Lactiplantibacillus plantarum ATCC 8014 fermentation of pearl millet: impacts of autoclaving modes and fermentation time on the nutrient content, bioactive compounds, and antioxidant potential

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Abstract

The study aimed to investigate the effects of mixed-mode autoclaving (MMA; sterilization of flour–water mixture; as control) and single-mode autoclaving (SMA; sterilization of flour and water separately) on the potential of the fermenting microorganism *Lactiplantibacillus plantarum* ATCC 8014 (*L. plantarum*), focusing on nutrient content, bioactive compounds, and antioxidant activity in millet flour. Pearl millet (*Pennisetum glaucum* (L.) R.Br.) flour mixed with water (1:4, w/v) was fermented with *L. plantarum* (10^8 colony-forming unit [CFU]/g) in a fermentor at 37°C for 12, 24, 36, 48, 60, and 72 h, with continuous stirring at 120 rpm, following sterilization in an autoclave (121.1°C; 15 psi; 15 min). SMA and MMA had varied effects on micronutrients and minerals, and fermentation affected them. Fermentation of *L. plantarum* results in substantially higher glucose production in SMA compared to MMA, whereas the opposite is true for fructose. SMA had higher glucose levels and lower fructose levels than MMA. The SMA *L. plantarum* and MMA-fermented samples showed similar trends in glucose and fructose changes. SMA samples contained higher total phenolic content (TPC) than MMA, while total flavonoid content (TFC) and total tannin content (TTC) remained unchanged. TPC and TFC increased gradually, while TTC decreased, after 72-h *L. plantarum* fermentation. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging rate in sterilized raw millet flour (0MMA) was lower than in raw millet flour, and it was also lower in SMA than in MMA. The DPPH scavenging rate of 0MMA increased significantly after 12 h of fermentation, reaching its peak at 36 h, with the MMA surpassing SMA in scavenging activity. High-performance liquid chromatography–mass spectrometry (HPLC-MS) analysis detected bioactive substances in SMA- and MMA-fermented millet, with some variations in their nature and levels. Overall, these results indicate that sterilization method and fermentation time are key factors in shaping the nutrient and bioactive compound content of millet flour, highlighting the need to optimize them to develop nutritious, health-promoting fermented millet products.

Keywords: antioxidant activity; autoclaving; bioactive compounds; fermentation; *Lactiplantibacillus plantarum* ATCC 8014; nutrients; pearl millet

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Introduction

Lactic acid bacteria (LAB) play a crucial role in the food industry, where they primarily ferment carbohydrates into lactic acid for food preservation purposes. LAB also break down proteins and fats, yielding organic acids, amino acids, and flavor compounds that enhance the quality, safety, and nutritional value of food (Yang *et al.*, 2024). LAB have traditionally been used for natural leavening, improving fermentation control, enhancing product quality, and extending shelf life while conserving energy (Aguirre-Garcia *et al.*, 2024). The rise in demand of fermented foods is linked to increased health awareness and decrease in disease occurrence (Zdziobek *et al.*, 2023). Approximately 20 strains of LAB, considered generally recognized as safe (GRAS), are used in fermentation because they play a vital role in medical and food applications by modulating gut microbiota and improving metabolic health, thereby shaping the future of functional foods (Yang *et al.*, 2024). They produce organic acids that extend shelf life of bread, inhibit spoilage, and improve various properties, including rheology, mineral bioavailability, protein digestibility, flavor, and glycemic index (Şerban *et al.*, 2021; Woo *et al.*, 2020). *Lactiplantibacillus plantarum*, including the strain ATCC 8014, can effectively ferment various cereal substrates, including wheat, barley, sorghum, millet, and rice, making it a widely used LAB in production of non-dairy food products. It provides significant technological and nutritional benefits, converting cereals into more nutritious, safer, and tastier food products (Arya *et al.*, 2025; Paventi *et al.*, 2024). It also produces bioactive compounds, contributing to the preservation of food and human health (Cui *et al.*, 2021; Şerban *et al.*, 2023).

Millet is an essential, gluten-free cereal crop mainly grown in Asia and Africa. It originated in Africa and is cultivated in Saudi Arabia's Jazan region (El-Hashash *et al.*, 2023; Food and Agriculture Organization [FAO], 2021). Nutritionally, it is a rich source of calories, dietary fiber, protein, vitamins, and minerals. It is also valued for its beneficial fatty acids and bioactive compounds, such as polyphenols, making it a popular functional food ingredient (Amadou, 2022; Goudar *et al.*, 2023). While pearl millet offers a good amount of calories, it is also gluten-free and rich in dietary fiber, non-starch polysaccharides, polyphenols, proteins, fatty acids, minerals—including higher levels of iron, zinc, and calcium—and vitamins, such as vitamin E, riboflavin, thiamine, and niacin. It also contains significant amounts of unsaturated fatty acids such as linoleic acid, linolenic acid, and oleic acid as well as saturated fatty acids, such as palmitic acid and stearic acid (Amadou, 2022). Additionally, pearl millet is a good source of bioactive compounds, such as phenolics, flavonoids, total anthocyanins, and polymeric tannins (Goudar *et al.*, 2023).

Fermentation is the most cost-effective and energy-efficient method for improving food preservation, and various changes occur in the nutritional and functional properties of grains (Balli *et al.*, 2023; Suma and Urooj, 2017). Fermentation is performed using specific starter cultures (controlled fermentation), intrinsic microorganisms, or spontaneous processes (Balli *et al.*, 2023). Compared to traditional fermentation, immobilized bacteria fermentation helps to control the process and enhance yield (Yang *et al.*, 2024). Utilizing specific strains with desired properties as starter cultures in controlled fermentation is a cost-effective and straightforward approach to improve the nutritional value and health benefits of cereal substrates (Sidari *et al.*, 2020; Yépez *et al.*, 2019). Sour dough/paste, a fermented mixture of flour, water, and LAB, is popular among bakers for its ability to improve bread quality (Şerban *et al.*, 2023). Moreover, fermentation produces various metabolites, including antioxidants and vitamins, which enhance their bioactivity or introduce new exogenous bioactive compounds, thereby increasing the bioactivity of fermented flour and providing health benefits (Melini *et al.*, 2019). Furthermore, millet fermentation enhances the availability of calcium, iron, phosphorus, and zinc (Yousaf *et al.*, 2021).

In fermentation, using an inoculum of single or mixed microorganisms, the culture medium must be sterilized. Therefore, autoclaving, a wet-heat sterilization method, effectively eliminates microorganisms by using pressurized steam, making it much more effective than dry-heat sterilization (Agrawal, 2024; Jayashree *et al.*, 2024). However, sterilization can enhance the fermentability of solid substrates by modifying nutrients such as polysaccharides, thereby improving solid-state fermentation (Zhao *et al.*, 2015). Nevertheless, employing a high-temperature, short-duration method during sterilization may lessen nutritional value while simultaneously eradicating bacteria (Mann *et al.*, 2001). Accordingly, a comprehensive understanding of how *L. plantarum* interacts with the components of millet flour during fermentation, especially following various autoclaving methods used to sterilize paste mix, is necessary (Şerban *et al.*, 2023). However, the importance of pearl millet lies in its natural composition, which provides a nutrient-rich environment that LAB utilizes, promoting strong microbial growth and activity (Adebo *et al.*, 2022; Mudau and Adebo, 2025). *L. plantarum*-fermented pearl millet is shown to improve antioxidant capacity, bioactive compounds, and nutrient content, positioning it as a promising functional food for nutritional enhancement. Research indicates that its enhanced properties can lead to health benefits, including anti-inflammatory effects

(Gabriele *et al.*, 2024; Srivastava *et al.*, 2024). Fermented pearl millet offers a cost-effective, health-promoting solution for communities that rely on millet as a staple food (Srivastava *et al.*, 2021). On the other hand, autoclaving used to sterilize pearl millet slurry before inoculation with *L. plantarum* is a crucial pre-processing step that significantly affects the substrate's physicochemical properties by quickly releasing fermentable sugars; this impacts the bioaccessibility of compounds metabolized by *L. plantarum*, thereby influencing the efficiency of fermentation process (Cui *et al.*, 2021; Zheng *et al.*, 2023). This is reflected in enhanced starch and carbohydrate digestibility, reduced antinutritional factors, improved mineral accessibility, and improved microbial safety and quality of the end fermented product (Chu *et al.*, 2025; Yehuala *et al.*, 2025). While extensive research exists on moist autoclaving treatments (i.e., autoclaving the mixture of flour and water) (Moiseenko *et al.*, 2024; Ntsamo *et al.*, 2020), understanding how different autoclaving methods affect the substrate and, in turn the fermentation process by *L. plantarum* is a significant knowledge gap. To address this, the novelty of this study lies in pretreating a millet flour–water mixture (1:4, w/v) or flour and water separately to autoclaving (121°C; 15 psi) for 15 min before fermentation. The first type of autoclaving is known as mixed-mode autoclaving (MMA), while the second is referred to as single-mode autoclaving (SMA). After cooling, the *Lactiplantibacillus* (LPB) inoculum was added aseptically. Therefore, this study aims to assess the impact of SMA and MMA treatments on the ability of fermenting *L. plantarum* to enhance the nutrient content, bioactive compounds, and antioxidant activity of millet flour.

Materials and Methods

Chemicals

Agar powder and de Man, Rogosa, and Sharpe (MRS) broth were obtained from EMDA International Trading Establishment (EITE; Riyadh, Saudi Arabia). The following supplies were purchased from Sigma-Aldrich (St. Louis, MO, USA): Folin–Ciocalteu reagent, gallic acid standard, quercetin standard, and tannic acid standard. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Merck (Darmstadt, Germany).

Millet flour preparation

Millet grains were purchased from Al-Baha market in Saudi Arabia. The grains were cleaned of debris, washed, and left to dry at room temperature. They were then milled into powder, passed through a 60-mesh sieve, and stored at 4°C for further use.

Fermentation process

Preparation of L. plantarum ATCC 8014 inoculum

Lactiplantibacillus plantarum ATCC 8014 was procured from the microbiology laboratory of College of Food and Agriculture Sciences, King Saud University, Saudi Arabia. The stock strain of *L. plantarum*, preserved in 10 mL of MRS broth with glycerol at -80°C, was inoculated into MRS broth and incubated at 37°C for 48 h. Grown culture, 1 mL, containing approximately 10^8 colony-forming unit (CFU)/mL, was inoculated into 500-mL sterilized conical flasks containing 100 mL of sterilized MRS broth and incubated at 37°C for 48 h. The cells of *L. plantarum* were centrifuged (2,300 ×g; 4°C; 10 min) and washed for three times with sterile water to remove any remaining medium. The total number of vegetative cells and spores was counted by using the colony count technique according to ISO 7932 (International Organization for Standardization [ISO], 2004). To enumerate *L. plantarum*, a serial dilution in sterile sodium chloride (0.85%) solution and the nutrient agar pour plate method were used; the plates were incubated at 37°C for 24 h. The CFU/mL was counted with a laser colony scanner model 500A.

Fermentation of millet flour

A 1-L Manifor fermentor (Lambda Laboratory Instruments, Sihlbruggstrasse 105, Switzerland), equipped with dissolved oxygen (DO), temperature, and pH probes, was used to ferment millet flour. *L. plantarum* served as an inoculum. The fermentor received a specified weight of millet flour, 490 mL of deionized water, and 10 mL of inoculum (*L. plantarum*, 10^8 CFU/g) to create fermentation mixture. The millet flour–water ratio was 1:4. Before adding the inoculum (1 mL; 10^8 CFU/mL of *L. plantarum*), the flour and water were sterilized separately in an autoclave (121.1°C; 15 psi; 15 min) in a process called SMA, or they were sterilized together, referred to as MMA, a commonly used autoclaving method for sterilization; thus, it was chosen to serve as a control. Subsequently, the fermentation process was conducted at a constant agitation speed of 120 rpm and 37°C for various durations (12, 24, 36, 48, 60, and 72 h). The DO level was maintained above a set saturation point (e.g., >10%) using a computer-controlled feedback system that adjusts agitation speed, airflow rate, and oxygen level in the incoming gas mixture based on real-time DO sensor data (Zheng and Pan, 2019). After completion of fermentation, the pH was measured, the sample was removed from fermentor, and then heated in a boiling water bath for 15 min to halt fermentation. The samples were dried in an oven at 50°C, packed in sample bags, and stored at -20°C for later analysis. For safety, we fermented millet flour samples from 6 h to 72 h, each in a separate conical flask, to eliminate the possible risk of contamination during withdrawing of samples at desired time intervals (Figure 1).

