



Heavy Metal and Shelf-life Analysis of Milk and Millet-based Calcium Rich Dietary Supplement (Choco Chips)

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10.18805/ajdfr.DR-2510

ABSTRACT

Background: The global burden of bone related musculoskeletal issues and osteoporosis is rising due to ageing populations and widespread micronutrient deficiencies, particularly calcium and vitamin D. In India, occupational groups with limited sun exposure and poor dietary intake are at increased risk of compromised bone health, which indicates the need for practical, food-based interventions.

Methods: Calcium rich and micronutrient-rich milk and millet based dietary supplements (Choco chips) were formulated using locally available ingredients through standardized processing techniques. The formulated and developed milk and millet-based supplement was evaluated for sensory acceptability using a 9-point hedonic scale, followed by analysis of heavy metal, microbial and shelf-life study.

Result: The observed results indicated that formulation I of the developed supplement showed the highest mean acceptability score (39.05±4.23). One-way ANOVA for sensory evaluation among three formulation showed 1% level of significant difference in flavour and among the groups, while colour and overall acceptability of the formulated supplement were significant at 5% level of significance. Assessment of microbial safety indicated that the product remained stable and safe during storage which is safe for consumption. Heavy metals such as cadmium, chromium, lead, copper and nickel (BLQ (LOQ: 0.01) were within safe limits and the shelf life during 30 days of storage indicated, moisture content between 3.65% and 4.21% and fat acidity between 0.44% and 0.49%, confirming acceptable shelf stability.

Key words: Calcium and vitamin D deficiency, Dietary supplement, Heavy metal, Micronutrient deficiency, Milk and millet, Nutritional quality, Occupational risk, Shelf life.

INTRODUCTION

According to global burden of disease 2019 the study by international osteoporosis foundation project that population growth and aging would result in a large increase in fracture incidence, prevalence of osteoporosis and Disability-Adjusted Life Years-DALYs (Zeng *et al.*, 2025). Globally, there were approximately 178 million new fracture cases in 2019, 133 times more than in 1990 with 25.8 million Years of Disability (YLD), a 65.3% increase from 1990 to 2019 (Wang *et al.*, 2023). In addition, people are experiencing acute, excruciating pain, fracture patients frequently experience long-term functional impairments, secondary osteoporosis, deep vein thrombosis and psychosomatic complications like anxiety and depression (Rundgren *et al.*, 2020).

India is the second most popular country in the world with a steady rise in life expectancy where a proportion of the ageing population is increasing rapidly. It is also projected that approximately 20% of the Indian population will be aged ≥60 years which results in osteoporotic fractures, particularly hip fractures, where the male-to-female ratio is estimated to be 1:3. Loss of bone mass increases the incidence of fragility fractures, particularly in the elderly, by decreasing the compressive and torsional strength of bone. Therefore, it is essential to detect osteopenia and osteoporosis early in order to lower the risk of fractures and the related morbidity and death (Saravanan *et al.*, 2025).

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How to cite this article: Karuppusamy, K.S. and Varadharajan, P.P. (2026). Heavy Metal and Shelf-life Analysis of Milk and Millet-based Calcium Rich Dietary Supplement (Choco Chips). *Asian Journal of Dairy and Food Research*. **45(5)**: 875-883. doi: 10.18805/ajdfr.DR-2510.

Submitted: 09-06-2026 **Accepted:** 09-07-2026 **Online:** 29-07-2026

Beyond Iron, deficiencies in vitamin B12, folate, calcium and vitamin D are common. Macrocytic anaemia and neuropathies associated with vitamin B12 deficiency contributes to low consumption of animal products (Killeen *et al.*, 2025). Similarly, inadequate calcium and vitamin D leads to musculoskeletal problems that are already aggravated by repetitive mechanical work. Females are more susceptible to osteopenia and early osteoporosis due to lower dairy intake and higher physiological requirements. As pointed out by Lakhani *et al.* (2024) still gaps exist in the assessment of the complete burden of micronutrient

deficiencies since biochemical investigations shows only deficiencies and very few explicitly connect them to clinical consequences like fractures or long-term impairment.

Garment industry workers, particularly women, are considered a nutritionally vulnerable occupational group due to long working hours, repetitive physical tasks, inadequate dietary intake and limited access to workplace health interventions. Studies have reported a high prevalence of work-related musculoskeletal disorders among garment workers, which may lead to calcium and vitamin D deficiency that adversely affects bone and muscle health. Inadequate calcium intake, along with increased physiological requirements among both men and women, may predispose this garment workers to osteopenia, osteoporosis and fracture risk (Gebrye *et al.*, 2025). Therefore, the development of an affordable, nutrient-dense, calcium-rich dietary supplement using locally available ingredients represents a practical nutritional strategy to support bone health and reduce the burden of musculoskeletal complications among garment workers.

A study by IIFPT, 2025 claims behavioural interventions to have strong long-term potential, but the available evidence is still limited and inconsistent. Workplace-based nutrition education programs showed measurable improvements in dietary choices and hygiene practices. Most importantly, education alone may not be sufficient unless supported by structural improvements in dietary practices, eventually there should be improvement in wages and also availability to food choices.

Millet-based composite food mixes are nutritionally rich and provide a balanced combination of macro- and micronutrients (Geetha *et al.*, 2023). Millets such as ragi (364 mg/100 g), bajra (27.35 mg/100 g) and jowar (27.6 mg/100 g) serve as excellent base ingredients, with ragi being particularly rich in calcium. Oilseeds like sesame

(1664 mg/100 g) and garden cress seeds (318 mg/100 g) provide exceptionally high calcium along with essential minerals such as iron and zinc, while nuts like almonds contribute additional calcium, protein and healthy fats (IFCT, 2017). Finger millet is a highly nutritious cereal grain traditionally incorporated into South Indian diets and complementary food formulations because of its rich content of dietary fibre, calcium, iron and essential amino acids (Fathimaa *et al.*, 2026). Pearl millet is a nutrient-dense cereal rich in protein, dietary fibre, essential fatty acids, minerals such as calcium, iron, zinc, copper and magnesium, as well as vitamins E and B-complex, making it a valuable ingredient for the development of nutrient-enriched food products (Bansal *et al.*, 2022).

Hence, based on the local availability of ingredients, their nutritional potential and functional suitability, we planned to develop calcium and micronutrient rich milk and millet based dietary supplement and to study their safety in terms of heavy metal concentration and shelf life was attempted as an interventional strategy to address calcium deficiency and its related health setback on bones. Fig 1 presents the conceptual framework of the present study.

MATERIALS AND METHODS

Ingredient selection and procurement

Identification of ingredients (cereals/millets, milk and milk products, sugars, nuts and oilseeds) was undertaken based on the nutrient content particularly calcium content along with their economic feasibility and local availability to ensure sustainability. The selected ingredients were procured from local farmers and local supermarket of Coimbatore, Tamil Nadu, India. Procured ingredients were cleaned for dust and dirt, meticulously checked for

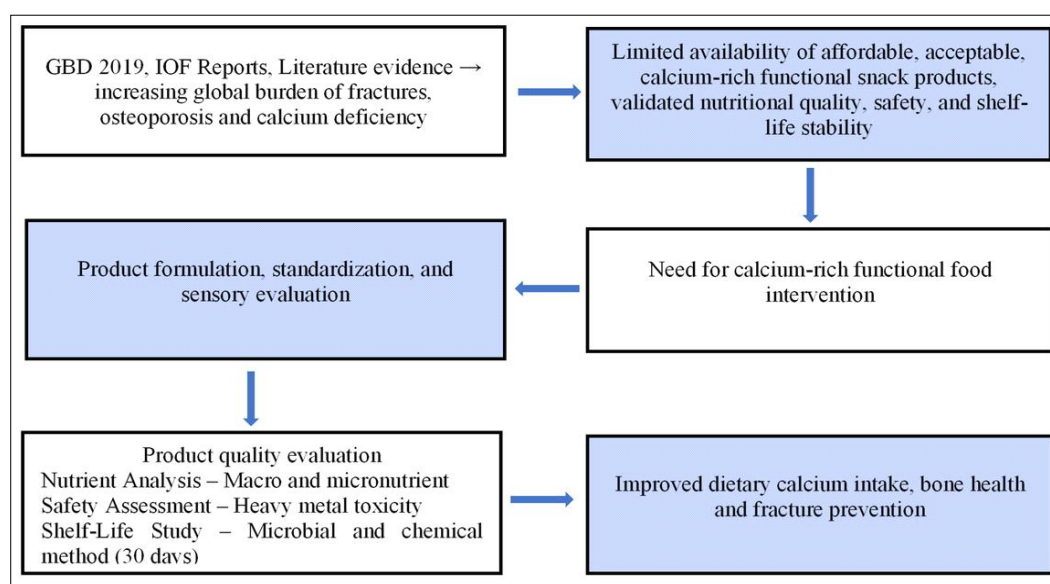


Fig 1: Conceptual framework of the study.

infestation and were stored under controlled room temperature maintained between 27°C to 30°C.

Formulation and development of recipe

A total of five trial formulations were developed and evaluated for sensory acceptability with respect to taste, texture and nutritional quality. Among the five trials, Trial IV showed the highest overall acceptability score and was therefore selected for the formulation. Thus, three formulations were developed, each with a unique value-added ingredient (Formulation I-pulses, Formulation II-green leafy vegetables and formulation III-underutilized seed). The formulation comprised ingredients from four food groups, namely millets (35-60%), milk and milk products (20-40%), nuts and oilseeds (10-20%) and sugars (10-20%). Millets constituted the major proportion of the formulation, contributing to its nutritional and functional properties.

Sensory evaluation

Sensory evaluation of formulated milk and millet-based calcium rich dietary supplement was carried out by a panel of 30 semi trained members using 9-point hedonic scale to ensure the acceptability of the formulated supplement. Based on acceptability score formulation 1 (Choco chips) was subsequently selected and subjected to nutritional, heavy metal and microbiological analyses.

Nutrient composition of the calcium rich product

The milk and millet-based calcium rich dietary supplement was calculated for nutrients such as energy, carbohydrate, protein, fat, fibre, iron, calcium, magnesium, zinc and phosphorous content using Indian food composition Table 2017. The nutritive value was expressed per 100 g of the formulated supplement and the nutrient composition of the developed supplement was calculated using the formula:

Nutrient value per 100 g of the product =

$$\frac{\text{Ingredient quantity used in grams}}{100} \times \text{IFCT value per 100 g}$$

Analysis of formulated milk and millet based dietary supplement (Choco chips)

Analysis for heavy metal toxicity

Heavy metal analysis was performed to ensure the safety of the supplement by detecting the presence of contaminants such as lead, cadmium and arsenic within permissible limits. The formulated dietary supplement sample was analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) following standard digestion procedures. The obtained results were expressed in mg/kg of the sample and the results are expressed as BLQ-below the limit of quantification indicating negligible or non-detectable levels.

Microbial safety of the formulated supplement

The microbiological quality of the formulated calcium rich dietary supplement was conducted to assess the safety of

the formulated choco chips. Total plate count was determined using IS 5402 (Part 1): 2021, while yeast and mould were analysed as per IS 5403: 1999. The presence of specific pathogens such as *Bacillus cereus* (MYP agar medium) coliforms (VRBA) and *Salmonella* (XLD agar) was evaluated using respective BIS method under controlled incubation conditions (30°C for 24 hours) to allow microbial growth and enumeration. The results were expressed as colony forming units per gram (CFU/g) for quantitative analysis, while pathogen detection such as *Salmonella* was reported qualitatively as presence or absence (P/A) per 25 g of sample.

Shelf life of the formulated supplement

Further microbial quality of the formulated supplement was carried out to determine products safety and shelf stability for a period of 30 days at regular storage intervals of 10 days and microbial load was expressed as colony forming units per gram (CFU/g).

Shelf-life evaluation was also carried using chemical method by storing the product under defined conditions mainly temperature (25°C), humidity (60% RH), airtight packing and dry storage conditions and periodically assessing changes in quality attributes such as moisture and acidity of extracted fat was analysed at four intervals (0th day, 10th day, 20th day and 30th day) using standard methods (IS 12711:1989). The obtained results were expressed in percentage showing the chemical stability and suitability for consumption during the storage period.

The research design adopted for the formulation and evaluation of the milk and millet-based calcium-rich dietary supplement is presented in the above Fig 2. The present study was carried out at the Department of Food Service Management and Dietetics, Avinashilingam Institute for Home Science and Higher Education for Women during the time period between January 2025 to December 2025.

RESULTS AND DISCUSSION

Sensory evaluation

Overall, Trail I consistently showed the lowest mean scores across all the parameters, especially in appearance, flavour, taste and texture. Trail IV obtained the overall mean score of 33.4±6.90 indicating enhanced sensory attributes (Table 1).

Based on the overall sensory attributes, Trail IV was the most preferred formulation, while Trail I was least accepted. These results directed us to make suggestive changes in the formulated recipe to enhances the sensory quality of the product for better consumer acceptance. The study by Daniel and Sokale (2024) on standardized cookie recipes using healthy alternatives reported that incorporation of nutrient-rich substitutes significantly influenced sensory attributes such as appearance, flavour, taste and texture. Their findings reported that optimized formulations achieved higher acceptability scores compared to control formulations, mainly due to improved

balance of ingredients and better textural characteristics developed during baking. These observations are in strong agreement with the present study, where Trail IV recorded the highest overall mean sensory score (33.4 ± 6.90) and it is comparable to the standard product prepared, indicating a high level of consumer acceptability while Trail I showed the lowest acceptability across all parameters. Hence the trial with the least score (Trail I, II, III and V) were withdrawn among which Trail IV with highest overall mean score was selected for formulation.

Thus, the above web diagram Fig 3 shows the sensory evaluation of formulated milk and millet-based calcium rich dietary supplement (Choco chips) across three formulation which showed noticeable changes in their acceptability score. Each formulation was incorporated with a novel value-added functional ingredient, to enhance organoleptic and nutritional quality. Thus, the formulation I was developed with a pulse with high protein and calcium

content, formulation II with a low-cost green leafy vegetables and formulation III with an underexploited plant seed.

The mean score of formulation I ranged between 7.37 ± 0.83 and 7.4 ± 0.93 for appearance, flavour, taste, texture and colour with an overall acceptability score of 39.05 ± 4.23 which suggests that the formulation I was highly acceptable by majority of the semi trained panellists. Overall findings revealed that incorporation of value-added ingredients influenced the sensory attributes of the milk and millet-based calcium rich dietary supplement with formulation I (Choco chips) showing higher overall acceptability and further formulation I was subjected to nutrient analysis, heavy metal analysis, microbial safety and shelf-life analysis.

The one-way ANOVA for sensory evaluation Table 2 showed highly significant differences in flavour and taste among the groups, while the overall acceptability of the formulated supplement was significant at 5% level of significance. But in contrast the appearance and texture of the formulated milk and millet-based calcium rich dietary

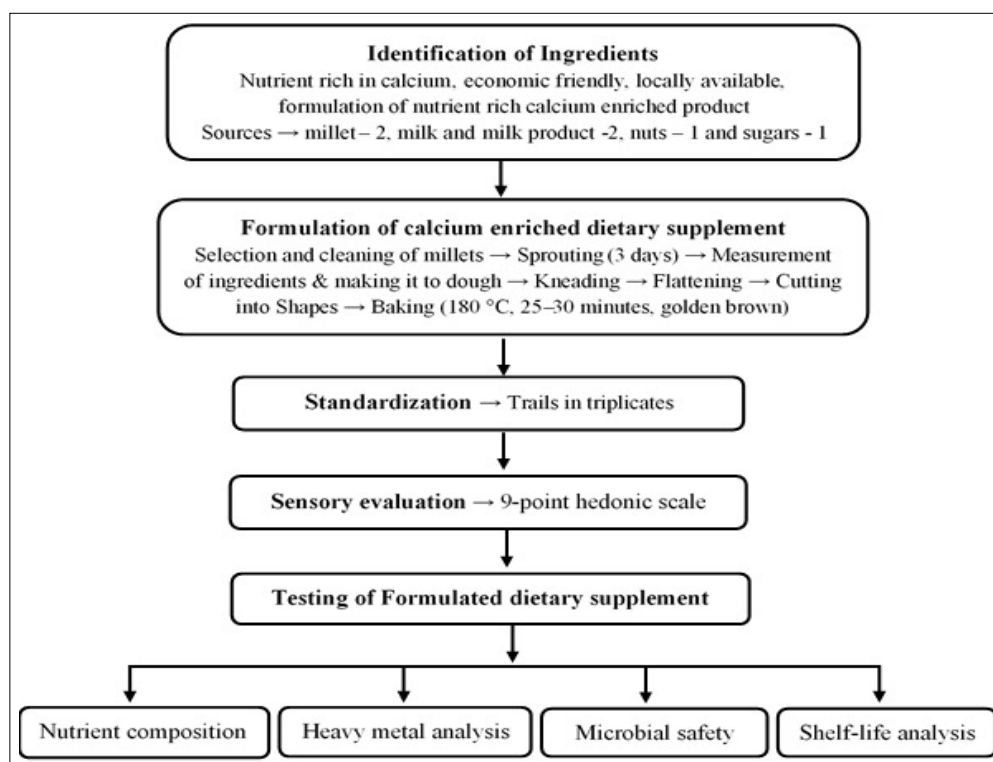


Fig 2: Research design of formulated milk and millet-based calcium dietary supplement.

Table 1: Mean acceptability score of formulated milk and millet-based calcium rich dietary supplement (Trail).

Trails	Appearance	Flavour	Taste	Texture	Colour	Overall acceptability
Standard	6.13 ± 0.6	6.6 ± 0.94	7.06 ± 0.92	7 ± 0.89	6.5 ± 1.3	33.4 ± 4
Trail I	5.26 ± 0.99	5.06 ± 1.33	4.73 ± 1.56	4.73 ± 1.80	5.26 ± 1.33	25.06 ± 6.11
Trail II	6.33 ± 1.49	6.2 ± 1.42	5.73 ± 1.87	5.53 ± 1.82	6 ± 1.75	29.8 ± 7.80
Trail III	5.13 ± 1.62	6.26 ± 1.52	6.53 ± 1.49	6.46 ± 1.58	5.53 ± 1.89	29.93 ± 7.37
Trail IV	5.66 ± 1.53	6.73 ± 1.38	7.2 ± 1.37	7.2 ± 1.37	6.6 ± 1.89	33.4 ± 6.90
Trail V	5.66 ± 1.81	6.66 ± 1.49	6.86 ± 1.30	6.8 ± 1.16	5.8 ± 1.78	31.86 ± 6.46

supplement did not exhibit significant differences. Further findings indicated that three different formulations had notable impact particularly on flavour, taste and overall acceptability of the formulated milk and millet-based calcium rich dietary supplement

Table 3 shows the nutrient composition of three formulated milk and millet-based calcium rich dietary supplement (Choco chips) and it showed minimal differences in both macro and micro nutrients. Slight difference was observed among the three formulations for calcium (Formulation I was 76 mg higher than formulation II and 84 mg higher than formulation III) and iron (formulation I was 3 mg higher than formulation II and 4 mg higher than formulation III), while magnesium, zinc and phosphorous remained relatively consistent. Overall, the formulated

supplement indicated balanced nutrient profile with very less differences across the formulation.

The analysed nutrient composition of the formulated and developed calcium rich dietary supplement (Formulation I) provided with 471 kcal/100 g with protein (10.9 g), fibre (11.6 g) and high calcium (570 mg) and iron (15.9 mg) and it is evaluated for heavy metal toxicity, microbial safety and shelf-life analysis. Considering the high prevalence of inadequate calcium intake, musculoskeletal complaints and compromised bone health among garment workers, particularly women, regular consumption of the developed calcium rich dietary supplement may contribute to improved calcium intake, support bone mineralization and potentially reduce the risk of osteopenia and osteoporosis. The protein content (10.9 g/100 g) of the supplement may help maintain muscle mass and musculoskeletal function, while the iron content (15.9 mg/100 g) helps in addressing micronutrient inadequacies commonly reported among industrial workers.

Table 4 on heavy metal content for cadmium, chromium, lead and nickel was very negligible and was below the limit of quantification. Therefore, the above finding confirmed that the formulated milk and millet-based calcium rich dietary supplement does not possess any potential health risk. Copper (Cu) was detected at 4.05 mg/kg, which was within permissible limits and was considered safe, as copper is an essential trace element required for various physiological functions, including enzyme activity and iron metabolism. The observed results confirm that formulated supplement was within the permissible limit and can be considered safe for regular consumption.

The findings of the present study fall in line with the study conducted by Chowdhury *et al.* (2025), which evaluated

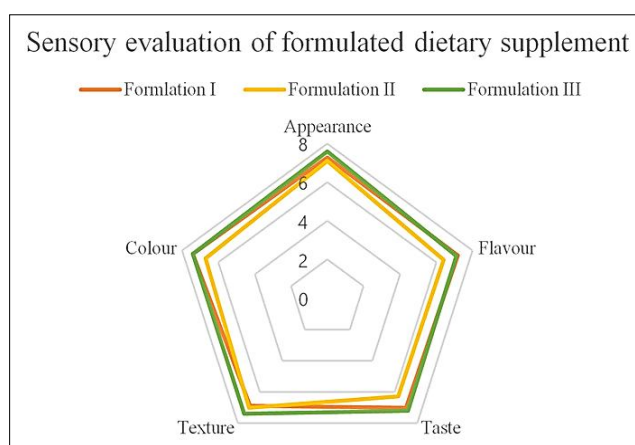


Fig 3: Web diagram-sensory evaluation of three formulations (Choco chips).

Table 2: One-way ANOVA across three formulated milk and millet-based calcium rich dietary supplement.

Attributes (Across three variations)		Sum of squares	df	Mean square	F value	p value
Appearance	Between groups	3.65	2	1.82	1.624	0.204 ^{NS}
	Within groups	114.8	102	1.126	3.874	
	Total	118.51	104			
Flavour	Between groups	12.133	2	6.067	3.874	0.01**
	Within groups	159.714	102	1.566		
	Total	171.848	104			
Taste	Between groups	14.762	2	7.381	4.204	0.01**
	Within groups	179.086	102	1.756		
	Total	193.848	104			
Texture	Between groups	3.962	2	1.981	1.328	0.270 ^{NS}
	Within groups	152.171	102	1.492		
	Total	156.133	104			
Colour	Between groups	9.657	2	4.829	3.175	0.04*
	Within groups	155.143	102	1.521		
	Total	164.800	104			
Overall acceptability	Between groups	167.390	2	83.695	3.086	0.05*
	Within groups	2766.457	102	27.122		
	Total	2933.848	104			

(**Significant at 1% level; *Significant at 5% level; NS- Not significant).

Table 3: Nutritive value of formulated milk and millet-based calcium rich dietary supplement for all three variations.

Nutrients	Formulation I nutrient/100 g	Formulation II nutrient/100 g	Formulation III nutrient/100 g
Energy (Kcal)	417	416	415
Carbohydrate (g)	49	49	47
Protein (g)	12	12	12
Fat (g)	18	18	19
Fibre (g)	8	7	7
Iron (mg)	8	5	4
Calcium (mg)	468	392	384
Magnesium (mg)	111	116	123
Zinc (mg)	2	2	2
Phosphorous (mg)	184	194	186

As per IFCT 2017 calculations.

Table 4: Heavy metals analysis of formulated milk and millet-based calcium rich dietary supplement.

Parameter	Method	Result
Cadmium (Cd)	Inhouse method	BLQ (LOQ: 0.01)
Chromium (Cr)	Inhouse method	BLQ (LOQ: 0.1)
Lead (Pb)	Inhouse method	BLQ (LOQ: 0.1)
Copper (Cu)	Inhouse method	4.05 mg/kg
Nickel (Ni)	Inhouse method	BLQ (LOQ: 0.1)

heavy metal contamination and health risk of biscuits. In their study, heavy metals were presented in small quantities in cookies (0.2 mg/kg) and dry cake (0.4 mg/kg) and it was not detected in toast and salted biscuits. For lead, the maximum permissible limit in food ranges from 0.1 to 0.2 mg/kg as it is a carcinogenic metal known to adversely affect the human brain and kidneys, but previous studies by Karimi *et al.* (2023) reported lead in higher concentration than the recommended limit in some animal products.

Microbial analysis of the formulated supplement Table 5. indicated excellent hygienic quality for safety and consumption and the total plate count was reported to be <10 CFU/g, reflecting a very low microbial load. Similarly, yeast and mould counts were also <10 CFU/g, indicating minimal risk of spoilage and extended shelf stability. The presence of *Bacillus cereus* and Coliform was found to be <10 CFU/g, which was well within safe limits and does not cause any risk of foodborne illness. *Salmonella* was absent in 25 g of the sample, confirming that the product is microbiologically safe and free from major pathogenic bacteria.

A study by Mukherjee and Uppaluri 2025 indicated freshly prepared cookies exhibited total plate counts and yeast and mould counts below detectable limits ($\approx$ <10 CFU/g), showed excellent microbiological quality and safety. Similarly, research on functional and fortified cookies reported coliforms <10 CFU/g and low yeast and mould counts, assuring that such products remain within permissible safety limits and it poses minimal risk for spoilage or contamination (Avila-Nava *et al.*, 2022).

Vatwani and Ravat (2025) in their recent study reported no microbial growth in fortified ragi cookies during storage which falls in line with the present study and stated that the permissible microbial limit for baked products is less than 10^5 CFU/g confirming their microbial safety, good storage stability and suitability for consumption.

Table 6. shows the shelf-life analysis of formulated product which belongs to the category of low-moisture baked foods, which generally possess reduced water activity and limited available moisture for the growth of enteric pathogens such as *Salmonella* and coliform bacteria. In low-moisture bakery products, spoilage is predominantly associated with fungal growth (yeasts and moulds) and spore-forming bacteria such as *Bacillus cereus*, which can survive baking temperatures and subsequently affect product quality during the time of storage. Therefore, TPC, yeast and mould count and *Bacillus cereus* were considered more relevant indicators for assessing the microbiological stability and it remained stable for 30 days as TPC, yeast and mould count and *Bacillus cereus* were observed below detectable levels (<10 CFU/g).

Similar observations were reported in the study conducted by Mukherjee and Uppaluri (2025) on cookie samples developed using indigenous grains of Northeast India, where the microbial count for TPC, yeast and mould, *E. coli*, *S. aureus*, *B. cereus* and *Salmonella* was undetected in the cookies. This suggests that the microbial quality of cookies during storage for 60 days remained good. Another study on value-added cookies incorporated with modified rice starch reported that total plate count and fungal counts were absent or below detectable levels during the initial storage period and remained within safe limits up to 45 days of storage, confirming the shelf stability of baked products (Muttagi and Ravindra, 2020).

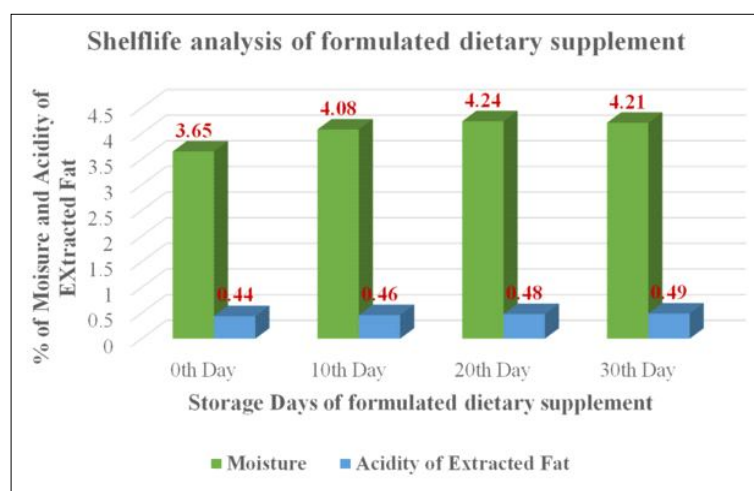
Shelf life and texture of the biscuits usually depend on moisture percentage, thereby formulated supplement on shelf-life analysis Fig 4 revealed gradual changes in their physiochemical properties over the 30 days period. The moisture content on 0th day of formulated supplement

Table 5: Microbial analysis of the formulated milk and millet-based calcium rich dietary supplement.

Parameter/unit	Method	Result
Total plate count (CFU/g)	IS 5402 (Part 1): 2021	<10
Yeast and mould (CFU/g)	IS 5403: 1999	<10
Bacillus cereus (CFU/g)	IS 5887 (Part 6): 2012	<10
Coliform (CFU/g)	IS 5401 (Part 1): 2012	<10
Salmonella (P/A0)	IS 5887 (Part 3) Sec: 1: 2020	Absent/25 g

Table 6: Shelf-life analysis of pathogens in the formulated milk and millet-based calcium rich dietary supplement using microbial method.

Parameter	Method	Unit	0 th day	10 th day	20 th day	30 th day
Total plate count	IS 5402 (Part 1): 2021	CFU/g	<10	<10	<10	<10
Yeast and mould	IS 5403: 1999	CFU/g	<10	<10	<10	<10
Bacillus cereus	IS 5887 (Part 6): 2012	CFU/g	<10	<10	<10	<10

**Fig 4:** Shelf-life analysis of the formulated dietary supplement using chemical method.

showed (3.65%) and a slight increase up to 4.21% on the 30th day. The increase in moisture may be attributed to variations in environmental temperature during the storage. However, this increase in moisture did not affect the texture of the formulated dietary supplement (Choco chips). Similarly, the acidity of extracted fat showed gradual increase from 0.44% at 0th day and 0.49% by the 30th day. A study by Koh *et al.* (2023) on moisture content of non-gluten shortbread cookies for 7 days ranged between 6.2% and 2.19% which is comparable to the findings of the present study. A lower moisture content in the formulated milk and millet-based calcium rich dietary supplement will provide a crunchy structure and a longer shelf life to the cookies. The study by Merlino *et al.* (2022) reported that fat composition and baking conditions significantly influence lipid stability in cookies, where higher fat content and processing conditions can accelerate lipid oxidation and hydrolytic reactions at the time of storage.

The shelf-life study was limited to 30 days due to time constraints and an extended shelf-life study (e.g., 180 days or 6 months) was not carried out, limiting the evaluation of

long-term storage stability of the formulated dietary supplement.

LIMITATIONS

The present study was limited to the development and short-term shelf-life analysis of the formulated supplement. The bioavailability of calcium and other nutrients, as well as long-term storage stability under varying environmental conditions, were not assessed in this paper. Future studies should focus on comprehensive proximate analysis with replicate measurements, bioavailability studies, extended shelf-life evaluation and intervention trials to determine the effectiveness of the supplement in improving nutritional and bone health outcomes among the targeted population.

CONCLUSION

To conclude the formulated and developed supplement (Choco Chips), serves as a supplementary dietary source of calcium and other micronutrients for individuals who have inadequate calcium intake. Also, the enhanced mineral

content of the cookie contributes to improved overall nutritional quality, as the presence of essential minerals and vitamins has been reported to support general function and bone health.

ACKNOWLEDGEMENT

The authors express sincere gratitude to the laboratory staff and technical personnels for their support and assistance in carrying out the analysis performed in the study. Their expertise and cooperation greatly contributed to the successful completion of the analytical work. Author greatly acknowledges the University Grants Commission for providing the financial assistance for the study.

Disclaimers

The views and conclusions of the study expressed are solely those of authors and does not necessarily reflect the opinions of the affiliated institution. The authors had made accuracy and completeness of the information provided and disclaim any responsibility or liability for any direct or indirect loss arising from the use of this content.

Informed consent

The researcher obtained informed consent from all the panellist prior to sensory evaluation and study was approved for ethical clearance from Institutional Human Ethics Committee [Approval No: IHEC/22/23/AUW/IHEC/FSMD-22/FHP-5].

Conflict of interest

Both the authors do not have any conflict of interest for publishing this article.

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