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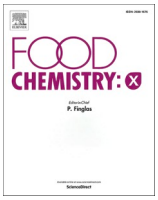

Effect of selective fermentation on nutritional parameters and techno-functional characteristics of fermented millet-based probiotic dairy product

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ABSTRACT

The primary goal of this study was to assess the effect of selective fermentation on the nutritional and techno-functional characteristics of fermented millet-skim milk-based product. The product was made with HHB-311 biofortified pearl millet (PM) flour, skim milk powder, and isolated cultures (either alone or in combination) of *Limosilactobacillus fermentum* MS005 (LF) and *Lactobacillus rhamnosus* GG 347 (LGG). To optimize fermentation time, time intervals 8, 16, and 24 h were explored, while the temperature was kept 37 °C. Results of protein digestibility showed that LF (16 h) and LGG (24 h) fermented samples had significantly higher ($P < 0.05$) protein digestibility of 90.75 ± 1.6% and 93.76 ± 3.4%, respectively, than that of control (62.60 ± 2.6%). Further, 16 h fermentation with LF showed enhanced iron (39%) and zinc (14%) bioavailability. The results suggested that LF with 16 h fermentation is most suitable for making millet-based fermented products with superior techno-functional attributes and micronutrient bioavailability.

1. Introduction

Fermentation is a type of metabolic processing that oxidizes carbohydrates to release energy when no external electron acceptors are present (Rollán, Gerez, & LeBlanc, 2019). As per the expert panel report of The International Scientific Association for Probiotics and Prebiotics (ISAPP), fermented food is "*food made through desired microbial growth and enzymatic conversions of food components*" (Marco et al., 2021). Traditional/indigenous fermented foods can contribute to enhanced nutritional state of the consumers and are connected to cultural relationships across other civilizations. Fermentation has grabbed the interest of academics seeking to optimize the process to improve specific nutritional qualities and health benefits of fermented products (El Sheikha & Hu, 2020). Microorganisms employed in the process of fermentation can create a variety of enzymes (i.e., amylase, lipase, protease, phytase, etc.) that hydrolyze carbohydrates, proteins, and lipids into easily digested elements with good texture and flavor (Dhull et al., 2020). During food fermentation, endogenous enzymes are activated due to lower pH, which contributes to the reduction of phytic acid. Fermentation improves mineral (i.e., Ca, Zn, and Fe) bioavailability by producing phytases that reduce phytic acid constituents in plant-based foods (García-Mantrana, Yebra, Haros, & Monedero, 2016; Gupta, Gangoliya, & Singh, 2015). Studies have shown that natural fermentation and strain-specific fermentation provide better mineral bioavailability through a more effective degradation of the plant food's phytic acid content (Nkhata, Ayua, Kamau, & Shingiro, 2018). Nutri-cereals, such as millet, are nutritionally equivalent to cereals because they are abundant sources of nutrients like dietary fiber and phytochemicals that benefit all (Kumar, Tomer, Kaur, Kumar, & Gupta, 2018; Rasane, Jha, Kumar, & Sharma, 2015) and could be utilized to make functional

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products. Fermented milk cereal flour products like *Raabadi*, Boza, Mahewu, and Togwa are widely consumed throughout Africa and the Indian subcontinent (Sudha, Devi, Sangeetha, & Sangeetha, 2016). Combining cereals and milk can improve nutrition and create a more functional product. Incorporating cereal into dairy products might compensate for the lack of fiber in milk and the low digestibility and amino acid deficiencies in cereal (Ganguly, Sabikhi, & Singh, 2022). Most probiotic food items available in global markets are milk-based, with very few developed employing alternative substrates. Cereals are considered the most affordable sources of nutrition and protein for a considerable portion of the human population, especially in underdeveloped countries (Di Stefano et al., 2017). The combination of cereal and milk will result in a synergistic impact, providing improved nutrition and eventually leading to a value-added functional food (Ganguly, Kumar, Singh, & Sabikhi, 2014). Research is being done to prepare millet and milk-based fermented products/substrates to improve nutritional health. Several such kinds of products were prepared previously, such as a composite dairy-cereal substrate (Ganguly & Sabikhi, 2012), *Rabadi*-like fermented milk beverage using pearl millet (Modha & Pal, 2011), fermented millet milk based curd (Sheela, Moorthy UmaMaheswari, Kanchana, Kamalasundari, & Hemalatha, 2018), pearl millet based fermented skim milk product (Basu & Tomar, 2016), whey skim milk-cereal based probiotic beverage (Ganguly, Sabikhi, & Singh, 2021), fermented finger millet-based yogurt-like beverage (Vila-Real et al., 2022), milk-cereal (millet) based composite substrate (Ganguly et al., 2022) and probiotics enriched rabadi beverage (PERB) (Yadav, Shukla, Kumari, Dhewa, & Kumar, 2024). Selective fermentation of millet and skim milk-based blend with desired culture (phytate degrading bacteria) could be a potential strategy to make micronutrient-enriched products with improved bioavailability. There are minimal studies on millet (biofortified) and milk-based composite food items made using desired culture (phytate degrading bacteria) specifically for improving micronutrient bioavailability. In the current study, we have used two lactobacilli, i.e., *Lactobacillus rhamnosus* LGG 347 (LGG) and *Limosilactobacillus fermentum* MS005 (LF). LGG is a well-known probiotic culture that has several health-beneficial properties. LF is one of the best phytate degrading cultures out of seven indigenous cultures isolated from dairy-fermented foods and characterized in our lab. Previous studies showed that fermentation improves the bioavailability of cereal and milk-based fermented products (Basu & Tomar, 2016; Ganguly et al., 2021; Ganguly et al., 2022). However, they did not perform selective fermentation with desired cultures at different durations.

Several indigenous biofortified pearl millet (PM) varieties have been released with high iron and zinc contents, e.g., the HHB-311. However, there is a dearth of micronutrient bioavailability data on these biofortified millet varieties. Therefore, in the present study a millet-based skim milk fermented food developed using the HHB-311 variety in combination with LGG and LF bacteria. The main reason for selecting LF in the present study was its phytate degrading potential, and LGG was chosen due to its well-established health-beneficial attributes. The study also investigated the effects of fermentation on nutritional attributes and techno-functional properties of the millet-based fermented substrate.

2. Materials and methods

2.1. Procurement and processing of raw materials/cultures for product development

HHB-311 PM variety was procured from the Bajra Section of Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar, Haryana. Skim milk was procured from a grocery store in New Delhi, India. *Lactobacillus rhamnosus* GG (LGG) probiotic culture was purchased from the National Centre for Dairy Cultures (NCDC) at ICAR-National Dairy Research Institute-Karnal, Haryana, India. Indigenous LF probiotic culture was selected from the LAB cultures isolated in our lab. Both cultures were used in the present investigation for product development purposes. MRS broth media was used to maintain both lactobacilli. PM seeds were cleaned and washed with double distilled water, then dried at 40 °C for 16 h. PM seeds were ground into flour using a hammermill and then sieved using a 200 μm mesh. After sieving, the flour was placed in a ziplock pouch and stored in a cool and dry place for further use.

2.2. Development of fermented millet-skim milk product (FMSMP)

Fermented products based on PM flour and skim milk powder were developed in the Animal Product Technology Lab, Department of Food Science and Technology, National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonepat, Haryana, India. Fig. 1 describes treatments used for the selective fermentation to prepare FMSMP. In the first step, 12 g of skim milk powder was dissolved in 100 mL distilled water, followed by the addition of 10 g of PM flour (HHB-311), and then the blended sample was continuously stirred and heated at 95 °C for 10–15 min until the mixture was properly gelatinized (Basu & Tomar, 2016). Then, the samples were cooled to 40–42 °C and inoculated with 2% each of LF and LGG, or a combination of both (LF + LGG, 1% each). The inoculation was performed with desired probiotic bacterial cultures at their log phase (16 h of activation). At the time of inoculation, CFU/mL level of probiotic bacteria was~10^{10}. All three experimental samples were incubated in a biochemical oxygen demand (BOD) incubator at 37 °C temperature for different periods, i.e., 8, 16, and 24 h. After each incubation, the fermented samples were removed and stored in the refrigerator (4–6 °C) for further analysis. The nutritional analysis was conducted within 24 h. A sample without culture/fermentation was prepared and used as a control.