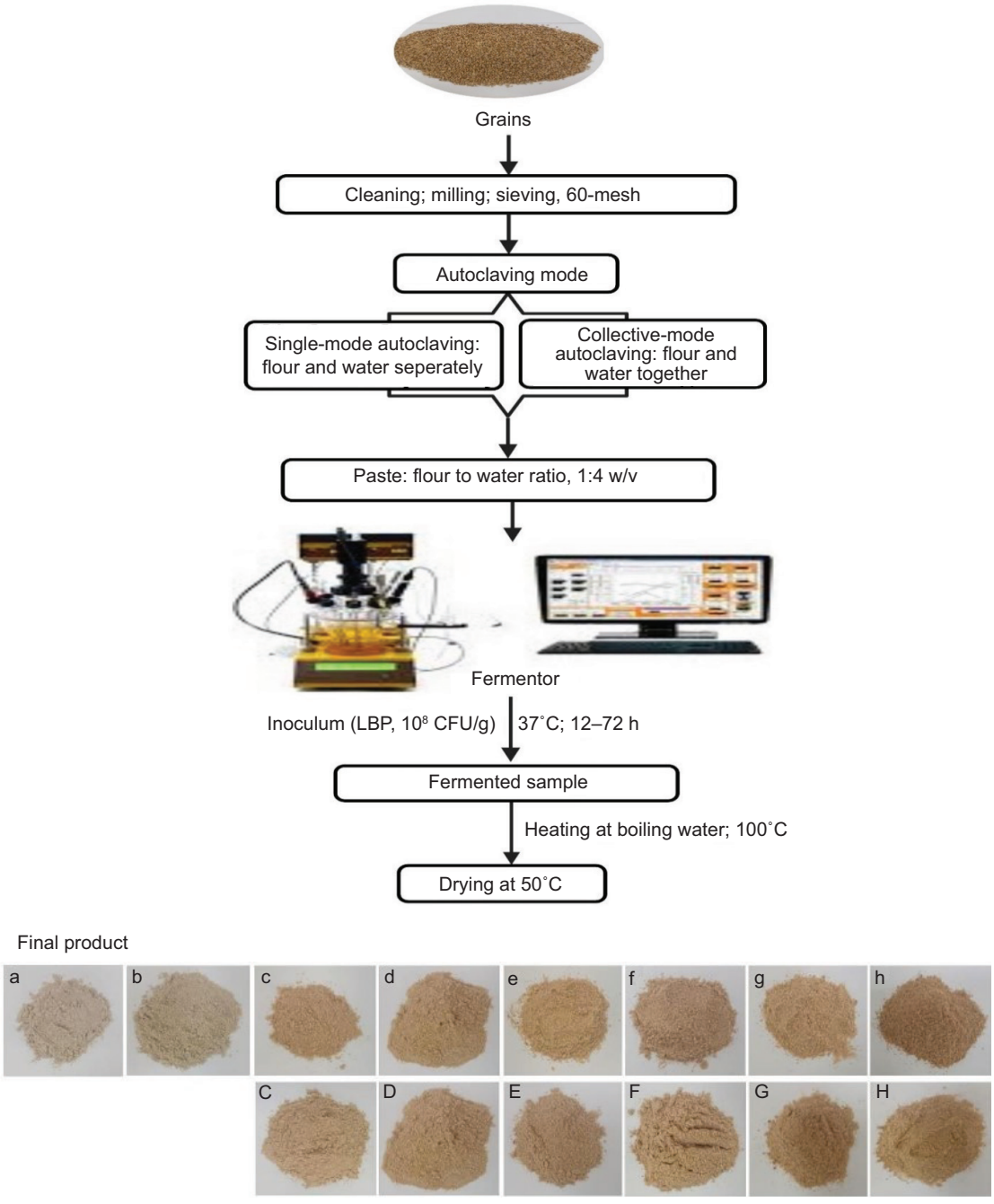

Figure 1. Overview of the pearl millet flour fermentation process using *L. plantarum* ATCC 8014. Notes. SMA: single-mode autoclaving; MMA: mixed-mode autoclaving. a: raw; b: raw millet flour sterilized with MMA; c: 12-h SMA-fermented millet; d: 24-h SMA-fermented millet; e: 36-h SMA-fermented millet; f: 48-h SMA-fermented millet; g: 60-h SMA-fermented millet; h: 72-h SMA-fermented millet; C: 12-h MMA-fermented millet; D: 24-h MMA-fermented millet; E: 36-h MMA-fermented millet; F: 48-h MMA-fermented millet; G: 60-h MMA-fermented millet; H: 60-h MMA-fermented millet.

Proximate composition analysis

The proximate composition of millet flours was determined using AOAC methods without modifications (AOAC, 2023). Moisture content was measured by drying overnight at 105°C (method 930.15), ash content was determined by incineration at 600°C (method No. 942.05), and fat content was estimated using the ether extraction Soxhlet method (No. 966.06). Total nitrogen percentage was determined by the Micro

Kjeldahl acid digestion/distillation method (No. 984.13), and crude protein was calculated as N% × 6.25. Although a more accurate method exists for measuring protein from amino acids, it has not yet been validated by AOAC, leading to the continued use of the 6.25 conversion factor (Krul, 2019). The carbohydrate content was calculated using the difference method (Pehrsson *et al.*, 2015).

Determination of contents of glucose and fructose

Glucose and fructose levels in fermented samples were determined by high-performance liquid chromatography with a refractive index detector (HPLC-RID; Shimadzu, Kyoto, Japan) using Kirsten's method with some modifications (Weiß and Alt, 2017). The procedure involved mixing 5 mL of HPLC-grade water with 0.5 g of sample, vortexing for 1 h, and then filtering the mixture through a 0.45-µm cellulose acetate syringe filter. Standard stock solutions of glucose and fructose at different concentrations were prepared and used for external calibration. The HPLC system, equipped with an LC-NH2 column (150 × 4.6 mm, 5 µm), an RID-10A detector, an LC-10AB binary pump, and an SIL-20A autosampler (Shimadzu), was used to analyze fructose and glucose. Separation conditions were as follows: a mobile phase of 90% aqueous acetonitrile (HPLC grade) with an isocratic flow rate of 1 mL/min, a column temperature of 85°C, an injection volume of 1 µL (diluted 10-fold) for sample extracts and standards, and a 50-min run time. Additionally, a spiked sample was used to ensure the accuracy and quality of results.

Determination of nutritional and heavy metal levels

A mixture of 0.5 g of sample, 0.5 mL of HCl, and 9.5 mL of HNO_3 was digested in a microwave adjusted as follows: step 1: 50°C, 2 min, and 1,000 W; step 2: 30°C, 3 min, and 0 W; step 3: 189°C, 25 min, and 1,000 W; step 4: 150°C, 1 min, and 0 W; step 5: 180°C, 4 min, and 1,000 W; step 6: 180°C, 15 min, and 1,000 W (ETHOS UP, 2024). For analysis, the sample (diluted 50-fold with 2% HNO_3) was injected in an ICP-MS-2030. Key parameters included a sampling depth of 5 mm, an output power of 1.2 kW, a plasma gas flow rate of 9.0 L/min, an auxiliary gas flow rate of 1.10 L/min, a spray chamber temperature of 5°C, and a carrier gas flow rate of 0.70 L/min. Calibration curves were plotted using element standards, allowing the calculation of sample element concentrations, expressed in mg/kg (ppb × 0.001) for nutritional elements and µg/kg (ppb) for heavy toxic elements.

Determination of TPC, TFC, TTC, and DPPH scavenging activity

Preparation of methanol extract

A total of 1 g of fermented millet flour and 20 mL of 95% methanol were placed in a conical flask and wrapped in aluminum foil. The flask was shaken for 4 h and then filtered using Whatman filter paper No. 42, followed by filtration through a 0.2-µm membrane filter. The filtrate was packed in amber glass bottles and stored at 5°C until used. This filtrate was used to analyze TPC, TFC, TTC, and DPPH scavenging rate.

Total phenolic content

The TPC of millet methanol extract was determined according to the method reported by Mouhoubi *et al.* (2023). A volume of 100 µL of extract was mixed with 100 µL of Folin–Ciocalteu reagent. After 5 min, 300 µL of 20% sodium carbonate solution was added to the mixture. Next, the sample was incubated in a dark place for 30 min at ambient temperature. Then absorbance of the sample was measured at 765 nm using a ultraviolet (UV)-visible spectrophotometer (UV-3200; Shimadzu). The TPC was calculated using a linear regression equation: $y = 0.0049x - 0.038$ ($R^2 = 0.9981$), derived from a gallic acid standard curve created with varying concentrations (25–400 µg/mL). The TPC findings are expressed as mg gallic acid equivalent (GAE)/g dry weight (dw).

Total flavonoid content

The TFC of sample methanol extract was determined using the method described by Hussain *et al.* (2024, 2025). A 0.5-mL aliquot of the extract was mixed with the same volume of 2% aluminum chloride ($AlCl_3$) solution. After 1 h, the wavelength was read at 420 nm. The TFC was quantified using the regression equation: $y = 0.0031x - 0.0294$ ($R^2 = 0.9975$). This equation was derived from a quercetin calibration curve prepared using different concentrations ranging from 25 to 400 µg/mL. The TFC results are expressed as mg of quercetin equivalents (QE)/g of sample (mg QE/g dw).

Total tannin content

The TTC of methanol extract was determined by following the method described by Rodrigues *et al.* (2007), with minor changes. A total of 0.1 mL of extracted sample was added to a 2-mL Eppendorf tube containing 1.5 mL of milli-Q water and 0.1 mL of Folin–Ciocalteu phenol reagent for 8 min. Then, 0.3 mL of 35% Na_2CO_3 solution was added to the mixture for neutralization. Next, the mixture was shaken well and kept in the dark at room temperature for 20 min. Absorbance of the mixture was recorded at 700 nm. To determine TTC, a calibration curve was created using various concentrations of tannic acid standard, and the following equation was

used: y = 0.005x - 0.016 (R^2 = 0.9992). The TTC results are expressed as mg tannic acid equivalents (TAE)/g dw.

DPPH radical scavenging rate

The methanol extract of fermented millet was evaluated for its antioxidant activity using the DPPH scavenging test, as described earlier (Alshammari *et al.*, 2025). Briefly, 200 µL of fermented millet sample was mixed with 2 mL of 0.1-mM DPPH solution in ethanol. The mixture was vortexed and left in the dark at room temperature for 30 min before measuring its absorbance at 517 nm. Methanol was also evaluated for its DPPH scavenging rate as a blank. The DPPH scavenging rate of the extract was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = ([A_0 - A_s]/A_0) \times 100,$$

where A_0 is the absorbance of the blank, and A_s is the absorbance of the sample.

Untargeted LC-MS/MS analysis of the composition of pearl millet flour

The analysis of the composition of SMA- and MMA-pretreated pearl millet, followed by fermentation for 72 h, was conducted using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. The analytes were separated using a Phenomenex Luna C18 PS column (100 Å, 100 × 2.1 mm, 1.7-µm particle size) with gradient conditions involving water with 0.01% formic acid (solution A) and acetonitrile (solution B), both modified with 10-mM ammonium acetate, at a flow rate of 0.4 mL/min. The column oven was 40°C, and the injection volume was 10 µL. Mass spectrometry analysis used the X500R Quadrupole time-of-flight (QTOF) system with electrospray ionization in positive mode and sequential window acquisition of all theoretical mass spectra (SWATH) acquisition. Time-of-flight mass spectrometry (TOF-MS) scans were conducted from 50 to 1,000 Da with specific voltage and gas parameters (Sun *et al.*, 2017). For detection and identification, the LC-MS/MS data were processed using the MZmine 2.53 software (Boutet *et al.*, 2022; Chambers *et al.*, 2012).

Statistical analysis

The data were presented as means ± standard deviation (SD) from triplicate experiments. One-way analysis of variance (ANOVA) and two-way ANOVA were employed to identify significant differences between treatment groups. Tukey's test (one-way ANOVA) and pairwise comparisons (two-way ANOVA) were used to assess significant differences between mean values ($p < 0.05$). IBM SPSS Statistics, version 28.0 (IBM Corporation, NY, USA), was used for statistical analysis.

Results and Discussion

Viability growth dynamics of *L. plantarum* ATCC 8014 during millet fermentation under various autoclaving regimes

Assessing the kinetic behavior of microorganisms is crucial for planning industrial production, understanding responses to environmental variables, and designing the most technologically efficient reactors (Üçok and Sert, 2020).

In Table 1, the growth data show the population changes of *L. plantarum* ATCC 8014 during a 72-h fermentation, using SMA- and MMA-pretreated millet flour as substrates. A control MRS substrate, simulating ideal laboratory conditions, was used as a baseline for comparison. All groups began with statistically similar, high inoculum levels (~1.00–1.03 × 10^8 CFU/g), confirming a consistent starting point. First, a lag/log phase (lag/early exponential, 0–24) at which an adaptation period occurs, followed by exponential growth. It has been reported that the lag phase for *L. plantarum* in cereal dough generally happens between 3 h and 4 h under conditions of 30–37°C and pH 5.5–6.5, immediately followed by the late exponential (Log) phase, and then the death phase (Popova-Krumova *et al.*, 2024; Śliżewska and Chlebicz-Wójcik, 2020).

In detail, the most notable observation is the superior early growth with SMA treatment. This is evident in the higher population count for SMA (3.53 × 10^8) at 0–12 h, which increased by 249.5%, compared to the initial bacterial count (1.01 × 10^8). In contrast, increments of 70% and 99.03% in bacterial count were observed for MRS control and MMA-fermented samples, respectively, during the same period. At 12–24 h of fermentation, compared to the initial inoculum count, the population of SMA-treated millet increased by 71%, followed by MMA-treated millet, which increased by 51%, and then MRS, with an increase of 42.2%.

Differences in the lag/early exponential phases observed in fermented millet substrates, particularly those treated with SMA, may result from variations as to how the flour matrix is affected. This difference probably results from the Maillard reaction and other heat-induced chemical changes. MMA (mixing flour and water during sterilization) may cause more extensive non-enzymatic Maillard reactions or starch gelatinization, which temporarily reduces nutrient bioavailability for fermenting bacteria, leading to a slightly more extended lag phase because the

Table 1. Changes in *L. plantarum* ATCC 8014 count (CFU/g) over time during millet flour fermentation subjected to single- and mixed-mode autoclaving.

Fermentation time	*L. plantarum* fermentation		
	MRS (CFU/g)	SMA-millet (CFU/g)	MMA-millet (CFU/g)
0 h	$1.00\pm0.098\times10^{8c}$	$1.01\pm0.028\times10^{8d}$	$1.03\pm0.113\times10^{8e}$
12 h	$1.70\pm0.071\times10^{8c}$	$3.53\pm0.177\times10^{8c,d}$	$2.05\pm0.212\times10^{8d}$
24 h	$2.40\pm0.141\times10^{8c}$	$6.05\pm0.354\times10^{8c}$	$3.10\pm0.424\times10^{8c}$
36 h	$9.45\pm0.989\times10^{8b}$	$12.53\pm1.59\times10^{8b}$	$11.05\pm0.212\times10^{8b}$
48 h	$1.65\pm0.212\times10^{9a}$	$1.90\pm0.283\times10^{9a}$	$1.90\pm0.127\times10^{9a}$
60 h	$9.74\pm0.820\times10^{8b}$	$10.61\pm1.44\times10^{8b}$	$10.15\pm0.021\times10^{8b}$
72 h	$2.63\pm0.488\times10^{8c}$	$2.21\pm0.057\times10^{8d}$	$1.29\pm0.035\times10^{8e}$

Note: Data are displayed as means ± SD (n = 3). Data were analyzed statistically using one-way ANOVA. According to Tukey's test ($p < 0.05$), mean values accompanied by different superscript alphabets differ significantly within a column.
MRS: de Man, Rogosa, and Sharpe agar; SMA: single-mode autoclaving (sterilizing each flour and water separately); MMA: mixed-mode autoclaving (sterilizing flour and water together); 12–72 h, fermentation time.

bacteria need to adapt to a more complex or nutrient-depleted environment (Pontonio *et al.*, 2020; Xiang *et al.*, 2019).

Moreover, SMA reduces direct contact and reactions between flour components and water at high temperatures. This helps to maintain the native structures of starches, sugars, and proteins, making them more accessible to enzymatic breakdown and to *L. plantarum* during the early stages of fermentation (Verni *et al.*, 2019). Additionally, SMA pretreatment may enhance starch gelatinization compared to the MMA method, as MMA's inconsistent hydration can result in partial gelatinization. SMA, by using pre-sterilized flour and sterile water, ensures a uniform gel that enhances the accessibility of glucose and dextrins (Chiodetti *et al.*, 2024). This efficient starch hydrolysis is crucial for LAB growth (Pontonio *et al.*, 2020).

Furthermore, at 24–48 h (late exponential phase), *L. plantarum* in all samples reveals rapid growth during 24–36 h of fermentation, increasing by 293.8% in MRS control, 256.5% in MMA-pretreated millet, and 106.94% in MMA-pretreated millet. The second part of the exponential phase (36–48 h) shows slower growth, as shown by 74.6%, 71.95%, and 51.64% increase in bacterial counts in MRS control, MMA-, and SMA-pretreated samples, respectively. Although *L. plantarum* grew faster in MRS control during the exponential phase, the bacterial counts of SMA- and MMA-pretreated fermented samples reached a peak of 1.9×10^9 CFU/g, compared to 1.65×10^9 CFU/g in the control. These findings aligned with the changes observed in glucose and fructose during the fermentation of millet samples (Figure 3).

Finally, the stationary/death phase (48–72 h) exhibits a significant reduction in cell viability (41–46.6%) across all samples (48–60 h), with a more pronounced decrease noted after 60 h of fermentation (73–87.4%). As shown, bacterial death occurred more rapidly in millet samples compared to the control. This is probably due to environmental stressors, including the production of bacteriocins and organic acids, and a decrease in pH (Anumudu *et al.*, 2024; Yoon *et al.*, 2024).

Interestingly, despite decrease in counts at 72 h, *L. plantarum* levels in all fermented SMA- and MMA-treated millet samples remain above the original inoculum, with values of 2.21×10^8 and 1.29×10^8, respectively, compared to 2.63×10^8 for the control MRS. However, higher bacterial death in MMA samples could be due to faster depletion of glucose and fructose than in SMA, consistent with the findings of simple sugar analysis (Figure 3; Supplementary Table S2). In general, *L. plantarum* exhibits higher acid tolerance than other LABs (Paramithiotis, 2025); therefore, the millet samples retained moderately viable cells after 72 h of fermentation. This is supported by the analysis of pH values of the studied samples (Figure 2).

Besides, in addition to lactic acid, LAB, including *L. plantarum*, can produce other antimicrobial compounds, including acetic acid, ethanol, and bacteriocins, under specific conditions. In the nutrient-depleted and acidic environment, metabolic pathways can undergo significant changes. The production of acetic acid, which is more toxic to microbial cells than lactic acid, might increase. Besides, *L. plantarum* is known to produce bacteriocins (e.g., plantaricin), which are protein-based compounds that

inhibit the growth of closely related bacterial strains (Ismael *et al.*, 2024; Zhao *et al.*, 2025). While primarily targeting competitors, at high cell densities, these bacteriocins can also cause auto-inhibition, a phenomenon known as "chemical warfare," where some cells undergo autolysis (self-digestion). The release of hydrolytic enzymes (autolysins) degrades the cell wall (Kawai *et al.*, 2023), leading to the observed decrease in CFU. Nevertheless, autolysis, followed by apoptosis, is believed to be a survival strategy for the population, as lysed cells release intracellular nutrients that are absorbed by the remaining live cells, helping the culture survive longer (Allocati *et al.*, 2015; Mohiuddin *et al.*, 2021). A sharp decline between 60 h and 72 h is probably due to a combination of ongoing acid stress and substantial autolytic activity. The above data suggest that the sterilization method (SMA vs. MMA) had a slight but significant impact, especially during the early- to mid-fermentation phases.

In summary, the impressive growth potential of *L. plantarum* in both SMA- and MMA-pretreated millet, reaching ~1.0×10^9 CFU/mL, demonstrates that millet flour is an effective substrate for LAB fermentation, supporting the trend toward plant-based fermented foods and functional components (Das *et al.*, 2022).

Changes in pH of millet flour during *L. plantarum* fermentation

As shown in Figure 2, raw millet flour has a pH of 6.9, which decreases to 6.56 after MMA (i.e., sterilized raw millet flour, 0MMA), indicating the release of organic acids into the medium. Autoclaving can increase the acidity of cereal flour by releasing adhered phenolic acids through hydrolysis in the high-temperature and high-moisture environment (Rico *et al.*, 2020; Solmaz *et al.*, 2025). Fermentation of both SMA and MMA samples results in a gradual decrease of pH over time, reaching its lowest point at 72 h (pH = 3 and 4, respectively). The most rapid decline in pH occurred between 12 h and 36 h, corresponding to the period of the most vigorous bacterial growth and sugar acidification (Table 1). Lactic acid, a byproduct of sugar metabolism, accumulates during *L. plantarum* fermentation of cereals, lowering the medium's pH (Adisa *et al.*, 2024; Moiseenko *et al.*, 2024). Additionally, it has been reported that autoclaving can render a more favorable substrate, thereby enhancing LAB fermentation and lowering pH (Isembart *et al.*, 2025).

It is also worth noting that fermented SMA-treated millet samples have lower pH values than fermented MMA-treated millet samples, indicating a higher presence of *L. plantarum* in SMA, compared to MMA. This is probably due to the greater effect of moist heat and pressure exerted by MMA on millet components, mainly starch, which may lead to the formation of resistant starch. It is discovered that autoclaving causes resistant starch type III to form via retrogradation, a process in which short-chain amylose molecules bond via hydrogen bonds after cooling (Chuwech *et al.*, 2023). As observed previously, SMA and MMA treatments resulted in a decline in pH from 6.9 in the raw sample to 3.0 (SMA) and 4.0 (MMA) after 72 h of fermentation. However, it is reported that millet fermentation resulted in higher acidification with milder initial heat treatments (Janiszewska-Turak *et al.*, 2024). Homofermentative *L. plantarum* primarily produces lactic acid, which aids in food preservation and flavor, but can inhibit bacterial growth at high concentrations (Popova-Krumova *et al.*, 2024). A notable decrease in pH in SMA, compared to MMA, may be

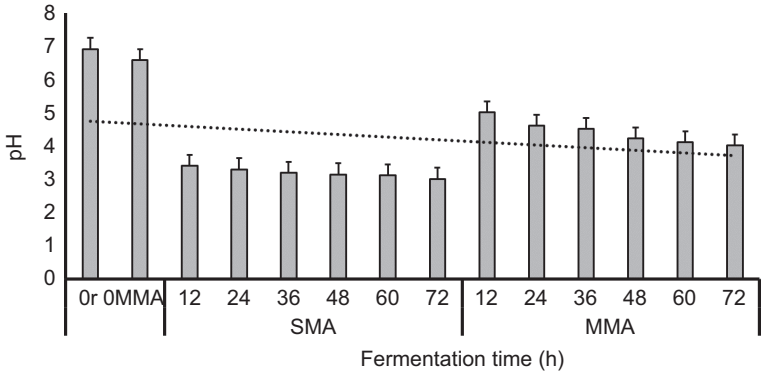

Figure 2. Changes in pH in millet flour following *L. plantarum* ATCC 8014 fermentation. Note: 0r: raw millet flour; 0MMA: raw millet flour sterilized with MMA (mixed-mode autoclaving: sterilizing flour and water together; control); SMA: single-mode autoclaving.

due to high accumulation of lactic acid, which enters bacterial cells and dissociates at neutral pH, releasing protons and lowering internal pH. Accordingly, cells expend energy to pump out protons via adenosine triphosphate (ATP)-dependent transporters to maintain homeostasis, which is called "uncoupling effect." This effect and subsequent acidification lead to energy depletion as ATP is diverted, reducing enzyme activity and potentially causing cell death when pH balance is disrupted (Martinez *et al.*, 2012; Sionek *et al.*, 2024). Our data show that the population decreased by 41% between 48 h and 60 h, clearly indicating severe acid stress.

Overall, the MMA treatment produced less acid than the SMA treatment because its harsher processing created more resistant starch, which fermented less effectively. In contrast, the SMA treatment created a much more acidic environment, which was toxic to *L. plantarum*, leading to greater apoptosis than MMA by the end of fermentation.

Proximate composition of millet flour fermented with *L. plantarum* ATCC 8014

The proximate composition of *L. plantarum*-fermented millet flours subjected to different autoclaving methods (SMA and MMA) is detailed in Table 2 and Supplementary Table S1. The levels of ash, fat, protein, and carbohydrates in the raw millet flour were 1.89, 7.76, 6.19, and 84.23 g/100 g dw, respectively. Ash levels significantly increased to 2.25 g/100 g dw, while carbohydrate and protein levels remained unchanged at 85.07 and 5.68 g/100 g dw, respectively, in the sterilized raw millet flour (0MMA), compared to the untreated flour. Meanwhile, fat levels decreased to 7.00 g/100 g dw ($p < 0.05$) (Supplementary Table S1).

Regardless of the fermentation method, using two different autoclaving modes, SMA and MMA, prior to *L. plantarum* fermentation resulted in varying macronutrient contents. In SMA, the levels of fat and protein (total means of 5.96 and 6.95 g/100 g dw, respectively) were significantly higher ($p < 0.05$) than those in MMA. Conversely, ash and total carbohydrates were higher in MMA, with total mean values of 2.58 and 85.54 g/100 g dw, respectively (Table 2). The differences observed between SMA and MMA in changes in macronutrient suggested that they exhibit distinct autoclaving effects, reflected in their unique interactions with millet flour components. Previous studies reported an increase in total carbohydrate level of cereals after autoclaving (Faridah *et al.*, 2022; Udensi *et al.*, 2010).

Besides, regardless of the autoclaving mode used, the ash content increases significantly from a total mean of 2.25 g/100 g dw for the 0MMA sample as fermentation time progresses, reaching a total mean of 2.88 g/100 g dw at 48 h. It then decreases with further fermentation, reaching its minimum level at 72 h (a total mean of 2.25 g/100 g dw), and leveled off at the initial 0MMA value ($p < 0.05$). The fat content of 0MMA (7.0 g/100 g dw) is significantly higher than all fermented samples (12–72 h), with the 72-h sample showing the lowest value ($p < 0.05$). The protein content of the 0MMA sample increases significantly in all fermented samples, with the highest values observed at 24 h and 36 h (7.53 g/100 g dw and 7.22 g/100 g dw, respectively). Furthermore, the protein content of the remaining fermented samples does not differ significantly from that of the 36-h sample ($p < 0.05$). The carbohydrates content remains unaffected by fermentation for 36 h but decreases after 60 h, then increases after 72 h (the total mean of 88.29 g/100 g dw; $p < 0.05$) (Table 2).

As shown in Supplementary Table S1, both SMA and MMA demonstrate distinct behavior during *L. plantarum* fermentation. Specifically, *L. plantarum* fermentation has a more pronounced impact on MMA than on SMA, as indicated by the magnitude of changes in macronutrients. In particular, MMA shows higher increases/decreases in ash, fat, protein, and carbohydrates as fermentation progresses than SMA. This could result from the moist heat and pressure applied by MMA to millet components, which enhance the millet matrix's suitability for both growth and activity of *L. plantarum*.

Furthermore, increase in protein levels in millet after fermentation is probably due to the production of single-cell protein by the LAB culture (Yadav *et al.*, 2016), or the synthesis of specific amino acids, which subsequently elevates protein levels after the fermentation process (Uwaegbute *et al.*, 2000). In contrast, decrease in protein level is potentially due to changes in dry matter, which is probably the result of the hydrolysis of sugars and fats (Dangal *et al.*, 2024).

Reduced fat content in both SMA and MMA during fermentation could be due to the enhanced lipase activity produced by *L. plantarum*. It is reported that lipases could degrade triglycerides into fatty acids and glycerol, which are then metabolized for energy, potentially increasing nutrient bioavailability or altering fat content (Djorgbenoo *et al.*, 2023; Jan *et al.*, 2022; Ogodo *et al.*, 2019).

The rate of increase in carbohydrate content, particularly between 48 h and 72 h of fermentation, is slightly higher than in SMA (Supplementary Table S1). The rise

Table 2. Macronutrients (g/100 g, dw) of autoclave-treated pearl millet, followed by *L. plantarum* ATCC 8014 fermentation.

Fermentation time (h)	Ash			Fat			Protein			Carbohydrates		
	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)
0r	1.89 ± 0.048	1.89 ± 0.048	**1.89 ± 0.04^{e}**	7.76 ± 0.23	7.76 ± 0.23	**7.76 ± 0.23^{a}**	6.19 ± 0.11	6.19 ± 0.11	**6.19 ± 0.11^{d}**	84.16 ± 0.24	84.16 ± 0.24	**84.16 ± 0.24^{d}**
0 MMA	2.25 ± 0.08	2.25 ± 0.08	**2.25 ± 0.07^{d}**	7.00 ± 0.19	7.00 ± 0.19	**7.00 ± 0.19^{b}**	5.68 ± 0.28	5.68 ± 0.28	**5.68 ± 0.28^{d}**	85.07 ± 0.20	85.07 ± 0.20	**85.07 ± 0.20^{c,d}**
12	2.57 ± 0.11	2.82 ± 0.11	**2.70 ± 0.16^{b,c}**	6.45 ± 0.09	6.35 ± 0.16	**6.40 ± 0.13^{c}**	7.06 ± 0.33	6.45 ± 0.14	**6.76 ± 0.41^{b}**	83.92 ± 0.27	84.38 ± 0.23	**84.15 ± 0.31^{d}**
24	2.52 ± 0.05	3.03 ± 0.09	**2.77 ± 0.29^{a,b}**	6.07 ± 0.30	4.92 ± 0.08	**5.55 ± 0.66^{e}**	7.61 ± 0.18	7.46 ± 0.28	**7.53 ± 0.23^{a}**	83.80 ± 0.29	84.59 ± 0.31	**84.20 ± 0.43^{d}**
36	2.25 ± 0.13	3.03 ± 0.12	**2.64 ± 0.45^{b,c}**	6.50 ± 0.58	5.46 ± 0.14	**5.98 ± 0.68^{d}**	7.03 ± 0.11	7.41 ± 0.76	**7.22 ± 0.53^{ab}**	84.22 ± 0.10	84.10 ± 0.91	**84.16 ± 0.60^{d}**
48	2.65 ± 0.13	3.12 ± 0.10	**2.88 ± 0.28^{a}**	4.69 ± 0.08	4.69 ± 0.08	**5.25 ± 0.65^{e}**	7.43 ± 0.43	6.22 ± 0.14	**6.83 ± 0.72^{b}**	85.23 ± 0.29	85.97 ± 0.20	**85.60 ± 0.97^{c}**
60	2.61 ± 0.05	2.49 ± 0.10	**2.55 ± 0.10^{c}**	4.50 ± 0.17	3.54 ± 0.16	**4.02 ± 0.54^{f}**	7.44 ± 0.20	6.19 ± 0.19	**6.82 ± 0.698^{b}**	85.45 ± 0.02	87.78 ± 0.15	**86.62 ± 1.30^{a,b}**
72	2.50 ± 0.08	2.00 ± 0.10	**2.25 ± 0.28^{e}**	4.70 ± 0.08	3.60 ± 0.08	**4.15 ± 0.61^{f}**	7.12 ± 0.50	6.11 ± 0.20	**6.62 ± 0.62^{b,c}**	85.68 ± 0.04	88.29 ± 0.21	**86.99 ± 1.00^{a}**
Total mean (n = 24)	**2.41 ± 0.42^{b}**	**2.58 ± 0.42^{a}**		**5.96 ± 1.10^{a}**	**5.42 ± 1.24^{b}**		**6.95 ± 0.50^{a}**	**6.46 ± 0.70^{b}**		**84.69 ± 0.96^{b}**	**85.54 ± 1.67^{a}**	
Grand mean (n = 48)			**2.49 (SE = 0.015)**			**5.76 (SE = 0.036)**			**6.71 (SE = 0.046)**			**85.12 (SE = 0.049)**

Notes: Statistical differences among factor means were analyzed using Type III two-way ANOVA. According to pairwise comparisons, different superscript alphabets indicate that total mean values differ significantly ($p < 0.05$) within a row or a column.
0r: raw millet flour; 0 MMA: raw millet flour sterilized with MMA; MMA: mixed-mode autoclaving: sterilizing flour and water together; control; SMA: single-mode autoclaving (sterilizing each flour and water separately); dw: dry weight.
Carbohydrate content (including crude fiber) is obtained by difference (100 – protein + fat + ash).

in carbohydrates could be due to *L. plantarum* depleting dry matter during the hydrolysis and metabolism of oligosaccharides, simple sugars, and lipids. In this study, the calculated carbohydrate by difference includes crude fiber. Another explanation for increase in carbohydrates after *L. plantarum* fermentation is that because pearl millet contains 2.6–4.0% crude fiber, a lignin–carbohydrate complex (Abdalla *et al.*, 1998), laccases discovered in *L. plantarum* (Olmeda *et al.*, 2021), could probably contribute to breaking down lignocellulose molecules (Bao *et al.*, 2022; Zhou *et al.*, 2023), partially releasing cellulose and hemicellulose from the complex, thereby increasing the carbohydrate content.

Overall, the results indicated that MMA showed slightly better preservation of macronutrients prior to fermentation, compared to SMA, although variations were observed in fat and protein levels as well.

Sugar analysis

In this study, sucrose, glucose, and fructose were analyzed in millet samples. However, sucrose was not detected in these samples. As shown in Figure 3, glucose levels in 0MMA and raw millet (0r) are statistically similar (86.81 and 94.11 mg/kg dw, respectively; $p > 0.05$). In contrast, fructose levels are significantly higher in 0MMA than in raw millet (73.18 and 59.92 mg/kg dw, respectively; $p < 0.05$). This suggests that autoclaving may partially hydrolyze specific oligosaccharides, such as sucrose or maltose, into their constituent monosaccharides, glucose and fructose. This is supported by the fact that autoclaving can hydrolyze about 10% of sucrose (Druart and Wulf, 1993). Furthermore, regardless of the fermentation time, data in Supplementary Table S2 reveal that SMA has a significantly ($p < 0.05$) higher glucose level (total mean of 192.57 mg/kg) than MMA (total mean of 53.79 mg/kg), indicating the difference between the two autoclaving modes in their interactions with substrate material (see Section 3.1).

Regarding the impact of fermentation, two distinct phases are observed in glucose and fructose levels in millet samples. An initial phase (0–36 h), aligning with the lag–log exponential stage, and a second phase (36-72 h) aligning with the log–stationary–death stage (Figure 3; Supplementary Table S2). The SMA-treated millet shows a significantly higher glucose level (181.58 mg/kg dw) after 12 h of fermentation compared to that of 0MMA (control: 86.81 mg/kg dw), while it remains unchanged in MMA-treated millet (71.72 mg/kg dw; Figure 3). Increasing the fermentation time from 12 h to 36 h resulted in a gradual increase in glucose content from 86.81 to 388.25 mg/kg dw in SMA samples, after which it declined. MMA samples exhibit a similar decreasing trend as that of SMA-treated samples, with glucose reaching zero level after 36 h of fermentation (Figure 3). After 48 h of fermentation, glucose levels decreased in both SMA and MMA ($p < 0.05$), with SMA showing a

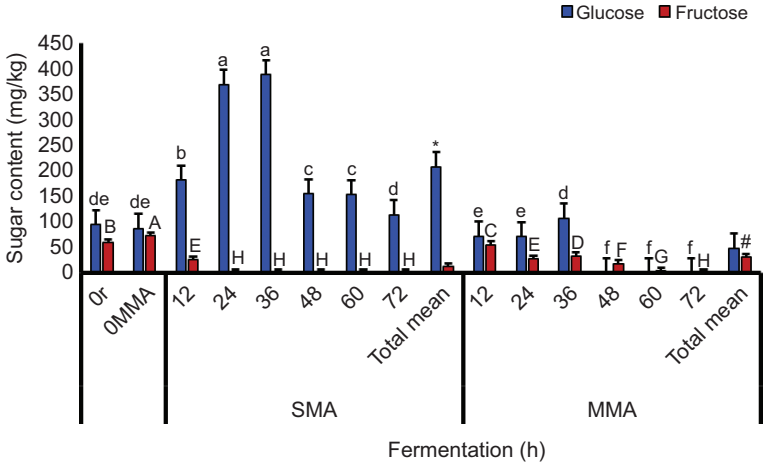

Figure 3. Glucose and fructose contents in autoclave-treated pearl millet, followed by fermentation with *L. plantarum* ATCC 8014. Means ± SE (n = 3) were statistically analyzed using one-way ANOVA. According to Tukey's test ($p < 0.05$), mean values with different superscript alphabets differ significantly. Total mean values (n = 24) were analyzed using a two-way ANOVA. According to pairwise comparison ($p < 0.05$), the total mean values marked with (*) or (#) are statistically different. Notes: 0r: raw millet flour; 0MMA: raw millet flour sterilized with MMA; MMA: mixed-mode autoclaving: sterilizing flour and water together; control; SMA: single-mode autoclaving. Lowercase and uppercase alphabets indicate significant differences among the mean values of glucose and fructose in millet flour during fermentation of samples, respectively. Error bars indicate the standard error (SE) of mean values.

gradual decline to its lowest level at 72 h, while glucose in MMA is entirely depleted after 48 h. Conversely, fructose in SMA and MMA behaved differently during *L. plantarum* fermentation compared to glucose, as its levels consistently decreased in MMA samples over 12–72 h of fermentation, reaching zero by 72 h. In contrast, *L. plantarum* fermentation of SMA-treated millet causes fructose to drop to zero after 24 h ($p < 0.05$; Figure 3).

Generally, fructose level in the MMA sample, regardless of fermentation, is significantly higher ($p < 0.05$) than in the SMA sample. Fermentation of millet, regardless of the autoclaving method used, exhibited a significant and gradual decrease ($p < 0.05$) in fructose levels with increasing incubation time, reaching a minimum at 72 h (Supplementary Table S2).

However, *L. plantarum* strains demonstrated superior carbohydrate utilization compared to most LABs, attributed to their sugar biosynthesis pathways and sugar-specific phosphate transport systems, enabling metabolism of a range of sugars, such as glucose and fructose (Zhao *et al.*, 2023). Increase in glucose in fermented SMA-treated millet samples could be due to the breakdown of complex sugars into simpler forms through starch hydrolysis (Mohapatra *et al.*, 2024). In contrast, gradual decrease of glucose by *L. plantarum* is probably attributed to the fact that LAB are characterized by more robust carbohydrate utilization system, commonly discovered in most LAB strains, which supports cellular growth (Mohapatra *et al.*, 2024). These results coincided with the change in population count over 72-h fermentation of autoclaved-pretreated millet flour (Section 3.1). However, decrease in fructose during fermentation could be due to its consumption through the glycolysis pathway (Krahn *et al.*, 2021) or its conversion to mannitol by *L. plantarum* (De Vuyst *et al.*, 2009; Krahn *et al.*, 2021). Generally, the severe nutrient limitation is a primary driver of the transition into the death phase (Section 3.1), as the cells no longer have the energy to maintain viability (Li *et al.*, 2023).

Finally, millet fermentation, following SMA and MMA, variably affects glucose and fructose levels, suggesting that sterilization and microbial processes interact to influence these levels.

Analysis of nutritional and toxic heavy element levels

For the quality control (QC) of the ICP-MS-2030 used for element analysis, a certified multi-element solution containing 36 inorganic standard elements (10 mg/L each of Ag, Al, As, Ba, B, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Na, Nd, Ni, P, Pb, Rb, Se, Sm, Sr, Tl, Tm, V, and Zn) and a separate 10 mg/L Hg standard were used for calibration. Spike samples were analyzed to assess matrix effects and method accuracy, yielding recovery rates of 80–120%. The method was validated for heavy toxic metals with limits of detection (LOD) and quantitation (LOQ) values for Fe (0.33 and 1.125 ppb), Na (19.97 and 66.58 ppb), Mn (1.54 and 5.25 ppb), Zn (0.22 and 0.744 pb), Ca (32.57 and 108.56), K (19.44 and 64.81 ppb), Mg (2.26 and 7.55), As (2.80 and 9.34 ppb), Cd (0.54 and 1.81 ppb), Hg (1.54 and 5.14 ppb), Pb (1.09 and 3.63 ppb), and Sb (3.50 and 11.66 ppb), in accordance with ISO (2004, 2016) standards (ISO and International Electrotechnical Commission [IEC], 2017).

The raw millet flour contains both nutritional macroelements and microelements as well as heavy toxic metals (Tables 3 and 4; Supplementary Tables S3 and S4). K, Mg, Na, Ca, Cr, Cu, Fe, Ni, Mn, and Zn have levels of 3432.5, 1011.1, 127.4, 201.8, 1.23, 6.3, 1.04, 3.59, 19.8, and 46.2 mg/kg dw, respectively. Additionally, it contains heavy toxic metals such as As, Cd, Hg, Pb, and Sb, with levels of 6.62, 66.87, 33.74, 16.59, and 1,256.62 μg/kg dw, respectively. All nutritional elements and heavy toxic metals in millet flour remain unchanged, compared to 0MMA, except for K, Na, and Sb, which showed significantly increased or decreased levels, respectively ($p < 0.05$; Supplementary Table S3). Regardless of fermentation, SMA resulted in significantly higher total mean levels of Cr, Cu, Fe, Cd, Hg, and Sb compared to MMA, and significantly lower mean levels of Mn, Ca, Mg, and Na ($p < 0.05$), while Ni, As, and Pb remained unchanged ($p > 0.05$) (Tables 3 and 4). This suggests that MMA and SMA treatments may interact differently with the millet flour matrix (Figure 3), leading to varied mineral content. Previous studies have reported fluctuations in the mineral content of cereals and legumes after autoclaving (Karaçoban *et al.*, 2023; Kemal *et al.*, 2025).

The mineral content results of millet flour after *L. plantarum* fermentation, regardless of SMA and MMA, are shown in Table 2. Total mean levels of several nutritional minerals, including Cu, Mn, Ca, K, Mg, and Na, increased significantly during the fermentation period of 12–36 h ($p < 0.05$). After that, their levels either decreased gradually until 72 h (Mg), fluctuated (Na) ($p < 0.05$), or remained stable ($p > 0.05$). In contrast, other nutritional elements varied differently over the 72 h of fermentation. For example, Cr gradually decreased significantly until 24 h of fermentation, then remained stable for rest of the period. While the total mean levels of Zn and Ni remained unchanged over the entire 72 h, but Fe showed variable changes (Table 3; Supplementary Table S3). It is worth noting that decrease in Mg during later fermentation stages may result from its uptake by *L. plantarum* as an essential cofactor for microbial

Table 3. Nutritional element analysis (mg/kg, dw) of autoclave-treated pearl millet, followed by *L. plantarum* ATCC 8014 fermentation.

Fermentation time (h)	Cr			Cu			Fe			Ni			Mn (mg/100 g)		
	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)
0r	1.23±0.01	1.23±0.01	**1.23±0.01^{a}**	6.30±0.71	6.30±0.71	**6.3±0.7^{b,c}**	110.40±1.30	110.40±1.30	110.40±1.30^{d}	3.58±0.02	3.58±0.02	3.58±0.02^{a}	19.8±0.12	19.8±0.12	**19.8±0.12$^{a–c}$**
0MMA	1.19±0.04	1.19±0.04	**1.19±0.03^{a}**	6.20±0.012	6.20±0.012	**6.2±0.1^{b,c}**	111.6±1.31	111.6±1.31	111.6±1.31^{d}	3.61±0.54	3.61±0.54	3.61±0.54^{a}	18.9±0.09	18.9±0.09	**18.9±0.1^{b,c}**
12	1.14±0.13	1.22±0.11	**1.18±0.10^{a}**	5.40±1.85	6.50±0.24	**6.0±0.07^{c}**	114.2±0.87	122.0±0.030	**118.1±4.5^{c}**	3.61±0.43	3.60±0.07	**3.61±0.2^{a}**	20.4±0.10	20.3±0.13	**20.4±1.1^{a,b}**
24	1.07±0.12	1.14±0.23	**1.11±0.04^{b}**	7.3±0.83	5.50±1.69	**6.4±1.1$^{a–c}$**	104.7±0.29	108.5±0.53	**106.6±2.2^{e}**	3.62±0.72	0.362±0.022	**3.62±0.1^{a}**	20.5±0.22	21.7±0.11	**21.1±1.6^{a}**
36	1.04±0.03	1.14±0.01	**1.09±0.10^{b,c}**	8.70±1.22	5.30±0.411	**7.0±0.19^{a}**	143.0±0.42	12.70±0.113	**135.0±8.8^{a}**	3.60±0.30	3.74±0.08	**3.67±0.2^{a}**	17.9±0.13	1.85±0.14	**18.2±0.4^{c}**
48	1.13±0.04	0.91±0.03	**0.99±0.11^{c}**	7.60±0.70	5.80±1.76	**6.7±1.0^{a,b}**	130.0±0.55	105.4±0.120	**117.7±14.0^{c}**	3.41±0.14	3.50±0.09	**3.46±0.1^{a,b}**	18.3±0.05	18.7±0.09	**18.5±3.0^{c}**
60	1.16±0.02	0.93±0.09	**1.03±0.20^{c,d}**	6.90±0.024	5.80±2.85	**6.4±0.7^{b,c}**	127.3±0.84	111.8±0.59	**119.6±8.5^{c}**	3.31±0.54	3.57±0.43	**3.44±0.2^{a,b}**	17.9±0.14	20.9±0.07	**19.4±1.8^{b,c}**
72	1.06±0.02	0.87±0.10	**0.97±0.12^{c}**	7.30±0.60	5.60±2.40	**6.5±0.9$^{a–c}$**	144.3±1.13	103.4±1.23	**123.9±22.4^{b}**	3.40±0.06	3.29±0.25	**3.35±0.1^{b}**	18.9±0.09	18.2±0.05	**18.6±0.5^{c}**
Total mean (n = 24)	**1.12±0.07^{a}**	**1.08±0.15^{b}**		**6.96±1.00^{a}**	**5.88±0.53^{b}**		**123.2±15.0^{a}**	**112.5±8.3^{b}**		**3.52±0.2^{a}**	**3.57±0.2^{a}**		**19.1±2.0^{b}**	**19.6±1.5^{a}**	
Grand mean (n = 48)			**1.10 (SE = 0.010)**			**6.44 (SE = 0.050)**			**117.9 (SE = 0.300)**			**3.54 (SE = 0.020)**			**19.4 (SE = 0.130)**
RDA*/AI# for adults (mg/day)			**0.025–0.035#**			**0.90***			**8.0–18.0***			**0.07–0.40***			**1.8-2.3#**

Fermentation time (h)	Zn (mg/100 g)			Ca (mg/100 g)			K (mg/100 g)			Mg (mg/100 g)			Na (mg/100 g)		
	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)
0r	46.2±0.20	46.2±0.20	**46.2±0.2^{a}**	201.8±0.10	201.8±0.10	**2018.±0.1^{d}**	3432.5±12.1	3432.5±12.1	**3432.5±12.1^{h}**	1011.1±1.3	1011.1±1.3	**1011.1±17.7^{e}**	127.4±1.0	127.4±1.0	**127.4±1.0^{e}**
0MMA	45.8±0.20	45.8±0.20	**45.8±0.2^{a,b}**	201.7±0.10	201.7±0.10	**201.7±0.1^{d}**	3772.8±11.2	3772.8±11.2	**3772.8±11.2^{g}**	1039.0±2.9	1039.0±2.9	**1039.0±13.0^{d}**	115.9±0.4	115.9±0.4	**115.9±0.4^{e}**
12	45.9±0.20	46.0±0.30	**46.0±6.8^{a,b}**	202.1±0.2	202.2±0.10	**202.2±0.5^{d}**	4422.4±7.6	4153.4±16.6	**4287.9±74.0^{a}**	1199.8±1.8	1286.1±1.3	**1243.0±47.8^{a}**	146.3±0.3	145.4±0.3	**145.9±4.2^{d}**
24	46.3±0.70	45.8±1.10	**46.1±11.5^{a}**	204.0±0.10	205.5±0.10	**204.8±1.0^{c}**	3942.4±7.6	4287.6±12.4	**4115.0±63.4^{e}**	1022.9±5.8	1254.7±2.0	**1138.8±127.4^{b}**	151.1±0.6	180.9±0.4	**166.0±16.4^{a}**
36	46.2±0.60	46.4±0.30	**46.3±4.7^{a}**	203.5±0.10	211.2±0.10	**207.4±4.4^{b}**	3935.2±14.8	4243.7±13.9	**4089.5±76.9^{f}**	958.9±3.32	1226.2±4.2	**1092.6±46.8^{c}**	142.6±0.2	173.2±0.6	**157.9±16.9^{b,c}**
48	46.1±0.30	46.3±0.50	**46.2±5.7^{a}**	204.8±0.30	211.6±0.10	**208.2±3.8^{a,b}**	3770.5±29.5	4521.4±18.6	**414.6.0±138.2^{d}**	1004.2±4.7	991.1±1.9	**997.7±14.2^{e}**	157.5±0.4	165.3±0.2	**161.4±4.6^{b}**
60	45.7±0.30	45.8±0.20	**45.8±0.6^{a,b}**	207.7±0.10	211.6±0.30	**209.7±2.4^{a}**	3756.6±7.8	4741.1±21.1	**4248.9±118.9^{b}**	812.9±2.3	912.9±2.2	**862.9±54.8^{f}**	119.7±0.2	190.1±0.4	**155.2±38.9^{c}**
72	45.8±0.30	45.5±0.20	**45.7±1.1^{b}**	209.3±0.20	21120±0.02	**210.3±0.7^{a}**	3709.7±13.6	4718.1±21.9	**4213.9±139.5^{c}**	861.8±4.2	832.4±2.8	**847.1±17.0^{f}**	122.0±0.5	178.4±0.7	**150.2±30.9^{d}**
Total mean (n = 24)	**46.0±8.7^{a}**	**46.0±3.0^{a}**		**204.4±12.7^{b}**	**207.1±14.3^{a}**		**3842.8±422.7^{b}**	**4233.8±584.7^{a}**		**988.8±119.2^{b}**	**10692±170.4^{a}**		**135.3±14.4^{b}**	**159.7±24.4^{a}**	
Grand mean (n = 48)	**46.00 (SE = 0.190)**			**205.8 (SE = 0.150)**		**4225.79 (SE = 2.650)**			**1029.0 (SE = 1.800)**			**147.5 (SE = 0.400)**			
RDA*/AI# for adults (mg/day)	**8.0–11.0***			**1,000***		**2,600–3,400#**			**310–400***			**1,500#**			

Notes: Statistical differences among factor means were analyzed using Type III two-way ANOVA. According to pairwise comparisons, different superscript alphabets indicate that total mean values differ significantly ($p < 0.05$) within a row or a column.
0r: raw millet flour; 0MMA: raw millet flour sterilized with MMA; MMA: mixed-mode autoclaving: sterilizing flour and water together; control); SMA: single-mode autoclaving (sterilizing each flour and water separately); dw: dry weight.

Table 4. Heavy metal contents (µg/kg, DW) of autoclave-treated pearl millet, followed by *L. plantarum* ATCC 8014 fermentation.

Fermentation time (h)	As			Cd			Hg			Pb			Sb		
	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean (n = 6)
0r	6.62±0.31	6.62±0.31	**6.62±0.31^{b}**	66.87±1.57	66.87±1.57	**66.87±1.57^{e}**	33.74±1.22	33.74±1.22	**33.74±1.22^{a}**	16.59±0.23	16.59±0.23	**16.59±0.23^{d}**	**1256.62±33.88**	**1256.62±33.88**	**1256.62±30.30^{c}**
0MMA	6.67±0.04	6.67±0.04	**6.67±0.04^{b}**	67.18±0.42	67.18±0.42	**67.18±0.42^{d,e}**	32.76±2.93	32.76±2.93	**32.76±2.93^{b,c}**	17.31±0.36	17.31±0.36	**17.31±0.36^{c,d}**	**1247.49±34.42**	**1247.49±34.42**	**1247.49±30.79^{c}**
12	6.80±0.20	6.83±0.15	**6.82±0.16^{a}**	67.67±0.13	66.92±0.52	**67.30±1.81^{d}**	31.42±0.68	32.99±0.31	**32.21±1.55^{a}**	17.37±0.23	17.47±0.13	**17.42±1.71^{b,c}**	**1246.37±33.54**	**1253.86±34.61**	**1250.12±59.13^{c}**
24	6.83±0.11	6.85±0.46	**6.84±0.60^{a}**	69.23±0.90	76.93±0.27	**73.08±4.30^{c}**	34.20±0.50	33.61±0.12	**33.91±1.85^{b}**	17.34±0.12	17.56±0.44	**17.45±0.73^{b,c}**	**1317.21±52.13**	**1300.48±70.01**	**1308.85±126.95^{b,c}**
36	6.79±0.07	6.87±0.13	**6.83±0.29^{a}**	76.26±0.44	77.34±0.84	**76.80±0.84^{a}**	33.88±0.18	30.92±1.08	**32.40±1.76^{c}**	17.57±0.23	18.31±0.50	**17.94±1.18^{a}**	**1352.05±50.41**	**1263.19±31.84**	**1307.62±61.57^{b,c}**
48	6.81±0.19	6.78±1.10	**6.80±0.71^{a}**	76.25±0.35	76.49±0.21	**76.37±1.25^{a,b}**	34.94±0.06	32.50±0.50	**33.72±2.52^{a}**	18.02±0.34	17.15±0.32	**17.59±0.51^{a,b}**	**1345.49±83.95**	**1300.78±15.49**	**1323.14±118.84^{a,b}**
60	6.83±0.09	6.85±0.04	**6.84±0.06^{a}**	75.65±0.35	75.89±0.35	**75.77±0.63^{b}**	31.34±0.06	33.43±0.63	**32.39±1.72^{a,b}**	18.32±0.38	17.19±0.11	**17.76±0.66^{a}**	**1325.51±10.30**	**1356.59±37.36**	**1341.05±182.99^{a}**
72	6.77±0.06	6.81±0.12	**6.79±0.58^{a}**	75.38±0.62	76.19±0.11	**75.79±1.59^{b}**	33.09±0.91	32.59±0.41	**32.84±2.54^{a,b}**	18.12±0.12	17.77±0.23	**17.95±0.25^{a}**	**1370.36±53.86**	**1388.09±12.08**	**1379.23±215.76^{a}**
Total mean (n = 24)	**6.77±0.59^{a}**	**6.78±0.23^{a}**		**71.81±4.40^{b}**	**72.98±6.31^{a}**		**33.17±6.07^{a}**	**32.82±5.25^{b}**		**17.58±1.64^{a}**	**17.42±0.86^{a}**		**1307.64±132.32a**	**1295.89±113.72^{b}**	
Grand mean (n = 48)			**6.775 (SE = 0.052)**			**72.395 (SE = 0.081)**			**32.996 (SE = 0.081)**			**17.50 (SE = 0.070)**			**1301.76 (SE = 6.250)**
MPL (µg/kg)			**200 (inorganic)**			**100**			**10**			**20**			**20 (inorganic)**

Notes: Statistical differences among factor mean values were analyzed using Type III two-way ANOVA. According to pairwise comparisons, different superscript alphabets indicate that total mean values differ significantly ($p < 0.05$) within a row or a column.
0r: raw millet flour; 0MMA: raw millet flour sterilized with MMA; MMA: mixed-mode autoclaving: sterilizing flour and water together; control; SMA: single-mode autoclaving (sterilizing each flour and water separately); dw: dry weight; MPL: maximum permissible limit (Mititelu *et al.*, 2025).

enzymes involved in glycolysis and nucleic acid stability (Sun *et al.*, 2025). Considering that adults can consume, on average, 1.5 servings of cooked millet (1 serving = 1 cup; 174 g) (Gleim, 2025), raw and autoclaved/fermented samples can supply Fe, Mn, Zn, Cu, Cr, and Ni that meet the recommended dietary allowance (RDA) or adequate intake (AI) daily requirements.

In Table 4, SMA shows significantly higher levels of Hg and Cd (total means of 33.17 and 1,307.64 µg/kg dw, respectively) and lower Cd levels (total mean: 71.81 µg/kg dw) than MMA ($p < 0.05$), while As levels remain similar ($p > 0.05$). These results confirmed the varied impacts of SMA and MMA on millet components. Moreover, regardless of the autoclaving method, the fermentation of autoclave-pretreated pearl millet with *L. plantarum* does not reduce the levels of analyzed heavy metals. Instead, it causes significant increase in Cd, Pb, and Sb ($p < 0.05$), while As and Hg levels remain unchanged (Table 4). Although cereals provide essential nutrients, they also contain toxic heavy metals that can harm humans. The European Regulation (EU) 2021/1323 and the US Food and Drug Administration (FDA) have set maximum permissible limits (MPL) for these harmful substances (Mititelu *et al.*, 2025). Except for Sb, all toxic heavy metals in raw and autoclaved-millet fermented samples are below the MPLs (Table 3 and Supplementary Table S4). Previous studies have reported contamination of millets with toxic heavy metals (Omeje *et al.*, 2021).

According to the above-mentioned data, variations in millet's mineral composition after autoclaving pretreatment, followed by *L. plantarum* fermentation, could be due to changes in dry matter content, as microbial metabolism alters millet composition by consuming energy-rich components.

In summary, processing methods for pearl millet flour significantly influence its mineral content. SMA and MMA result in varying changes in flour. At the same time, fermentation generally boosts levels of beneficial minerals but also raises certain toxic heavy metals, mostly to non-harmful levels.

Analysis of TPC, TFC, TTC, and DPPH scavenging activity

The raw millet sample exhibits TPC, TFC, TTC, and DPPH scavenging activity of 2.81 mg GAE/g dw, 2.20 mg QE/g dw, 2.04 mg TAE/g dw, and 76.82%, respectively (Table 5 and Supplementary Table S5). As observed, 0MMA has significantly lower TPC (2.73 mg GAE/g dw) and higher TFC (2.67 mg QE/g dw) than raw millet ($p < 0.05$), while TTC is similar in both cases (Supplementary Table S5). This decline in TPC of millet after autoclaving could be due to the breakdown of heat-sensitive phenolic compounds or their transformation into unextractable forms, as they form insoluble complexes with macromolecules (Wang *et al.*, 2022). Moreover, Rahman *et al.* (2021) reported that thermal treatment boosts flavonoid levels by releasing bound forms, but the effect depends on plant matrix, temperature, and duration. Furthermore, regardless of fermentation period, SMA samples have significantly higher TPC than MMA samples (total mean of 3.12 and 2.94 mg GAE/g dw, respectively), while TFC and TTC showed similar values (Table 5). This suggests that these molecules react differently under two different autoclaving modes (121°C, 15 psi, 15 min). However, the effect of autoclaving on food varies depending on factors such as material, duration, temperature, and the type of phenolic compounds present. The impact of autoclaving on TPC, TFC, and TTC varies across studies, with decrease, increase, or no significant change reported (Balyatanda *et al.*, 2024; Kemal *et al.*, 2025).

Regarding the impact of fermentation, TPC increases gradually and significantly after 12 h (total mean of 2.92 mg GAE/g dw), reaching a peak between 36 h and 48 h, then gradually declines from 48 h to 72 h ($p < 0.05$; Table 5). At 12–24 h, fermentation does not ($p < 0.05$) alter TFC significantly in 0MMA (total mean of 2.67 mg QE/g dw). However, 24-h fermentation results in a significant increase in TFC, peaking at 36 h and remaining nearly stable until 72 h. After 36 h, *L. plantarum* fermentation increases significantly ($p < 0.05$) the TFC of 0MMA to 3.87 mg QE/g dw, which remained almost unchanged until 72 h of fermentation (Table 5).

For TTC, results show that regardless of the sterilization method used, 24-h fermentation significantly reduces the TTC of 0MMA from 2.00 to 1.70 mg TAE/g dw, and further to 1.56 mg TAE/g dw after 36 h ($p < 0.05$), remaining unchanged until the end of the 72-h fermentation period ($p > 0.5$) (Table 4). However, controlled fermentation can partially restore or enhance phenolics, with the degree of recovery depending on the initial autoclaving intensity and fermentation time (Khalil *et al.*, 2025; Liangyu *et al.*, 2023). Jan *et al.* (2022) investigated the effects of autoclaving (at 121°C for 15 min), followed by fermentation of Finger millet flour for 12, 24, and 36 h on phenolic compounds. They discovered that the TPC of millet increased with fermentation period, while tannins decreased (by 53.6–56%), aligning with our findings.

Generally, gradual increase in TPC and TFC after 72 h of *L. plantarum* fermentation could be due to fermentation reactions, such as decarboxylation, hydrolysis, microbial oxidation, reduction, esterification, and release of conjugated phenols (Alshammari *et al.*, 2025). It is discovered that LAB produce β-glucosidase and decarboxylase. These enzymes can facilitate the

Table 5: Contents of total phenolic (TPC), total flavonoids (TFC), and total tannins (TTC), and DPPH scavenging rate of autoclave-treated pearl millet, followed by *L. plantarum* ATCC 8014 fermentation.

Fermentation time (h)	TPC (mg GAE/g, dw)			TFC (mg QE/g, dw)			TTC (mg TAE/g, dw)			DPPH scavenging rate (%)		
	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean	SMA	MMA	Total mean	SMA	MMA	Total mean
0r	2.81±0.02	2.81±0.02	**2.81±0.02^{b}**	2.20±0.18	2.20±0.18	**2.20±0.18^{d}**	2.04±0.12	2.04±0.12^{a}	**2.04±0.12^{a}**	76.82±0.736	76.82±0.736	**76.82±0.658^{d}**
0MMA	2.73±0.03	2.73±0.03	**2.73±0.03^{c}**	2.67±0.03	2.67±0.03	**2.67±0.03^{c,d}**	2.00±0.07	2.00±0.07^{a}	**2.00±0.07^{a}**	63.40±0.797	63.40±0.797	**63.40±1.39^{e}**
12	2.83±0.08	3.01±0.05	**2.92±0.11^{b}**	2.59±0.02	2.66±0.01	**2.62±0.04^{c,d}**	1.79±0.08	1.68±0.02	**1.73±0.08^{b}**	77.02±1.07	81.15±1.36	**79.08±2.51^{c}**
24	3.47±0.17	3.22±0.05	**3.34±0.17^{a}**	2.52±0.01	3.36±0.03	**2.94±0.46^{b,c}**	1.78±0.09	1.62±0.06	**1.70±0.11^{b}**	79.39±0.894	80.35±0.299	**79.87±0.793^{c}**
36	3.24±0.12	3.51±0.01	**3.37±0.17^{a}**	3.36±0.04	4.37±0.15	**3.87±0.32^{a}**	1.51±0.05	1.61±0.03	**1.56±0.07^{c}**	83.14±0.478	85.84±0.384	**84.49±1.53^{a}**
48	3.55±0.06	3.32±0.04	**3.43±0.14^{a}**	3.30±0.03	3.24±0.10	**3.27±0.07^{b}**	1.46±0.04	1.55±0.02	**1.51±0.06^{c}**	81.76±0.675	81.91±0.391	**81.83±0.500^{b}**
60	3.35±0.05	2.43±0.02	**2.89±0.50^{b}**	4.66±0.20	3.07±0.03	**3.87±1.36^{a}**	1.46±0.02	1.56±0.03	**1.51±0.13^{c}**	81.78±0.710	79.62±0.186	**80.69±1.27^{b,c}**
72	3.01±0.02	2.50±0.04	**2.75±0.28^{c}**	3.79±0.35	3.96±0.52	**3.88±0.41^{a}**	1.45±0.03	1.57±0.01	**1.51±0.07^{c}**	76.78±0.750	77.43±0.571	**77.10±0.694^{d}**
Total mean (n = 24)	**3.12±0.30^{a}**	**2.94±0.40^{b}**		**3.14±0.82^{a}**	**3.19±0.63^{a}**		**1.64±0.23a**	**1.65±0.16^{a}**		**77.51±6.09^{b}**	**78.32±6.47^{a}**	
Grand mean (n = 48)			**3.03 (SE = 0.010)**			**3.17 (SE = 0.029)**			**1.70 (SE = 0.101)**			**77.91 (SE = 0.217)**

Notes: Statistical differences among factor mean values were analyzed using Type III two-way ANOVA. According to pairwise comparisons, different superscript alphabets indicate that total mean values differ significantly ($p < 0.05$) within a row or a column. 0r: raw millet flour; 0MMA: raw millet flour sterilized with MMA; MMA: mixed-mode autoclaving: sterilizing flour and water together; control); SMA: single-mode autoclaving (sterilizing each flour and water separately); dw: dry weight; GAE: gallic acid equivalents; QE: quercetin equivalents; TAE: tannic acid equivalents.

release and transformation of phenolic compounds from the plant cell wall during pomace fermentation, thereby boosting TPC (Kalinowska *et al.*, 2023). In addition to improving TPC, *L. plantarum* metabolizes phenolic compounds into non-phenolic ones, such as converting protocatechuic acid (a hydroxybenzoic acid) into catechol through decarboxylation (Pulido-Mateos *et al.*, 2024). This may occur when the TPC of millet flour decreases after more than 60 h of fermentation (Supplementary Table S5). Reduction in antinutritional factor TTC after *L. plantarum* fermentation of pearl millet flour could be due to partial hydrolysis by tannase, a tannin acyl hydrolase produced by LAB, which reduces tannin polymerization, thereby lowering its content and anti-nutritional properties (Yang *et al.*, 2023). This decrease in tannin content was observed previously in finger millet fermentation, with a greater reduction with increased fermentation period (Endalew *et al.*, 2024). An increase in TPC and TFC after cereal fermentation by *Lactobacillus* spp., including *L. plantarum*, has been reported previously (Srivastava *et al.*, 2024; Yang *et al.*, 2024).

Post-autoclaving fermentation with *L. plantarum* has been reported to enhance DPPH antioxidant activity by releasing bound phenolics and flavonoids and by forming new antioxidant metabolites (Mutshinyani *et al.*, 2020).

The DPPH scavenging rate of raw millet is 76.82%, which significantly decreased to 63.4% in raw millet treated with MMA (0MMA) ($p < 0.05$; Supplementary Table S5). Moist heat and pressure during autoclaving may reduce antioxidant molecules' ability to neutralize free radicals (Chuwech *et al.*, 2023). However, SMA exhibits a lower DPPH scavenging rate than MMA (77.51% and 78.32%, respectively), regardless of fermentation period ($p < 0.05$; Table 5). The high moist heat, probably occurring during MMA treatment (with a millet flour–moisture ratio of 1:4) may create a more effective heat-transfer environment in MMA than in SMA, which involves autoclaving of millet flour and water separately before combining them. This could enhance the activity of specific antioxidant molecules and, in turn, improve the overall antioxidant activity (Popoola, 2022).

Research indicates that pressure and thermal treatment can alter the integrity of macromolecular structures, thereby affecting polyphenol dissolution rates and antioxidant efficacy (Zheng *et al.*, 2023). Furthermore, although SMA sample has a significantly higher TPC than MMA sample, the DPPH activity is significantly higher in MMA sample (Table 5). This rise in TPC may primarily result from the release of bound phenolics and the formation of Maillard reaction products (Carciochi *et al.*, 2016). Horvat *et al.* (2020) discovered that the DPPH scavenging rate decreases because the most potent, heat-sensitive antioxidants may be destroyed, and the newly available phenolics often become inactive through binding or transformation into less effective compounds. Moreover, Zhang *et al.* (2017) discovered that extrusion cooking of highland barley increased total phenolic content while decreasing DPPH activity. They attributed this to decrease in free phenolic acids, such as vanillic acid and syringic acid, which form insoluble complexes that reduce antioxidant activity. Han *et al.* (2023) stated that the impact of thermal processing on cereal antioxidant activity depends on a balance, as it may generate new antioxidant Maillard reaction products while also destroying native phenolics. The result is either an increase, a decrease, or no change in antioxidant activity, depending on specific processing conditions and cereal variety.

Additionally, regardless of the autoclaving method, the DPPH scavenging rate of 0MMA (63.40%) increases by 79.08% after 12 h of fermentation ($p < 0.05$). This rate continues to rise, reaching its peak at 36 h, and then slightly declines as fermentation progresses to 72 h (77.10%) ($p < 0.05$) (Table 5). As observed, *L. plantarum* fermentation of millet flour after MMA treatment shows better DPPH scavenging activity than SMA (Table 5; Supplementary Table S5). This is likely due to the effectiveness of moist heat from the MMA treatment, which enhances *L. plantarum*'s ability to produce low molecular phenolic metabolites with increased bioactivity, such as (+)-catechin, (−)-epicatechin, and others (Pulido-Mateos *et al.*, 2024). Studies indicate that *L. plantarum* produces enzymes, such as β-glucosidase, during fermentation that hydrolyzes phenolic glycosides into aglycones with radical-scavenging properties, thereby enhancing the antioxidant activity of the fermented product (Paventi *et al.*, 2025). The decrease in DPPH scavenging activity after 36 h of fermentation in millet samples could result from the conversion of phenolic compounds into non-phenolic ones, as mentioned above (Pulido-Mateos *et al.*, 2024). Additionally, an increase in lactate in the medium can cause cellular acidic stress, leading to a linear decrease in the growth rate of *L. plantarum*, which negatively impacts all metabolic activities (Giraud *et al.*, 1991). This is evident in the reduction of TPC and DPPH scavenging rates after 72 h of fermentation in millet samples (Table 5; Supplementary Table S5).

In conclusion, the 24–36-h fermentation period often marks a turning point, during which levels of phenolics, flavonoids, and tannins as well as antioxidant activity undergo significant changes. This corresponds to the findings of increased phenolics and DPPH activity at 38.86 h of fermentation by *L. plantarum* (Srivastava

et al., 2024). Additionally, higher concentrations of flavonoids, total phenols, and improved radical scavenging activity were observed when using *L. plantarum* and other strains (Yang *et al.*, 2024).

Untargeted LC-MS/MS profiles of processed pearl millet samples

Although untargeted LC-MS/MS is an effective method for identifying active ingredients in complex natural mixtures, it has several limitations. Owing to issues such as ionization bias and combined effects, results depend heavily on data processing. They can be influenced by false positives (detecting inert chemicals) and false negatives (missing potent and low-concentration compounds; Caesar *et al.*, 2019). Nevertheless, despite possible quantitative errors from using the percentage of total area, we used these data to preliminarily indicate that our fermented samples contained bioactive compounds, possibly from millet or microbes, which may contribute to their DPPH radical scavenging activity (Section 3.6) as well as some contaminants that could be impacted by autoclaving/fermentation processes.

A total of 18 bioactive compounds and contaminants were identified in SMA-pretreated fermented pearl millet samples after 72 h, compared to 15 in MMA-pretreated samples under the same conditions. These compounds mainly originate from millet, microbial sources, and contaminants. The SMA- and MMA-pretreated fermented samples share several compounds, such as indoxacarb, 3-carboxy-1-hydroxypropyl-thpp, methotrexate, absinthin, and metconazole (Tables 6 and 7; Supplementary Figures F1 and F2). The profile of bioactive compounds changed significantly over time. Some beneficial phytochemicals (e.g., salicin 6-phosphate and absinthin) and contaminants (e.g., insecticides, such as indoxacarb) decreased or were completely metabolized during the 72-h *L. plantarum* fermentation (Acebrón *et al.*, 2017; Wei *et al.*, 2022). Conversely, some compounds (e.g., inulicin and solasodine) are only detected later in fermentation (72 h), potentially released from conjugated forms by microbial enzymes (Kumar *et al.*, 2019). It has been reported that fermentation of millet flour with LAB can yield probiotic-rich products and enhance bioactive compounds (Tomar *et al.*, 2025). Moreover, fermentation effectively reduced levels of pesticides and fungicides (e.g., indoxacarb, cyfluthrin, and metconazole), with most disappearing within 60–72 h (Armenova *et al.*, 2023).

In conclusion, fermentation with *L. plantarum* modifies the bioactive profile of SMA- and MMA-pretreated pearl millet by increasing compound diversity and decreasing beneficial phytochemicals while effectively degrading pesticide and fungicide contaminants over 72 h.

Conclusions

This study demonstrates that SMA and MMA pretreatments affect millet matrix and *L. plantarum* fermentation, revealing their links to bacterial growth, sugar consumption, nutritional content, and antioxidant and bioactive compound production through a detailed systems-level analysis. Both pretreatments achieved the same peak cell density but differed in their effects. The data showed that SMA was favored for fast startup processes, such as producing fermented beverages or quick acidification, due to its rapid growth and acid production. MMA is more appropriate for developing functional ingredients that require higher antioxidant activity and mineral bioavailability, despite a slower initial fermentation. This shows that sterilization method is more than just a technical step; it is a key factor in developing effective fermentation processes.

However, the study has some limitations, including the method of carbohydrate measurement, which is calculated "by difference" and may not accurately reflect metabolizable carbohydrates. In addition, explanations for starch gelatinization, nutrient bioavailability, and compound release rely on indirect data, and direct measurements of resistant starch and key enzyme activities were absent. Moreover, LC-MS/MS data comparisons based on percentage of total area are prone to ionization bias, affecting the accuracy of concentration measurements.

Future research should assess the impact of autoclaving pretreatments on millet's metabolic and structural changes, evaluating starch and enzyme activity, quantifying metabolic products, and analyzing structural changes using techniques such as scanning electron microscope (SEM) and X-ray diffraction (XRD). Results should be validated through pilot- and larger-scale trials, as well as consumer sensory evaluations, to determine product quality and acceptability.

Data Availability Statement

The dataset used and analyzed during the current study are available from the corresponding author upon reasonable request.

Table 6. LC-MS/MS analysis of bioactive compounds in SMA-treated millet flour, followed by *L. plantarum* ATCC 8014 fermentation.

No.	RT	Compound	Molecular formula (g/mol)	Biological activity	Peak area (%)					
					12 h	24 h	36 h	48 h	60 h	72 h
1.	3.73	3-Carboxy-1-hydroxypropyl-ThPP	$C_{16}H_{25}N4O_{10}P_2S$	It is a human and mouse metabolite (PubChem National Center for Biotechnology Information (NCBI), 2004.	0.18	0.15	0.32	0.20	0.00	0.00
2.	3.73	Indoxacarb	$C_{22}H_{17}ClF_3N_3O7$	It is an organochlorine insecticide used against lepidopteran larvae (Jeschke *et al.*, 2019).	0.32	0.33	0.39	0.29	0.00	0.00
3.	3.85	1-(5'-Phosphoribosyl)-5-formamido-4-imidazolecarboxamide (Faicar)	$C_{10}H_{15}N_4O_9P$	Escherichia coli produces it and exhibits anti-inflammatory and anti-cancer activities (Brooks *et al.*, 2018).	0.82	0.79	0.21	0.00	0.00	0.00
4.	4.30	6-Methylpretetramide	$C_{20}H_{15}NO_6$	It is discovered in some plants and is functionally related to pretetramid, a tetracycline (PubChem National Center for Biotechnology Information (NCBI), 2005).	0.82	0.83	0.71	0.18	1.67	0.00
5.	4.43	Salicin 6-phosphate	$C_{13}H_{19}O_{10}P$	It is a glycoside phosphate produced by certain bacteria that inhibits inflammation, tumor growth, and angiogenesis (Kong *et al.*, 2014).	1.07	1.0	1.08	0.73	0.39	0.00
6.	4.70	(Sulfonamide-N)(L-isoleucyl) altemicidin	$C_{19}H_{31}N_5O_8S$	It is a monoterpene alkaloid that demonstrates potent anti-tumor activity (Hameed *et al.*, 2021).	0.47	0.23	0.62	0.00	0.29	0.00
7.	7.00	Inulicin	$C_{17}H_{24}O_5$	It is a terpene lactone, with anti-inflammatory properties (Yan *et al.*, 2024).	0.00	0.00	0.00	0.69	0.71	0.64
8.	7.07	Plumieride	$C_{21}H_{26}O_{12}$	It is a glycoside with antifungal, anti-virulence, and anti-inflammatory properties (El-Shiekh *et al.*, 2024).	0.87	0.88	0.87	0.87	0.96	1.04
9.	7.09	Limonin	$C_{26}H_{30}O_8$	It is a natural tetracyclic triterpenoid compound that exhibits anti-inflammatory, analgesic, antibacterial, antiviral, antioxidant, anticancer, and liver-protective properties (Fan *et al.*, 2019).	1.93	0.64	9.63	0.33	0.59	1.05
10.	7.47	Obacunone	$C_{26}H_{30}O_7$	It is a plant metabolite that acts as an anticancer agent (Zheng *et al.*, 2023).	0.23	0.24	0.21	0.18	0.14	0.00
11.	7.48	Rutaevin (a steroid lactone)	$C_{26}H_{30}O_9$	It is a phytochemical that inhibits nitric oxide production in lipopolysaccharide-stimulated RAW 264.7 macrophages (Cheenpracha *et al.*, 2010).	0.00	0.40	0.40	0.23	0.20	0.15
12.	7.63	Methotrexate	$C_{20}H_{22}N_8O_5$	It is an antimetabolite drug that acts as an anticancer agent by slowing the growth of cancer cells and treating psoriasis and diabetes (Sakhare, 2018).	0.0	0.0	0.0	2.80	2.13	3.12
13.	7.96	Paucin	$C_{23}H_{32}O_{10}$	It is a sesquiterpene lactone that suppresses the proliferation of nasopharyngeal cancer and lymphocytic leukemia (Hoffmann *et al.*, 1978).	3.34	3.38	3.40	3.34	3.08	0.00
14.	8.10	Iridodial glucoside tetraacetate	$C_{24}H_{34}O_{11}$	It is a terpene glycoside that effectively suppresses liver cancer and inhibits insect growth (Kim and Choi, 2021).	1.79	0.86	8.35	1.05	4.11	0.00
15.	8.21	Strictosamide	$C_{26}H_{30}N_2O_8$	It is a wound-healing compound of plant origin (Ming *et al.*, 2024).	0.29	1.41	1.67	0.29	0.39	0.00
16.	9.55	Cyfluthrin	$C_{22}H_{18}C_{l2}FNO_3$	It is an insecticide with less toxicity to humans (Hanson *et al.*, 2018).	0.38	0.34	0.23	0.29	0.00	0.00
17.	14.50	Absinthin	$C_{30}H_{40}O_6$	It is a plant metabolite, a dimeric sesquiterpene lactone, with anti-inflammatory properties (Talmon *et al.*, 2020).	0.23	0.29	0.29	0.24	0.00	0.00
18.	17.30	Metconazole	$C_{17}H_{22}ClN_3O$	It is a synthetic triazole fungicide (Fei and Hao, 2024).	0.86	0.94	0.94	0.90	0.65	0.60

Table 7. LC-MS/MS analysis of bioactive compounds in MMA-treated millet flour, followed by *L. plantarum* ATCC 8014 fermentation.

No.	RT	Compound	Molecular; molecular (g/mol)	Biological activity	Peak area (%)					
					12 h	24 h	36 h	48 h	60 h	72 h
1.	4.09	3-Carboxy-1-hydroxypropyl-ThPP	$C_{16}H_{25}N_4O_{10}P_2S$	It is a human and mouse metabolite (McNulty *et al.*, 2015).	0.0	0.0	0.86	0.0	0.0	0.12
2.	4.17	Indoxacarb	$C_{22}H_{17}ClF_3N_3O_7$	It is an organochlorineinsecticide used against lepidopteran larvae (Jeschke *et al.*, 2019).	0.30	0.26	0.29	0.0	0.0	0.0
3.	7.63	Methotrexate	$C_{20}H_{22}N_8O_5$	It is an antimetabolite drug that serves as an anticancer by slowing the growth of cancer cells, and treats psoriasis and diabetes (Mohammed, 2025).	0.0	0.0	0.0	2.80	2.13	3.12
4.	9.32	Celapanine	$C_{30}H_{35}NO_{10}$	It is a sesquiterpenoid with analgesic, anti-inflammatory, antioxidant, and antipyretic activities (Shashank *et al.*, 2017).	0.0	0.0	0.0	0.0	0.0	2.11
5.	9.92	Bisibuthiamine	$C_{32}H_{46}N_8O_6S_2$	It is a synthetic vitamin B1 analogue used to treat asthenia (MedChemExpress, 2024). The prolonged administration of the medication has been shown to enhance long-term memory formation in mice (DrugBank, 2024).	0.0	3.18	10.31	7.23	1.66	0.0
6.	17.30	Metconazole	$C_{17}H_{22}ClN_3O$	It is a synthetic triazole fungicide (Fei and Hao, 2024).	0.90	0.94	0.94	0.90	0.65	0.60
7.	18.86	Fexaramine	$C_{32}H_{36}N_2O_3$	It is a synthetic agonist of the farnesoid X receptor (Downes *et al.*, 2003).	0.0	0.44	10.91	15.85	7.79	2.11
8.	19.49	Absinthin	$C_{30}H_{40}O_6$	It is a sesquiterpene lactone with anti-inflammatory properties (Talmon *et al.*, 2020).	0.21	0.20	0.22	0.18	0.16	0.00
9.	19.51	Capsorubin	$C_{40}H_{56}O_4$	It is a xanthophyll pigment with antioxidant, antibacterial, anti-obesity, antidiabetic, and anti-inflammatory activities (Yuca, 2022).	0.0	0.0	0.0	0.0	0.0	9.88
10.	19.57	beta-Geranylfarnesene	$C_{25}H_{40}$	It is a sesquiterpene with a role as a bacterial metabolite (PubChem National Center for Biotechnology Information (NCBI), 2015). It is primarily used by plants and insects for communication (Bi *et al.*, 2022).	0.0	0.0	0.0	1.92	4.16	4.42
11.	19.67	Timosaponin A-III	$C_{39}H_{64}O_{13}$	A triterpene exhibits anticancer activity and is used as an antipyretic, antidiabetic, anti-inflammatory, antiplatelet-aggregation, and antidepressant agent (Han *et al.*, 2018).	0.0	0.0	0.0	0.0	0.0	3.12
12.	19.72	7beta-Hydroxytaxusin	$C_{28}H_{40}O_9$	It is a diterpenoid with a role as a metabolite (PubChem National Center for Biotechnology Information (NCBI), 2012).	0.0	1.57	1.82	2.10	3.38	0.0
13.	19.86	Auriculine	$C_{31}H_{45}NO_8$	It is a useful organic compound that has obvious effects on Gram-positive and Gram-negative bacteria (Guo *et al.*, 2016).	1.98	1.9	1.77	0.0	0.0	0.0
14.	19.94	Solasodine	$C_{27}H_{43}NO_2$	It is an alkaloid and exhibits antioxidant, hepatoprotective, immunomodulatory, cytotoxic, antinociceptive, anti-inflammatory, antiatherosclerotic, antimicrobial, and antiobesity properties (Kumar *et al.*, 2019).	0.0	0.0	0.0	0.0	0.0	8.32
15.	19.94	Oleanolate 3-O-beta-D-glucoside	$C_{36}H_{58}O_8$	Its precursor, oleanolic acid, demonstrated inhibition of HIV-1 replication in acutely infected H9 cells (Sultana and Ata, 2008).	0.0	0.0	0.0	0.0	0.0	1.79

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Author contributions

Mohammed A. Mohammed: project administration, conceptualization, methodology, software, writing original draft, editing, and reviewing. Abu El Gasim A. Yagoub: project administration, conceptualization, editing, reviewing, and supervision. Pandurangan Subash-Babu: supervision and validation. Laila Naif Al-Harbi: software, funding, and validation. Ghedeir M. Alshammari: methodology, software, and supervision; Hany M. Yehia: software and methodology; M.A. Osman: methodology and software. Abdullah Al Tamim: methodology. Mohammed A. Yahya: resources and data curation. All authors had read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declared no conflict of interest regarding this paper.

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References

Abdalla, A., El Tinay, A., Mohamed, B. and Abdalla, A. 1998. Proximate composition, starch, phytate and mineral contents of 10 pearl millet genotypes. Food Chem. 63(2):243–246. https://doi.org/10.1016/S0308-8146(97)00228-8

Acebrón, I., Plaza-Vinuesa, L., De Las Rivas, B., Muñoz, R., Cumella, J., Sánchez-Sancho, F. and Mancheño, J.M. 2017. Structural basis of the substrate specificity and instability in solution of a glycosidase from *Lactobacillus plantarum*. BBA Proteins Proteom. 1865(10):1227–1236. https://doi.org/10.1016/j.bbapap.2017.07.007

Adebo, J.A., Njobeh, P.B., Gbashi, S., Oyedeji, A.B., Ogundele, O.M., Oyeyinka, S.A. and Adebo, O.A. 2022. Fermentation of cereals and legumes: impact on nutritional constituents and nutrient bioavailability. Fermentation, 8(2):63. https://doi.org/10.3390/fermentation8020063

Adisa, A.M., Badejo, A.A., Ifesan, B.O.T. and Enujiugha, V.N. 2024. Phenotypic and molecular differentiation of lactic acid bacteria in fonio millet ogi fermentation and their potential as starter cultures. Food Humanity. 2:100230. https://doi.org/10.1016/j.foohum.2024.100230

Agrawal, R. 2024. Fermentation media and industrial sterilization. In: Textbook of Industrial Microbiology. Springer, New York, NY, pp. 87–96. https://doi.org/10.1007/978-981-97-9582-6_6

Aguirre-Garcia, Y.L., Nery-Flores, S.D., Campos-Muzquiz, L.G., Flores-Gallegos, A.C., Palomo-Ligas, L., Ascacio-Valdés, J.A., Sepúlveda-Torres, L. and Rodríguez-Herrera, R. 2024. Lactic acid fermentation in the food industry and bio-preservation of food. Fermentation. 10(3):168. https://doi.org/10.3390/fermentation10030168

Allocati, N., Masulli, M., Di Ilio, C. and De Laurenzi, V. 2015. Die for the community: an overview of programmed cell death in bacteria. Cell Death Dis. 6(1):e1609–e1609. https://doi.org/10.1038/cddis.2014.570

Alshammari, G.M., Mohammed, M.A., Al Tamim, A., Yahya, M.A. and Yagoub, A.E.A. 2025. Changes in nutrients, heavy metals, residual pesticides, bioactive compounds, and anti-oxidant activity following germination and fermentation of Sudanese and Yemeni sorghums. Int J Food Sci Technol. 60(2):vvaf069. https://doi.org/10.1093/ijfood/vvaf069

Amadou, I. 2022. Millet-based functional foods: bio-chemical and bio-functional properties. Functional Foods Book, Chapter 9, pp. 303–330). https://doi.org/10.1002/9781119776345.ch9

Anumudu, C.K., Miri, T. and Onyeaka, H. 2024. Multifunctional applications of lactic acid bacteria: enhancing safety, quality, and nutritional value in foods and fermented beverages. Foods. 13(23):3714. https://doi.org/10.3390/foods13233714

Armenova, N., Tsigoriyna, L., Arsov, A., Petrov, K. and Petrova, P. 2023. Microbial detoxification of residual pesticides in fermented foods: current status and prospects. Foods. 12(6):1163. https://doi.org/10.3390/foods12061163

Arya, P., Vaidya, D. and Gupta, A. 2025. Fermentation-driven bioactive enhancement in cereal grains: mechanisms, nutritional improvements, and functional food applications. Trends Food Sci Technol. 166: 105403. https://doi.org/10.1016/j.tifs.2025.105403

Association of Official Analytical Chemists (AOAC). 2023. The Official Methods of Analysis of AOAC International, 22nd edn. Oxford University Press, Oxford, UK. https://doi.org/10.1093/9780197610145.001.0001

Bajtai, A., Németi, G., Le, T. M., Szakonyi, Z., Péter, A., & