were taken on days 0, 14, and 28. Twenty-eight albino rats (130-180 g) were acclimatized for 7 days and divided into seven groups (n=4). Rats received oral doses of laboratory-fermented or locally-fermented whey at 1.0, 0.75, or 0.5 ml daily; the control group received distilled water. Animals were maintained at 24 ± 2°C, 45–64% humidity, with a 12-hours light/dark cycle and basal diet. Subsets of rats were sacrificed at days 14 and 28. Liver and kidney tissues were fixed in 10% formalin, processed, stained with H&E, and examined microscopically using a SCOPETEK DCM 500 imaging system.

Results

Laboratory-fermented whey showed a decrease in pH from 4.0 to 3.52 and an increase in acidity from 0.28 to 0.35 over 28 days of storage while the locally fermented whey exhibited sharper pH drop from 4.11 to 3.17 and acidity from 0.29 to 0.39, indicating uncontrolled fermentation as shown in Tables 1 and 2

Table 1: pH Levels Across Sampling Days for Different Treatments

Treatment	Day 0	Day 14	Day 28
A	4.0±0.007[a]	3.56±0.008[a]	3.52±0.008[b]
B	4.11±0.004[a]	3.50±0.007[a]	3.17±0.007[b]

Table 2: Acidity Levels Across Sampling Days for Different Treatments

Treatment	Day 0	Day 14	Day 28
A	0.28±0.02[a]	0.31±0.01[a]	0.35±0.02[b]
B	0.29±0.01[a]	0.33±0.33[a]	0.39±0.02[b]

Note: Values are presented as mean of (n=4) ± standard error. Means in the same column with different superscripts differ significantly. Key- A: Laboratory fermented cheese whey; B: Locally fermented cheese whey.

Laboratory-fermented whey had a total bacterial count of 1.7 × 10^3 cfu/ml with no fungal growth, whereas locally fermented whey had 2.3 × 10^4 cfu/ml bacteria and 9.0 × 10^3 cfu/ml fungi as shown in Table 3. The identified bacteria include: *Staphylococcus aureus*, *Lactobacillus* species, *Micrococcus* species, and *S. epidermidis* in laboratory-fermented whey while; a number of known pathogens and spoilage organisms such as; *Klebsiella spp*., *Salmonella spp*., *Shigella spp*., *Bacillus spp*., *Aspergillus niger*, *A. flavus*, *Mucor racemosus*, *Penicillium spp*., and *Saccharomyces cerevisiae* were found in locally-fermented whey as shown in Table 4.

Table 3: Total Microbial Count for Different Treatments

Treatment	Bacterial Count (cfu/ml)	Fungal Count (cfu/
A	1.7 x 10^3 ± 0.04^{a}	Nil
B	2.3 x 10^4 ± 0.05^{b}	9.0 x 10^3 ± 0.03

Note: Values are presented as means of (n=3) ± standard error. Means in the same column with different superscripts differ significantly. Key- A: Laboratory fermented cheese whey; B: Locally fermented cheese whey; Cfu/ml- Colony forming unit per millilitre.

Table 4: Microbial Isolates for Different Treatments

Microbial Isolates	A	B	Number of Occurrence	Percentage of Occurrence (%)
Staphylococcus aureus	+	+	2	18.18
Staphylococcus epidermidis	+	-	1	9.00
Escherichia coli	-	+	1	9.00
Lactobacillus specie	+	+	2	18.18
Klebsiella specie	-	+	1	9.00
Salmonella specie	-	+	1	9.00
Shigella specie	-	+	1	9.00
Bacillus specie	-	+	1	9.00
Micrococcus specie	+	-	1	9.00
Aspergillus niger	-	+	1	20.00
Aspergillus flavus	-	+	1	20.00
Mucor racemosus	-	+	1	20.00
Penicillium specie	-	+	1	20.00
Saccharomyces cerevisiae	-	+	1	20.00

Key- A: Laboratory fermented cheese whey; B: Locally fermented cheese whey; + Present of Isolate; - Absent of Isolate.

The histopathology observation of tissues at day 14 of administration appeared normal compared to controls. However, at day 28, dose-dependent hepatic necrosis, glomerular epithelial changes, and tubular degeneration were observed, particularly in high-dose groups as shown in Figure 1.

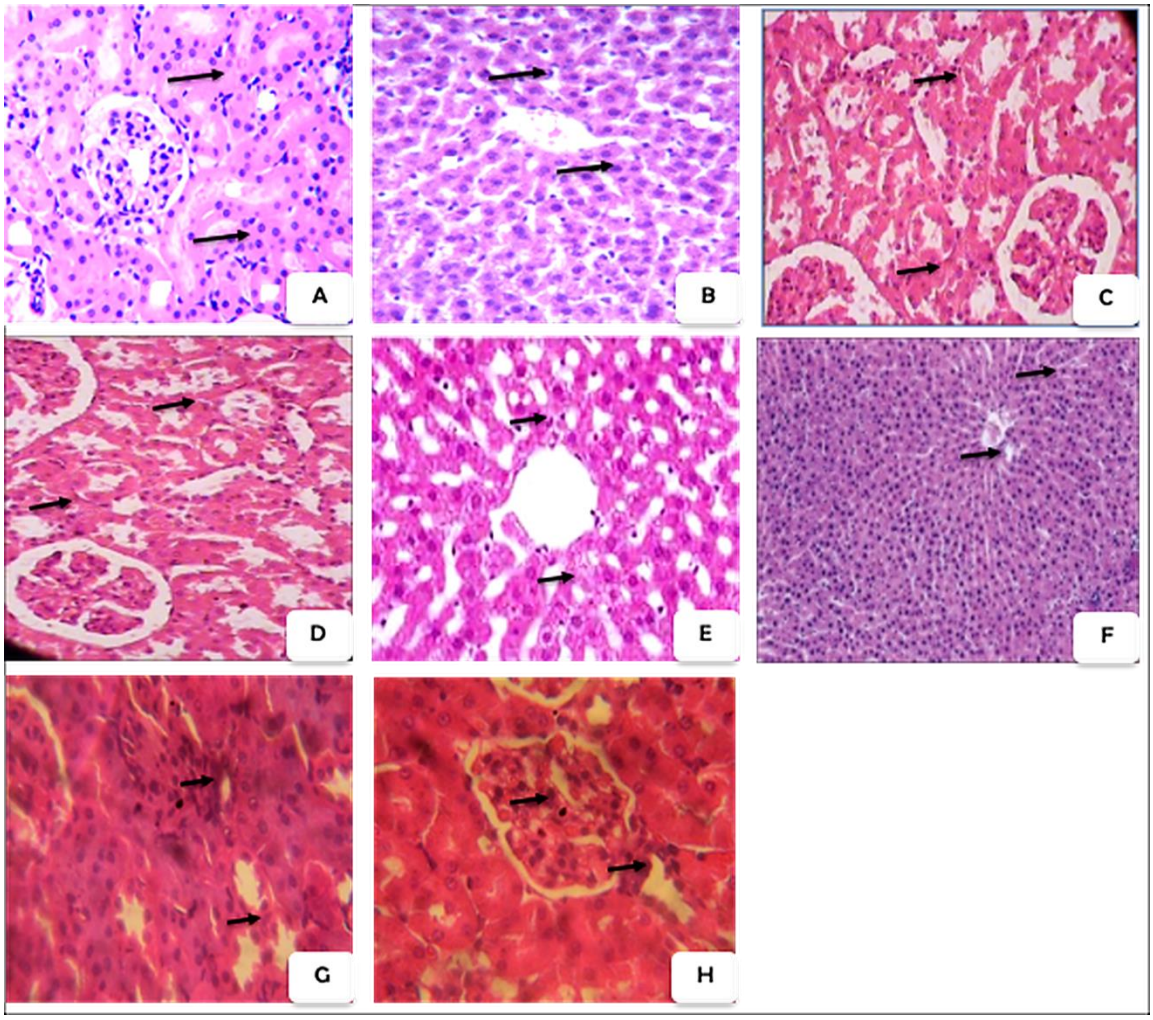

Figure 1: Photo-micrographs of liver and kidney tissues of Albino rats (×400)

(A) Liver sample of animals given laboratory fermented whey at day 14 (B) Liver sample of animals given locally fermented whey at day 14 (C) Kidney sample of animals given laboratory fermented whey at day 14 (D) Kidney sample of animals given locally fermented whey at day 14 (E) Liver sample of animals given laboratory fermented whey at day 28 (F) Liver sample of animals given locally fermented whey at day 28 (G) Kidney sample of animals given laboratory fermented whey at day 28 (H) Kidney sample of animals given locally fermented whey at day 28.

Discussion

The comparison between locally fermented and laboratory prepared using microbiological, physicochemical, and *in vivo* toxicological analysis in albino rats was

analyzed. The results highlight how crucial regulated fermentation conditions are for reducing the dangers of long storage, especially when it comes to turning cheese whey a nutrient-rich dairy byproduct into safe, valuable products. These findings highlight possible risks from unregulated processes typical in local (Fulani) settings and are consistent with the increasing focus on whey production for sustainable food applications.

The continuous lactic acid fermentation, which is caused by microbial metabolism of leftover lactose, is reflected in the gradual drop in pH and rise in acidity over a 28-day period in both fermented whey samples. Locally fermented whey showed alterations (pH from 4.11 to 3.17; acidity from 0.29% to 0.39%), laboratory-fermented whey showed a mild pH reduction from 4.0 to 3.52 and an increase in acidity from 0.28% to 0.35%; (Table 1 and Table 2). This discrepancy points to increased microbial activity and variety in the local sample, possibly as a result of poor hygiene and unclear starting cultures, which accelerated acidification. Recent research on fermented ovine whey-based fruit drinks that were refrigerated showed the pH gradually dropped, which was explained by the native microbiota's continuous generation of lactic acid. Optimal storage times were also found to preserve physicochemical stability in demineralized whey fermented with *Kluyveromyces marxianus*; excessive acidification after 14 days compromised sensory quality (Nedanovska *et al*., 2022). In addition, this report also conforms with (Anggara, 2025) study on blueberry cream cheese yogurt drinks found that the pH changed over the course of 21 days which highlighted how storage affects acidity and peroxide levels. These are in line with other extensive studies by (Altinay & Şanli, 2025) and (Sikombe *et al*., 2025) on whey cheese, which found that microbial interactions and whey content affect the pH during storage.

The microbial analysis revealed that laboratory-fermented whey had low initial bacterial counts (1.7×10^3 CFU/ml) and no

fungus, with beneficial *Lactobacillus spp*. predominating with *Staphylococcus aureus*, *Micrococcus spp*., and *S. epidermidis*. Meanwhile, locally-fermented whey showed higher levels of bacteria (2.3×10^3 CFU/ml) and fungi (9.0×10^3 CFU/ml), (Table 3). These include pathogens like: *Klebsiella spp*., *Salmonella spp*., *Shigella spp*., *Bacillus spp*., and *E. coli*, as well as fungi like: *Aspergillus niger*, *A. flavus*, *Mucor racemosus*, *Penicillium spp*., and *Saccharomyces cerevisiae* (Table 4). These results indicate that contamination hazards in local areas (Fulani) fermentation are probably caused by environmental factors or inadequate hygiene during the handling and manufacture of cheese. Findings have also reported whey has been susceptible to microbial contamination, especially in non-sterile conditions. Although whey promotes beneficial microbial development but unmanaged conditions can introduce pathogens, jeopardizing safety. According to (Uribe-Velázquez *et al*., 2026), whey has proved to be a fermentation substrate for lactic acid bacteria (LAB), yeasts, and fungi. Indigenous LAB is used in acid whey valorization to produce probiotic goods, however if hygiene is poor, coliforms and enterobacteria are still a risk as confirmed by our report. When cheese whey was produced under regulated settings, studies on fermented whey-based snacks revealed low pathogenic levels, guaranteeing microbiological safety (Cao *et al*., 2026). *Aspergillus spp*. contamination, as seen here, is consistent with reports of filamentous fungi creating hazards in dairy and pastry goods (Shi *et al*., 2025) requiring the use of natural preservatives.

At day 14, histopathological analysis revealed no notable changes in the liver or kidneys, indicating that fermented whey is not acutely hazardous. On day 28, however, those exposed to locally fermented whey experienced more severe renal tubular degeneration or destruction, glomerular epithelial alterations, and dose-dependent liver necrosis. This development suggests the accumulation of sub-chronic toxicity, which

may be connected to microbial pollutants and their metabolites. The protective characteristics of controlled fermented whey found in another toxicological research contrast with these negative consequences and this is due to long storage according to our findings. It was argued that fermented whey reduced oxidative stress and organ damage through bioactive peptides that controlled the hepatotoxicity and nephrotoxicity caused by aflatoxin B1 (AFB1) and ochratoxin A (OTA) *in vivo* (Frangiamone *et al*., 2023). This benefit remains intact as confirmed by our study due to no noticeable organ damage at day 14 of whey storage. However, (Cava *et al*., 2024) reported possible increases in liver or kidney toxicity indicators from whey protein due to prolong take without caution which is consistent with our findings that are dependent on dose and source. Mycotoxins produced by *Aspergillus flavus*, such as aflatoxins, which are known to contaminate dairy and cause hepatorenal toxicity, are probably the origin of the increased harm caused by local whey. *Aspergillus* species are the main producers of aflatoxin M1 (AFM1), which poses a concern during storage (Kovac Tomas *et al*., 2025). Endotoxins from pathogens like; *Salmonella* and *Klebsiella* may also contribute to the organ deterioration and inflammation (Roszak *et al*., 2025).

Conclusion

The result of our findings showed that although fermented cheese whey has potential as a source of nutraceuticals. However, long-term preservation particularly of locally produced varieties presents toxicological and microbiological hazards, requiring stringent quality standards. The use of rat models, which might not be entirely applicable to people and the use of no-sex identification effect are still limitations. Therefore, there is also need for further research on fermentations interactions with toxins and human clinical trials to confirm safety.

Declaration: *The authors declare no conflict of interest in the publication of this work*

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