2.3. Physico-chemical evaluation of FMSMP

For the estimation of physico-chemical quality of the product, parameters such as pH, total titratable acidity, and total soluble solids were measured for the control (unfermented), and samples (8 h, 16 h, and 24 h) fermented with different cultures.

2.3.1. pH value

The pH of fermented and control samples was estimated using a calibrated pH meter (Eutech Instruments) with a single junction pH electrode (ThermoFisher, Scientific).

2.3.2. Total titratable acidity (TTA)

The total titratable acidity of the fermented and control samples was estimated by standard titration, and results were expressed as percent lactic acid (LA). Calculation of lactic acid (%) was done by following formula:

$$TTA\ as\ LA\ (\%) = (V \times 0.1\ N\ \ NaOH\ \ \times\ \ 9)/M$$

where, V = Volume of sodium hydroxide used for titration; 0.1 N NaOH = Normality of NaOH used for titration; 9 = Factor for lactic acid; M = Mass of the sample used for the titration.

2.3.3. Total soluble solids (°Brix)

Total soluble solids (TSS) of the control (unfermented) and fermented samples were measured using a high-accuracy touchscreen digital refractometer (RX-7000i, ATAGO, Japan). For the assessment of the TSS content of samples, nearly 250 mg of sample was taken and put on the sample stage of the refractometer, and results were recorded as the percentage of Brix.

2.4. Proximate composition of FMSMP

For the estimation of moisture, protein, ash, and carbohydrate content of the control and fermented samples, the standard method of AOAC

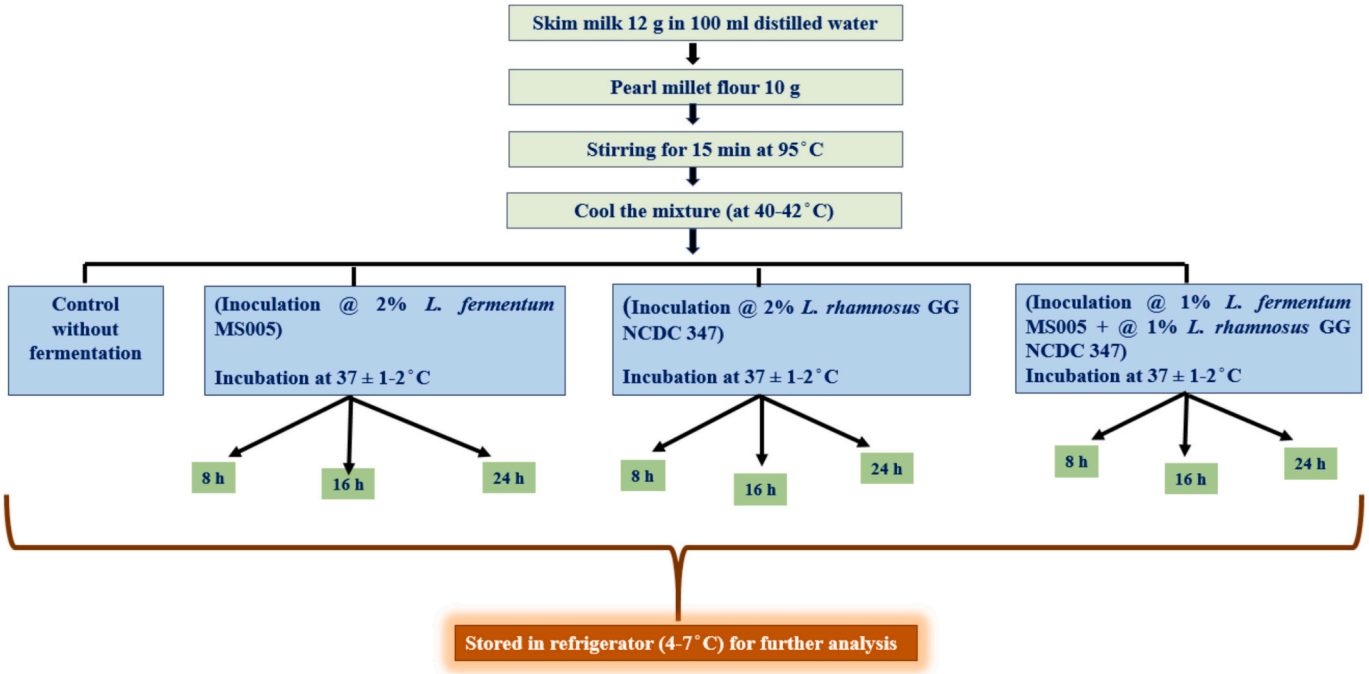

Fig. 1. Schematic illustration of fermentation treatments for selective fermentation.

(2005) was followed.

2.4.1. Estimation of fat

Estimation of fat in control and fermented samples was performed using the Mojonnier method (AOAC, Official Method 989.05, 2012). Fat content was measured using the given below formula:

$$Fat\ (\%) = [(WS - WB)/WP] \times 100$$

where WS = Weight (g) of beaker with fat content (after drying); WB = Weight (g) of empty beaker; WP = Weight (g) of sample.

2.5. Estimation of phytochemical content and antioxidative activity of FMSMP

2.5.1. Extraction

The control and fermented samples were extracted to evaluate phytochemical content and antioxidant activity with slight changes to the technique of Öztürk et al. (2018). For the extraction, 3 g samples were dissolved in 15 mL of 80% methanol and vigorously homogenized for 5 min. The samples were kept undisturbed for 1 h and were then homogenized for 5 min. After centrifugation (~7000 *g* for 10 min at 4 °C), the supernatant was passed through a 0.45 μm syringe filter, and the filtrate was collected in amber-colored falcon tubes (15 mL) and stored at 4 °C.

2.5.2. Phytochemicals

2.5.2.1. Total phenolic content (TPC). The total phenolic content of the samples was estimated according to the method of Kennas, Amellal-Chibane, Kessal, and Halladj (2020) with minor changes. Absorbance of the samples was read at 765 nm (Spectramax M2e system, Molecular Devices, USA). Findings were represented as mg of gallic acid equivalent (GAE)/100 g of sample.

2.5.2.2. Total flavonoid content (TFC). The total flavonoid content in the samples was estimated according to the colorimetric assay Ge et al. (2022) with slight modifications. After incubation, the mixture's absorbance was read at 510 nm, and results were recorded as mg quercetin equivalent (QE)/100 g of sample.

2.5.3. Antioxidative potential

2.5.3.1. Ferric reducing antioxidant power (FRAP) activity. With slight modifications, the FRAP activity was evaluated using a technique reported by Wu et al. (2020). The absorbance was read at 593 nm, and the results were expressed in mM Trolox equivalent (TE)/100 g sample.

2.5.3.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (RSA) assay. The 2,2-diphenyl-1-picrylhydrazyl RSA of samples was determined using the procedure of Singhal, Kumar, and Badgujar (2021), with slight changes. The absorbance was taken at 517 nm, and the control/blank of the assay was prepared by using DPPH and extraction reagent (methanol) only. The results were recorded as the DPPH RSA (%) and the following formula used for calculation:

$$RSA\ (\%) = [A_1 - (A_D - A_S]/A_1 \times 100$$

where A_1 = Control absorbance; A_D = DPPH solution absorbance with the sample; A_S = Sample extract absorbance without DPPH.

2.6. Anti-nutritional factors (ANFs) of FMSMP

2.6.1. Phytic acid

The phytic acid content of the samples was assessed using a phytate estimation kit (Megazyme, K-PHYT, Ireland). Sample (1 g) was taken into a 50 mL conical flask, and 20 mL 0.66 M HCl was added. The conical flask was covered with foil paper and incubated in an incubator shaker (Innova 42, New Brunswick Scientific, USA) at 250 rpm for 3 h at room temperature. A 1 mL aliquot of the extracted solution was transferred into a 1.5 mL tube and centrifuged for 10 min at 18,000 *g*. A portion (0.5 mL) of the resultant extracted supernatant was placed into a new 1.5 mL tube immediately, and subsequently, 0.5 mL of 0.75 M NaOH solution was added to neutralize it. Other components were added following the instructions provided in the kit. Phosphorus standard was prepared at different concentrations (0, 0.5, 2.5, 5, and 7.5 μg), and 0.5 mL of color reagent was added to each tube, following the same procedure as for the sample.

Calculation:

To determine the phytic acid content, the absorbance of "Free Phosphorus" sample was subtracted from the absorbance of "Total Phosphorus" sample, thereby obtaining $\Delta A_{phosphorus}$.

For the estimation of phytic acid, the below-given formula was used:

$$Phosphorousg/100\ g) = \text{mean M} \times 0.1112 \times \Delta A_{phosphorus} \quad (a)$$

where Mean M = Mean obtained from the standard curve of phosphorus; 0.1112 = Multiplication factor given in the kit; $\Delta A_{phosphorus}$ = Absorbance obtained after subtraction of absorbance of the "Free Phosphorus" sample from the absorbance of "Total Phosphorus" sample

$$Phytic\ acid\ (g/100\ g) = \frac{\text{Phosphorous (g/100 g)}}{0.282}$$

where Phosphorous (g/100 g) = Value taken from (a); 0.282 = Factor given in kit to calculate phytic acid.

2.6.2. Tannin

Tannin content of the samples was determined using Anaemene and Fadupin (2022) technique with few modifications. After color development of the standard and sample solutions, absorbance was taken at 760 nm, and results were discussed as mg of tannic acid equivalent (TAE)/100 g of sample.

2.7. In vitro protein digestibility (IVPD) assessment

IVPD of the samples was assessed by following the method of Verma et al. (2021) and Gong et al. (2022) with modifications. The digestion model included three phases: oral/mouth, gastric, and intestinal. The final mixture was kept undisturbed at 37 °C for 1.5 h; subsequently, the reaction was stopped by heating the mixture for 10 min in a 95 °C water bath. The supernatant was collected and kept at −20 °C for subsequent analysis after centrifugation at 5000 *g* for 15 min at 4 °C. Nitrogen content of the 1 mL supernatant was estimated using Kjeldahl apparatus (Gerhardt, Analytical Systems, Germany). The IVPD (%) was calculated using the below-given formula:

$$IVPD\ (\%) = \left(N_{supernatant} - N_{control}\right)/N_{sample} \times 100$$

where $N_{supernatant}$ = Nitrogen content in the sample supernatant (after digestion); $N_{control}$ = Nitrogen of control (following the same process, but without sample); N_{sample} = Nitrogen content in the original sample (without digestion).

2.8. In vitro bioavailability of iron (Fe) and Zinc (Zn)

Fe and Zn bioavailability of the control and fermented samples was estimated according to the approach of Kumar et al. (2017) with slight variations. The sample (5 g) was transferred to a 100 mL conical flask, and 30 mL of distilled water was added. The flask was then shaken in an incubator at room temperature for 16 h at 150 rpm. Then, 2 mL α-amylase solution (6.25 mg/mL) was added and placed in a water bath to incubate for 45 min at 37 °C. The mixture was adjusted to pH 4.0 using HCl solution, followed by addition of 8 mL pepsin (1.25 mg/mL) solution. A water bath was subsequently utilized for incubating samples (1 h at 37 °C). A NaOH solution was added to bring the mixture to pH 6.0, and 10 mL pancreatin solution (20 mg/mL) was added. The resulting mixture was centrifuged at 13,000*g* for 12 min at 4 °C after being left undisturbed at 37 °C for 1 h. The sample supernatant was passed through a 0.45 μm syringe filter and analyzed for the Fe and Zn content using ICP-OES (Optima 7000DV, PerkinElmer, USA) against a water blank (processed similarly). The undigested sample was first digested with acid, and micronutrients were estimated using ICP-OES. The micronutrient bioavailability was measured using the formula:

$$Bioavailability\ (\%) = (M_S - M_B)/M_U \times 100$$

where M_S = Micronutrient in the sample supernatant (after digestion); M_B = Micronutrient in water blank (processed similarly); M_U = Micronutrient in the sample (without enzymatic digestion/undigested).

2.9. Molar ratio of phytic acid/minerals

The molar ratio of minerals to anti-nutrients was determined using the method of Norhaizan, Ain, and A. W. (2009). The mole of phytic acid/minerals was evaluated by dividing the weight of phytic acid (PA) and minerals present in 100 g of sample with the atomic weight of PA (660 g/mol) and Fe (56 g/mol) or Zn (65 g/mol). The molar ratio between PA/mineral was determined after dividing the mole of phytic acid by the mole of minerals.

2.10. Techno-functional properties of FMSMP

2.10.1. Functional groups (FTIR) analysis

Functional groups of unfermented and fermented samples were determined using a Fourier-transform infrared spectrophotometer (Cary 630, Agilent Technologies, USA). The sample was placed on Attenuated Total Reflectance (ATR), which presented the peaks between the wavelength range of 4000–600 cm^{-1}, and the scan was completed in 1–2 min.

2.10.2. Texture profile analysis (TPA)

To analyze the texture profile of unfermented and fermented samples, the TA.HDplusC Texture Analyser (Stable Micro Systems, England, UK) was used. The TPA method was performed as it automatically calculates a range of food texture properties. Program conditions were: 1 mm s^{-1} of heat speed, 25-mm stainless steel cylinder probe with 5 mm s^{-1} moving speed, and 1 mm s^{-1} of test speed.

2.10.3. Apparent viscosity (rheological property)

The apparent viscosity of the samples was determined based on the approach given by Ge et al. (2022) using a rotating rheometer (MCR 52, Anton Paar Co. Ltd., Australia). The apparent viscosity was measured using operating parameters: measuring the temperature of 25 °C, PP50 detector, the gap between the flat and detector was set to 0.5 mm, shear rate ranged from 0.01 to 100 s^{-1} in 300 s, and 30 points were collected for a single sample.

2.10.4. Color profile analysis

Unfermented and fermented samples were assessed for their color properties in terms of L*, a*, and b* using a digital colorimeter (KONICA MINOLTA, INC., Japan).

2.11. Statistical analysis

Microsoft Office (version 2019) was used for raw data tabulation and descriptive statistical calculations. GraphPad Prism (version 5.01) was used for grouped and column statistics, and one-way analysis of variance (ANOVA) was used for the statistical analysis of data, followed by the Tukey post-hoc test to separate the mean ($P \leq 0.05$), which was considered statistically significant at 95% confidence level. The results were represented as mean ± SD (standard deviation).

3. Results and discussion

3.1. Effect of fermentation on physico-chemical properties and proximate composition of FMSMP

A fermented product's pH, acidity, and total soluble solids (TSS) affect its acceptability. As a result, TSS, pH, and acidity were used to optimize the probiotic bacterial culture and incubation duration in this investigation (Fig. S1A–C). The initial TSS value for the control

(unfermented, 0 h) was 15.51 ^{0}Brix; the pH was 6.3; and the acidity was 0.18 (% LA). LF and LF + LGG fermented products recorded a significant decrease ($P < 0.05$) in the TSS (11.35 and 10.27) and pH (4.68 and 3.94), respectively after 24 h of fermentation. Our findings are consistent with recent research by Vila-Real et al. (2022), who reported lower pH values in the finger millet slurries fermented with co-cultures than in single-culture fermentation. The microflora generates organic acid with an increased duration of fermentation, subsequently causing the pH level to drop (Ahmed, Xua, Sulieman, Mahdi, & Na, 2019). Furthermore, the lowest decrease in the TSS and pH was observed for the LGG substrate after 24 h of fermentation. Compared to the LF and LGG, the LF + LGG fermented product revealed a faster decline in TSS, pH, and a rise in acidity after 8, 16, and 24 h of fermentation. The results of our study corroborated those of Di Stefano et al. (2017), wherein the authors reported the strain-dependent fermentation capability of probiotic strains in terms of pH and lactic acid for PM-based probiotic products. Similarly, a reduction in the total solids content has been reported in the fermented skim milk/cereal combinations compared to their unfermented equivalents due to fermentation, corroborating the present investigation findings (Ganguly et al., 2022). Titratable acidity negatively correlated with the pH value, which was expected. For LF, LGG, and LF + LGG, respectively, there was a considerable increase in acidity during fermentation. The utilization of carbohydrates by lactic acid bacteria throughout the metabolic processes leads to a rise in the organic acids, especially lactic acid (Kuria, Matofari, & Nduko, 2021), which increases TTA, as seen in the present investigation.

Table 1 lists the fermented products' proximate composition (protein, fat, ash, carbohydrates, moisture). There were statistically substantial variations in the moisture content readings between the samples fermented with different cultures for varied fermentation times, with values ranging from 79.71 ± 0.18 to 81.67 ± 0.13%. Values for the ash content ranged from 0.90 ± 0.01 to 1.09 ± 0.07%. The control sample had the lowest value for ash content; however, the 8 h fermented sample (LF + LGG) recorded significantly ($P < 0.05$) higher ash content than that of control (unfermented). The fat content ranged between 0.28 ± 0.07 to 0.75 ± 0.10%, with the 8 h fermented sample (LGG) showing the highest fat. Recent research by Divisekera et al. (2021) supported our findings, who found that the moisture, ash, and fat contents of the control and fermented finger millet-based products ranged from 74.00 to 82.33%, 0.57 to 0.76%, and 0.08 to 0.14%, respectively. The present evaluation recorded varying protein content (4.27 to 5.59%) and carbohydrates (11.82 to 14.44%). Carbohydrate content was slightly decreased in most of the fermented substrates compared to the control. According to observations, the carbohydrate content decreased gradually during fermentation at different times in the process. These findings align with earlier results claiming that carbohydrates are a primary carbon source for bacteria to ferment sugars (Adams, 1990; Jan et al., 2022). The maximum protein level was found in the 8 h fermented product (LF), with values ranging from 4.01 ± 0.28 to 5.59 ± 0.22%. Our findings are consistent with those of Basu and Tomar (2016), who found that the PM-based fermented skim milk product contained protein (5.01%), ash (0.82%), fat (0.73%), and moisture (82.18%). The range of carbohydrate content values was 11.82 ± 0.02 to 14.44 ± 0.01%. Carbohydrate reduction could be related to the elevated efficiency of amylolytic enzymes, which results in complex carbohydrates being broken down into simpler forms of sugar. The same pattern of reduced carbohydrate content was observed resulting from the fermentation of maize (Gernah, Ariahu, & Ingbian, 2011), blends of maize-soybeans for weaning (Amankwah, Barimah, Acheampong, Addai, & Nnaji, 2009), 'Fura' (Inyang & Zakari, 2008), which are in consonance with data from present study. The biological activities of microorganisms could cause a decrease in dry matter during the fermentation process, which consumed a certain amount of the substrate nutrients, leading to an overall dry matter reduction (Wedad, El Tinay, Mustafa, & Babiker, 2008).

Table 1
Effect of selective fermentation on proximate composition of fermented millet-skim milk products.

Samples	Moisture (%)	Fat (%)	Ash (%)	Protein (%)	Carbohydrates (%)
Control	79.71 ± 0.18^{a}	0.45 ± 0.08^{ab}	0.90 ± 0.01^{a}	5.29 ± 0.16^{ac}	13.64 ± 0.25^{ade}
LF (8 h)	80.74 ± 0.14^{b}	0.28 ± 0.07^{a}	0.92 ± 0.01^{ab}	5.59 ± 0.22^{a}	12.48 ± 0.15^{bc}
LF (16 h)	81.51 ± 0.16^{cd}	0.49 ± 0.03^{ab}	1.01 ± 0.04^{ab}	4.39 ± 0.05^{bde}	12.60 ± 0.03^{abc}
LF (24 h)	81.18 ± 0.13^{c}	0.55 ± 0.06^{ab}	0.99 ± 0.11^{ab}	4.49 ± 0.39^{bde}	12.80 ± 0.35^{abc}
LGG (8 h)	79.87 ± 0.25^{a}	0.75 ± 0.10^{b}	0.97 ± 0.04^{ab}	4.00 ± 0.28^{be}	14.41 ± 0.67^{de}
LGG (16 h)	80.82 ± 0.12^{bc}	0.50 ± 0.05^{ab}	1.00 ± 0.01^{ab}	4.33 ± 0.01^{b}	13.35 ± 0.06^{abd}
LGG (24 h)	80.96 ± 0.17^{bc}	0.70 ± 0.12^{b}	0.10 ± 0.01^{ab}	4.50 ± 0.10^{bde}	12.83 ± 0.18^{abc}
LF + LGG (8 h)	80.59 ± 0.13^{bc}	0.52 ± 0.07^{ab}	1.09 ± 0.07^{b}	5.02 ± 0.22^{ade}	12.77 ± 0.21^{abc}
LF + LGG (16 h)	79.81 ± 0.11^{a}	0.45 ± 0.11^{ab}	1.03 ± 0.02^{ab}	4.27 ± 0.01^{bde}	14.44 ± 0.01^{e}
LF + LGG (24 h)	81.67 ± 0.13^{d}	0.74 ± 0.05^{b}	0.96 ± 0.04^{ab}	4.80 ± 0.13^{ce}	11.82 ± 0.02^{c}

Data expressed as mean ± SD.
At $p < 0.05$, mean values in the same column with distinct superscript letters (a, b, c …..) are statistically different.
Abbreviations: LF, *Limosilactobacillus fermentum* MS005; LGG, *Lactobacillus rhamnosus* GG 347.

3.2. Effect of fermentation on phytochemical and antioxidant of FMSMP

Phenolic substances are considered to provide several health advantages, primarily serving as potent antioxidants. Total polyphenol content (TPC) was higher in the control (unfermented) substrate compared to all fermented products except the 16 h and 24 h LGG fermentation (Fig. 2A). TPC ranged from 12.58 ± 0.53 to 53.91 ± 2.57 mg GAE/100 g sample. The 16 h and 24 h LGG fermentation products had TPC values of 39.68 ± 2.28 and 53.91 ± 2.57 mg GAE/100 g, respectively. Study results indicate that milk protein and phenolics from PM might interact, and phenolic releases during LGG fermentation were relevant. Sample fermented for 8 h, 16 h, and 24 h with LF or LF + LGG were found to have considerably ($p < 0.05$) lower TPC concentrations than the control sample, which may be due to the LF culture. LAB strain's hydrolytic enzymes degrade complex phenolic substances into simpler compounds, which results in a rise in TPC after LAB fermentation (Li et al., 2020). The results obtained in this study may be due to using LF culture for fermentation because the products from LF, singly or in combination, showed reductions in TPC. Polyphenol levels decreased during the fermentation of treated grains, indicating that the microflora can ferment phenolics (Bravo, 1998). Additionally, phenolic molecules might exhibit a prebiotic impact and promote the abundance of probiotics along with other beneficial bacteria, demonstrating a reciprocal link among phenolic substances and probiotics (de Llano et al., 2017; Gibson et al., 2017; Ozdal et al., 2016; Succi et al., 2017). Consequently, probiotics and starter cultures used in the present study might contribute to decreasing these bioactive substances by promoting the biotransformation of phenolic molecules during fermentation. The TFC overall trend in all samples differed from the TPC, as seen in Fig. 2B. TFC levels in fermented samples were generally higher in most categories than in control samples. However, only the 16 h and 24 h LGG fermented products demonstrated significant increases ($p < 0.05$) in TFC. TFC content ranged from 12.20 ± 1.24 to 20.70 ± 0.57 mg QE/100 g sample. Additionally, although the percentage of PM remained the same, TFC varied dramatically between samples fermented with different probiotic strains. This revealed that the growth of different cultures could considerably impact the TFC. The basis for this increase has been attributed to the LAB, which form compounds as metabolic byproducts

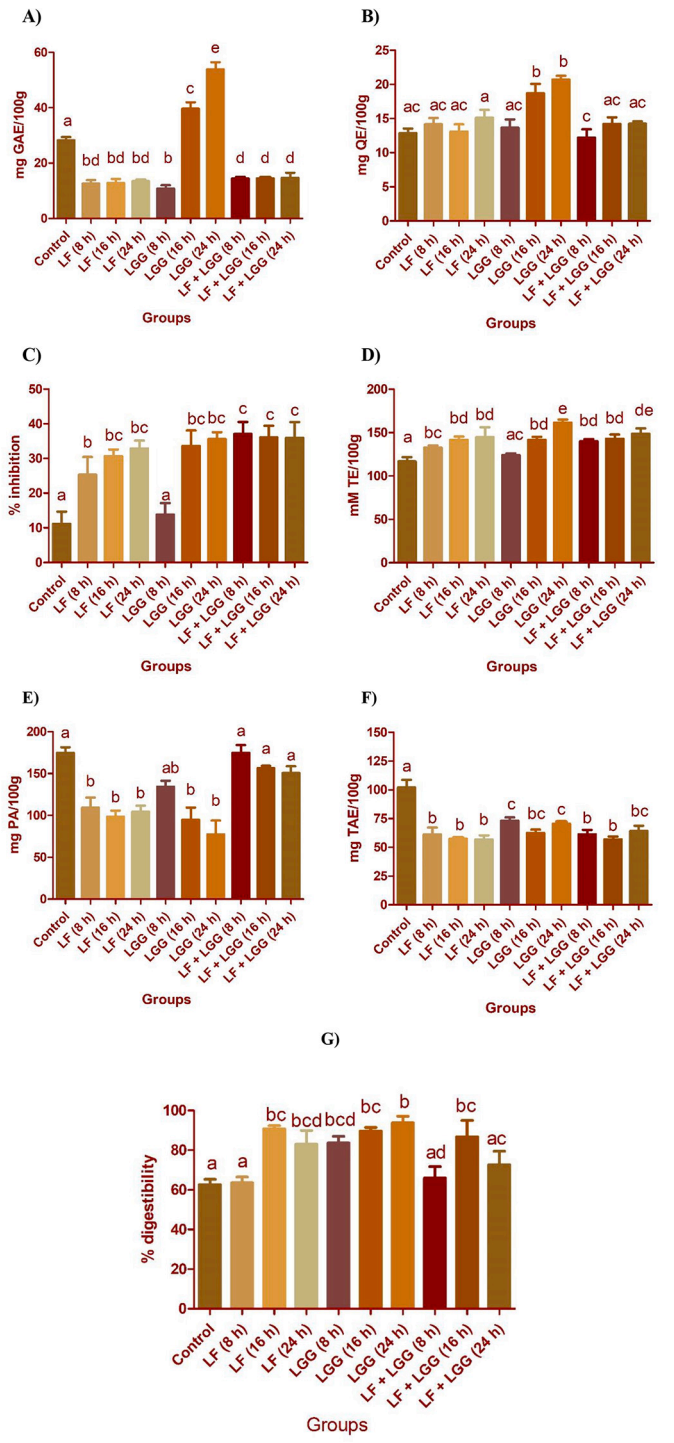

Fig. 2. Effect of selective fermentation on phytochemical content, antioxidative potential, anti-nutritional factors and protein digestibility (%) of fermented millet-skim milk products. Total Polyphenol Content (A); Total Flavonoids Content (B); 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (C); Ferric reducing antioxidant power (FRAP) (D); Phytic acid (PA) (E); Tannin Content (F); In vitro protein digestibility (IVPD %) (G).

Data expressed as mean ± SD,

At P< 0.05, Mean values bearing different letters (a, b, c, d) are significantly different.

Abbreviations: GAE, Gallic acid equivalent; QE, Quercetin equivalent; PA, Phytic acid; TE, Trolox equivalent; TAE, Tannic acid equivalent; LF, *Limosilactobacillus fermentum* MS005; LGG, *Lactobacillus rhamnosus* GG 347.

to increase the concentration of accessible bioactive molecules (Malik, Krishnaswamy, & Mustapha, 2022). The results are most likely related to the LAB's metabolic processes and may result in the breakdown of highly complex phenolic substances or the release of phenolic substances (such as flavonoids), which are linked to the structure of food materials (Dabbagh Moghaddam, Garavand, Razavi, & Dini Talatappe, 2018). According to a related work by Banwo, Asogwa, Ogunremi, Adesulu-Dahunsi, and Sanni (2021), the fermentation of millet and sorghum enriched with L. *fermentum* KL4, *L. plantarum* MOBL1, and *Candida tropicalis* OBY6 and MKY improved total flavonoids in the products.

The DPPH radical scavenging activity (RSA) and FRAP activity systems were used to test the antioxidant's effectiveness. Fig. 2C showed the results of DPPH activity (ranging from 11.14 ± 3.56% to 37.05 ± 3.51%) of all the samples fermented by different LAB cultures at varying fermentation times. Our findings are corroborated by Byresh et al. (2022), who observed an increase in antioxidative capability (DPPH) in a probiotic beverage (pineapple peel powder and white finger millet) fermented with L. *rhamnosus* GG NCDC 347. These results can be linked to a rise in TFC levels. In a fermented sample containing 10% PM flour and fermented with LF + LGG (24 h), the DPPH scavenging activity peaked at 37.05%, demonstrating that the millet has a high potential for free radical inhibition, which is enhanced by fermentation. Although it is worth noting that samples fermented by different strains of LAB had varying DPPH scavenging behavior even when the PM content was the same, this may be attributed to strain-specific effects and fermentation duration. As a result, fermentation with lactic culture influences the antioxidant potential of the products, which must be considered. However, observations also revealed that samples fermented with numerous LAB cultures exhibited substantial variations in DPPH potential even though the amount of sea buckthorn was still equal (Ge et al., 2022). According to the FRAP technique, an antioxidant might convert the Fe^{3+}-TPTZ complex into the violet-colored Fe^{2+}-TPTZ form. Fig. 2D showed the results of FRAP activity (ranging from 116.8 ± 4.95 to 161.4 ± 3.73 mM TE/100 g) of all the samples fermented with different culture conditions at various durations. The FRAP nearly followed the same pattern as the DPPH results showed. The FRAP peaked at 161.4 ± 3.73 mM TE/100 g in a sample fermented for 24 h with LGG, exhibiting that PM has a significant potential for free radical scavenging activity, which is improved by fermentation. All of the fermented products, except that of 8 h LGG, had considerably ($p < 0.05$) elevated reducing antioxidant power compared to the control sample. Therefore, it is essential to consider that fermentation influences the antioxidant activity. Several studies have demonstrated the breakdown of proteins into small peptides during LAB fermentation to enhance the effectiveness of antioxidants (Aloğlu & Öner, 2011; Xiao et al., 2015). Furthermore, an increase in the activity of antioxidants has been shown to be positively linked to the level of proteolysis. Moreover, lactic acid, free amino acids, and isoflavones generated by the fermentation of legumes can enhance the antioxidant capacity of the products (Gan, Shah, Wang, Lui, & Corke, 2017).

3.3. *Effect of fermentation on anti-nutritional factors and mineral molar ratio*

Anti-nutritional substances prevent proteins and minerals from being digested and assimilated by chelating metals and preventing their hydrolysis. Phytate and tannin contents of fermented substrate ranged from 77.47 to 174.80 mg/100 g and 56.91 mg to 102.10 mg/100 g, respectively. Fig. 2E represents the results of phytic acid in fermented and control (unfermented) products. The tannin content in all the fermented samples was much reduced after fermentation treatments when compared to the control (Fig. 2F). Samples fermented with LF for 8 h, 16 h, and 24 h showed tannin contents of 61.13 ± 2.43 mg TAE/100 g, 57.40 ± 0.24 mg TAE/100 g, and 56.65 ± 3.82 mg TAE/100 g, respectively. The primary mechanism of action involves microbial

breakdown by enzymes like tannin acyl hydrolases, and phytases. Under the present investigation, results showed that all the singly fermented treatments of LF and LGG led to considerable ($p < 0.05$) reductions in phytic acid quantity as compared to the control (unfermented). However, all the co-culture (LF + LGG) fermented substrates showed a slight decrease in phytic content but were not significant ($p > 0.05$) when compared to the control. Our findings for phytic acid decrease owing to fermentation align with those of Chaudhary and Mudgal (2020), who observed phytic acid and tannin content reductions in a milk-finger millet composite probiotic fermented product. Present observations are supported by Jan et al. (2022), who observed a substantial ($P < 0.05$) reduction in the anti-nutrient compounds in finger millet flour after diverse fermentation procedures. Similarly, a most recent investigation validated the influence of fermentation on phytic acid level reduction in fermented skim milk-cereal (PM) mixtures compared to unfermented substrates. Previous work also showed that fermentation produced a substantial ($p < 0.01$) 78% drop in phytic acid concentration (Ganguly et al., 2022). Under the present investigation, tannin content was significantly reduced in the fermented substrates (28.30 to 44.5%) when compared to the control (unfermented). Results suggest that the highest tannin reduction was observed in the product obtained from LF culture fermentation for 24 h, while the lowest tannin reduction was recorded for the LGG 8 h fermented product. Our findings are supported by a recent study that confirmed the fermentation of both proso and kodo millets, with initial tannin concentrations of 17.50 g/L and 16.45 g/L, which reduced to 3.01 g/L and 5.07 g/L, respectively (Malik et al., 2022). Likewise, Asres, Nana, and Nega (2018) demonstrated that spontaneous fermentation reduced the anti-nutritional components present in cereal grain products, subsequently improving the mineral's extraction level. Study outcomes confirmed that the FMSMP obtained from co-culture (LF + LGG) showed the lowest decrease in the phytic acid content, which suggests that combined fermentation is not as efficient as single-culture fermentation in enhancing the bioavailability of iron and zinc in millet-based functional beverages.

PA is recognized for its well-known negative impact on the availability of minerals (Frontela, Ros, & Martínez, 2011). Under the present investigation, PA/mineral molar ratios were determined for the iron and zinc content of control (unfermented) and fermented products (Table S1). The mineral molar ratio results correlated with findings of in vitro micronutrient bioavailability; thus, both demonstrate that co-culture fermented substrates have reduced micronutrient bioavailability and maximum PA/minerals for iron and zinc. In the present scenario, some crucial parameters of the phytate/mineral molar ratio have been interpreted as a marker of the possible bioavailability of minerals (Marin, Siqueira, & Arruda, 2009). Comparable prior investigations suggested the following anticipated critical values: (PA/Zn) > 15 for Zn, and (PA/Fe) > 1 for Fe (Hallberg, Brune, & Rossander, 1989; Turnlund, King, Keyes, Gong, & Michel, 1984), which were followed in the current study. The phytate content and its molar ratios are assumed to measure the product's dietary minerals bioavailability, and the quantities have been estimated to compare with the ratios recommended as crucial values. While the molar ratio of all the fermented products made from the PM-skim milk composite was considerably less ($p < 0.05$) than the unfermented control, the molar ratio of all the samples was still above the critical value of iron, i.e., >1 Fe. The outcomes are consistent with the recent finding by Ayub, Castro-Alba, and Lazarte (2021), who also reported a significant reduction in the PA/Fe molar ratio of quinoa fermented instant-mix probiotic beverage. However, the PA/Fe molar ratio was still above the critical value of iron, i.e., >1. Similarly, other studies reported mineral molar ratios of fermented groundnut flour samples above the critical values of iron (>1) and zinc (> 15) (Ijarotimi, Ogunmola, & Oluwajuyitan, 2022). Similarly, a recent investigation found that extending the fermentation period lowered the median molar ratio of PA/Fe of all kisra bread considerably ($p < 0.05$), but it was above the crucial value (>1) for iron (Ahmed, Xu, Sulieman, Na, & Mahdi, 2020), which is also in line with present investigation findings. In the present study, the molar ratio of PA/Zn for the 16 h and 24 h LF or LF + LGG fermented products were below or near 15, which suggests that these samples have high zinc bioavailability. Mainly, the molar ratios of PA/Fe and PA/Zn reduced considerably over the fermentation duration for all fermented substrates in the present investigation, even though the ratios of iron for all samples and ratios of zinc for most samples were above the proposed critical values.

3.4. *Effect of fermentation on in vitro protein digestibility and micronutrient bioavailability*

The nutrient's digestibility, in addition to the nutritional and bioactive constituents of food, is essential. As shown in Fig. 2G, the IVPD of most of the fermented PM-containing samples in the current investigation was significantly higher than the control (unfermented). Results confirmed that the digestibility of protein was significantly increased in all the fermented samples compared to control (unfermented), except for the LF (8 h) and LF + LGG (8 h and 24 h) fermented samples. The IVPD data showed that LF (16 h) and LGG (24 h) fermented samples had excellent digestibility, i.e., 90.75% and 93.76%, respectively, compared to 62.60% for the unfermented sample. Our results are supported by a recent study, which reported that a fermented finger millet yogurt-like beverage had a higher IVPD of 64% than 25% for unfermented flour (Vila-Real et al., 2022). Similarly, Sharma, Sharma, and Singh (2022) confirmed that the IVPD of fermented proso millet was significantly enhanced compared to untreated proso millet due to the fermentation process. Relative changes in dry matter loss occur during the fermentation process due to the activity of microbes that metabolize and hydrolyze lipids and carbohydrates as energy sources. Plant protein digestibility, on the other hand, increases with fermentation (Nkhata et al., 2018). Similarly, according to our findings, De Pasquale, Pontonio, Gobbetti, and Rizzello (2020) reported that fermentation with *L. brevis* and L. *plantarum* improved the nutritional characteristics of treated legume flour by increasing protein digestibility. Reasons for this possibly beneficial impact include the fact that a decrease in pH coupled with increased activity of bacteria's proteolytic enzymes causes the protein to be broken down into smaller peptides with elevated nutritional value as an outcome of fermentation (Sripriya, Antony, & Chandra, 1997). Data on IVPD also support the present investigation's results on anti-nutrients, as a recent study reported reduced amounts of tannins, which leads to increased protein digestibility (Joye, 2019). In the same way, fermentation improved protein digestibility in PM, which might be explained by the microflora's potential production of proteolytic enzymes. Anti-nutritional elements have been shown to negatively impact the digestibility of proteins because they can bind to proteases, restricting their actions and preventing them from being hydrolyzed (Cirkovic Velickovic & Stanic-Vucinic, 2018).

Fermentation also improves mineral bioavailability by producing a phytase enzyme that degrades phytic acid in plant-based foods. For instance, phytic acid reduction may enhance calcium, iron, and zinc levels several-fold (Samtiya, Aluko, Puniya, & Dhewa, 2021). The data show that the Fe level in control and fermented samples was in the 5.01 to 7.23 mg/kg range (Fig. 3A). The iron bioavailability findings for control and fermented samples are presented in Fig. 3A. As a result of fermentation compared to the control (unfermented), Fe bioavailability significantly improved in most of the fermented samples. The range of the Zn level in control and fermented samples was 5.72 to 7.21 mg/kg (Fig. 3B). The results of zinc bioavailability for control and fermented samples are presented in Fig. 3B. Blue column showing the micronutrient present in the unfermented (control) and fermented samples in mg/Kg, however, brown column presents micronutrient bioavailability (%) in unfermented (control) and fermented samples after in vitro micronutrient bioavailability assay. Results confirmed that iron bioavailability was considerably increased in all the fermented products using LF or LGG when compared to the control (unfermented). However, a considerable reduction in vitro micronutrient (Fe and Zn)

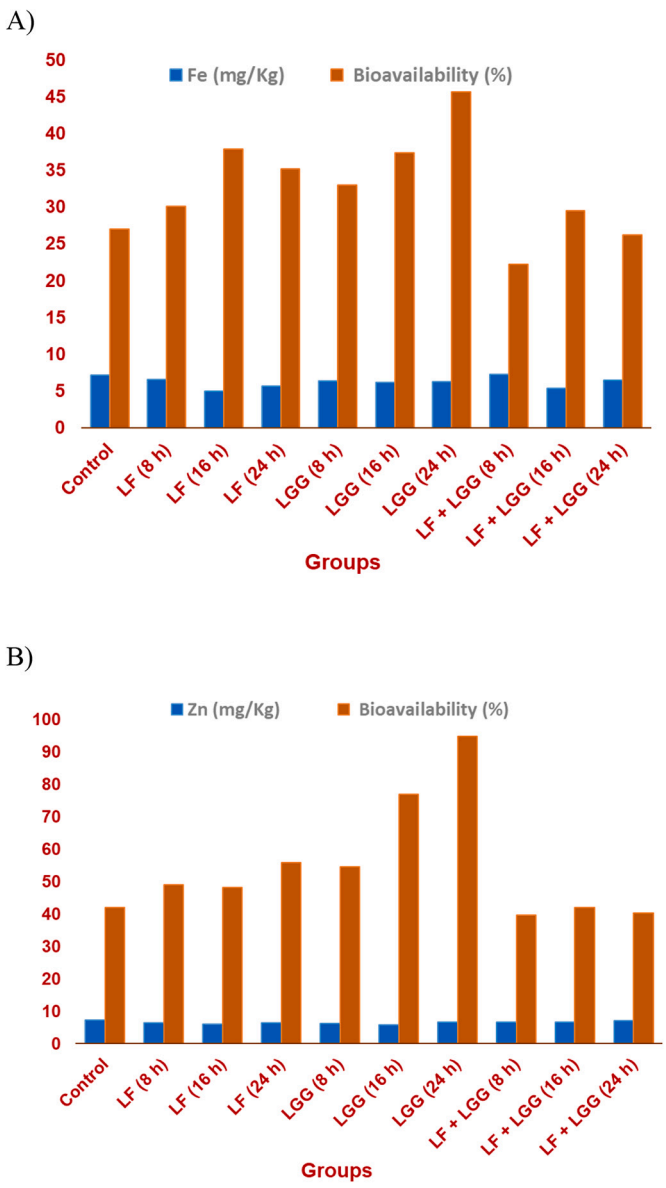

Fig. 3. Effect of selective fermentation on in vitro micronutrients bioavailability of fermented millet-skim milk products. Iron (mg/kg) and bioavailability (%) (A); Zinc (mg/kg) and bioavailability (%) (B).
Abbreviations: Fe, Iron; Zn, Zinc; LF, *Limosilactobacillus fermentum* MS005; LGG, *Lactobacillus rhamnosus* GG 347.

bioavailability was observed for the substrates fermented by co-culture (LF + LGG) treatment. These results are supported by the phytic acid content, where no considerable reduction was observed for all the samples fermented by co-culture (LF + LGG) treatment. The micronutrient bioavailability results of the present investigation agree with a previous study by Basu and Tomar (2016), who assessed the micronutrient bioavailability of PM-based fermented skim milk products. Results found that fermented products' micronutrient bioavailability (Ca, Fe, Zn, Mn, and Cu) was much improved compared to unfermented products. Similar to this, earlier research found that the bioavailability of iron in skim milk cereal-based fermented substrates was improved (Ganguly et al., 2022). The data concluded that LGG (24 h) fermented samples measured for the highest iron and zinc bioavailability compared to other fermented samples. However, LGG could not develop an organoleptically acceptable sour flavor during sample fermentation on its own and did not adequately ferment the sample even after 24 h. Conversely, LF (16 h) fermented samples were recorded for the highest iron bioavailability (37.82%) compared to all other fermented samples. Consequently, LF ferments samples properly and produces excellent organoleptic flavor, so LF (16 h) could be used to make millet skim milk based fermented products. The increase in bioavailability in the LF (16 h) sample compared to the control group was 39.8% and 14.5% for iron and zinc, respectively. Our findings are reinforced by previous studies, which reported that fermentation by lactobacilli strains decreased phytate levels and enhanced the availability of micronutrients (mainly Fe and Zn). Recently, a study reported that fermentation (4 or 10 h at 30 °C) of milled quinoa seeds using L. *plantarum* 299v reduced the phytic acid content significantly and enhanced the availability of micronutrients including zinc, iron, and calcium (Castro-Alba et al., 2019; De Castro, Cunha, Barreto, Amboni, & Prudencio, 2009). Consequently, the resultant reduction of the phytate level turned fermentation into a possible technique to enhance mineral bioavailability. Therefore, as an outcome, the ultimate nutritional value of the fermented item may be improved.

3.5. *Effect of fermentation on texture, functional groups, rheology, and color profile*

The phrase “food texture” refers to a broad range of textural features or attributes that consumers consider when assessing the quality and acceptability of food products (Paredes, Cortizo-Lacalle, Imaz, Aldazabal, & Vila, 2022). The parameters of hardness, chewiness, gumminess, cohesiveness, and springiness (Table 2) were evaluated for control and fermented samples; a non-significant ($P > 0.05$) difference was detected between both in terms of cohesiveness or springiness. Results of the study confirmed that non-significant ($P > 0.05$) variations were observed for the chewiness, gumminess, cohesiveness, and springiness of all the fermented samples compared to the control. However, all the co-culture (LF + LGG) samples fermented for 24 h showed considerable ($P < 0.05$) changes in chewiness in comparison to the unfermented sample. Adhesiveness represents the force needed to remove the material that attaches to the teeth while consuming food, which is an opposing force (Delikanli & Ozcan, 2014). Likewise, another study by Park et al. (2005) described the texture parameters (hardness, springiness, gumminess, and cohesiveness) in the yogurt-like products (prepared by skim milk and soymilk containing saccharified rice), which supported the results of the present study. Similarly, our results align with the previous investigation, which reported oat-based yogurt texture properties (Raikos, Juskaite, Vas, & Hayes, 2020). Our springiness, gumminess, and cohesiveness observations agree with the data reported by Mudgil, Barak, and Khatkar (2017), which recorded the same range of these texture parameters. Under the present study, fermented products' springiness was higher than the control sample, suggesting that fermented samples containing PM regained their initial form more quickly once the force deformed it was removed.

The FTIR technique identifies functional groups and structural variations at the molecular scale of several food samples. The chemical interactions that exist in the molecule's structure in the form of peaks contributed to identifying the functional groups in samples based on natural frequencies of vibration (Fanelli, Zimmermann, Totoli, & Salgado, 2018). Fig. 4 shows the FTIR spectra of the fermented and control samples. All the fermented and control (unfermented) samples showed a broad peak between 3300 and 3200 cm^{-1} and a sharp peak near 2930 cm^{-1}, which signifies that the stretching of O—H groups (H-bonds) and C—H were present in all the samples. Our findings were corroborated by the prior investigation, which revealed that the test and control samples displayed identical peaks around 3450.65 cm^{-1}. This would indicate that stretching the hydroxyl group (O—H) bond in alcohols could be responsible (Byresh et al., 2022). Similarly, in a fermented beverage, Zhao et al. (2021) indicated a distinctive peak at 2937 cm^{-1}, denoting the aromatic ester's existence. The present study recorded all the samples for other peaks near 2350, 2120, 2000, 1650 to 1550, and 1450 to 1000 cm^{-1}. Several absorption zones are associated with stretching the

Table 2
Effect of selective fermentation on texture parameters of fermented millet-skim milk products.

Samples	Hardness	Chewiness	Gumminess	Cohesiveness	Springiness
Control	19.15 ± 0.99^{ae}	2.51 ± 0.20^{ac}	7.78 ± 0.35^{ac}	0.41 ± 0.04^{a}	0.32 ± 0.012^{a}
LF (8 h)	24.16 ± 2.42^{ae}	3.01 ± 0.09^{ac}	8.39 ± 0.18^{abc}	0.35 ± 0.04^{a}	0.36 ± 0.003^{a}
LF (16 h)	25.63 ± 2.28^{ad}	2.46 ± 0.13^{ac}	7.35 ± 0.05^{ac}	0.29 ± 0.03^{a}	0.34 ± 0.015^{a}
LF (24 h)	34.85 ± 1.16^{bdf}	3.45 ± 0.00^{ad}	9.96 ± 0.17^{ab}	0.29 ± 0.01^{a}	0.35 ± 0.006^{a}
LGG (8 h)	19.07 ± 0.98^{ae}	2.49 ± 0.00^{ac}	7.84 ± 0.11^{ac}	0.34 ± 0.01^{a}	0.33 ± 0.010^{a}
LGG (16 h)	20.35 ± 4.39^{aed}	2.09 ± 0.56^{c}	6.39 ± 1.95^{ac}	0.31 ± 0.03^{a}	0.33 ± 0.013^{a}
LGG (24 h)	16.55 ± 0.12^{e}	2.44 ± 0.07^{ac}	6.18 ± 1.63^{c}	0.37 ± 0.10^{a}	0.41 ± 0.095^{a}
LF + LGG (8 h)	29.07 ± 3.64^{cd}	3.03 ± 0.31^{ac}	8.39 ± 0.68^{acd}	0.29 ± 0.01^{a}	0.36 ± 0.008^{a}
LF + LGG (16 h)	36.35 ± 0.82^{f}	3.38 ± 0.05^{ad}	9.93 ± 0.19^{ad}	0.27 ± 0.001^{a}	0.34 ± 0.002^{a}
LF + LGG (24 h)	39.33 ± 0.99^{cf}	4.44 ± 0.74^{bd}	11.73 ± 1.17^{bd}	0.30 ± 0.04^{a}	0.38 ± 0.025^{a}

Data expressed as mean ± SD.
At $p < 0.05$, mean values in the same column with distinct superscript letters (a, b, c …..) are statistically different.
Abbreviations: LF, *Limosilactobacillus fermentum* MS005; LGG, *Lactobacillus rhamnosus* GG 347.

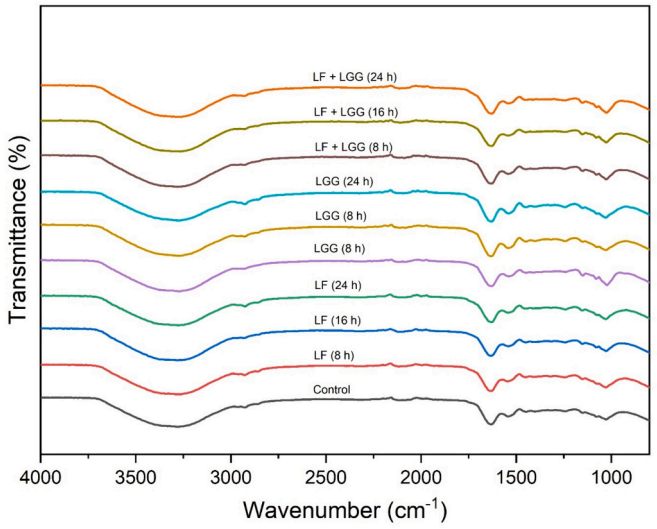

Fig. 4. Fourier transform infrared spectroscopy (FTIR) spectrum obtained from fermented millet-skim milk products.

CH and OH groups of molecules like organic acids and sugars, including glucose and fructose, that may be observed between 1500 and 960 cm^{-1} (Cozzolino, Cynkar, Shah, & Smith, 2011; Wu et al., 2015). Nevertheless, FTIR spectrum bands with certain variations within the wavenumber 1000–1500 cm^{-1} may be attributed to the binding to specific biologically active elements that exist (such as phenolic substances) and milk proteins' carboxyl groups (Mehanna et al., 2014). Similarly, another study's results are in line with our findings, reporting that the band between 1542 cm^{-1} and 965 cm^{-1} is assigned to a vibration of the C—O, C—N, and C—N, suggesting the presence of organic acids, sugars, and ethanol in this region. Stretching vibration of the C—C bond in the phenolic group absorption peak occurs between 1500 cm-1 and 1400 cm^{-1} while the peak bands at 1098 cm^{-1} and 995 cm^{-1} are assigned to OH deformation and C—O stretching in the phenolic group, in the fermented beverage (Ayed, Ben Abid, & Hamdi, 2017).

Apparent viscosity is an essential measure that could be utilized to evaluate the quality of dairy products developed using various procedures. The control (unfermented) and fermented samples were tested for rheological behavior, as shown in Fig. S2 (A to C). The apparent viscosity was initially high but gradually decreased as the shear rate increased. The rise in viscosity could be linked to the formation of a gel or network due to physicochemical and biochemical alterations after fermentation of the substrate. On the other hand, LGG fermented products had considerably lowest viscosity compared to LF and co-culture (LF + LGG). The results suggest that LGG culture could not singly ferment the substrate, which is also supported by the TSS, pH, lactic acid, and texture (hardness) data, as previously discussed in the present investigation. In agreement with current investigation results, Ge et al. (2022) reported apparent viscosity in similar ranges for the fermented milk product supplemented with sea buckthorn. Similarly, the effect of pattern on apparent viscosity against shear rate was demonstrated by a millet-based yogurt-like product (Song et al., 2020). Our results are supported by an earlier study, which reported that as the shear rate increased, the apparent viscosity of the skim milk cereal-based substrates (fermented and unfermented) decreased, representing a non-Newtonian fluid behavior. Both samples displayed shear thinning properties, as demonstrated by the fact that the apparent viscosity of both substrates reduced as the shear rate increased at a particular temperature (Ganguly et al., 2022). Similarly, symbiotic lactic beverage (De Castro et al., 2009) and inulin-enriched yogurt (Donkor, Henriksson, Vasiljevic, & Shah, 2007) obtained results aligned with the current findings. As shear time increased, viscosity decreased, demonstrating that the shear dilution condition was correlated with an interaction between apparent viscosity and shear duration. As a result, the FMSMP obtained from LF fermentation was more stable and suited for industrial dairy product manufacturing.

Table S2 shows the color parameters of control and fermented samples. Results showed that the lightness color parameter (L*) value was substantially ($p < 0.05$) increased in all the fermented substrates when compared to the unfermented sample, except the LF (8 h) fermented sample, which had a slightly high but not significant ($p > 0.05$) L* value. The increased L* value might be defined as the oxidation of pigmented components, which results in improved light color of the fermented products. Similar to our findings, Ganguly et al. (2022) reported a considerable rise in the L* value because of skim milk cereal-based composite substrate fermentation compared to the unfermented product. The findings are similar to the outcomes stated by Gong et al. (2020), who found that potato flour lightness intensity gradually rose with fermenting duration. Our findings are corroborated by a recent study by Navyashree, Buvaneswaran, Sunil, Rawson, and Natarajan (2022), who reported L* and b* value color profiles in the white finger millet probiotic beverage that are similar to the range obtained in the current study. In the present study, no significant changes were measured for a* value in fermented samples compared to the control, except in the LGG (8 h) sample. Numerous investigations reported color values for L*, a*, and b* in non-dairy or dairy functional products (Ge et al., 2022; Öztürk et al., 2018; Park et al., 2005; Sharma, Singh, Deshwal, Rao, & Kumar, 2021) that are similar to those obtained in the current study.

4. Conclusion

The present study was conducted to determine the best possible fermentation combination for biofortified HHB-311 PM and skim milk mixtures that yield fermented probiotic products with enhanced

micronutrient bioavailability, protein digestibility, and other techno-functional characteristics, in addition to reduced levels of anti-nutrients. Substrates were fermented with indigenous LF and a known probiotic culture LGG in three selective fermentation combinations. All the singly fermented treatments using LF or LGG resulted in significant ($p < 0.05$) reductions in phytate content as compared to the control (unfermented). Singly fermented products had significantly reduced phytic acid content with concomitant increment in in vitro protein digestibility and bioavailability of micronutrients when compared to the product from co-culture fermentation. A significant negative correlation was observed between phytic acid and in vitro micronutrient bioavailability. The highest tannin reduction was observed in the 24-h fermented product from LF. The results showed improved iron bioavailability for the fermented LGG (24 h) sample. However, at the time of sample fermentation, LGG could not singly produce an organoleptically good sour taste and did not properly ferment the sample even after 24 h. On the other hand, LF 16 h fermentation time showed improved iron (39% increase) and zinc (14% increase) bioavailability and proper fermentation of the sample. The results suggest that the LF 16 h fermentation is an excellent process to make millet-based fermented products as a result of the improved in vitro protein digestibility and micronutrient bioavailability as well as better techno-functional attributes. The study results concluded that fermentation improved the micronutrient bioavailability and protein digestibility by reducing anti-nutritional factors that negatively affect absorption. Overall, we conclude that various low-cost but nutritious millet-based probiotic foods can be produced from fermentation with different microorganisms that were used in this study.

Ethics approval and consent to participate

Not applicable.

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CRediT authorship contribution statement

Mrinal Samtiya: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Prarabdh C. Badgujar:** Writing – review & editing, Resources, Conceptualization. **Gauri A. Chandratre:** Writing – review & editing, Resources. **Rotimi E. Aluko:** Writing – review & editing, Data curation. **Ashwani Kumar:** Writing – review & editing, Data curation. **Bharat Bhushan:** Writing – review & editing. **Tejpal Dhewa:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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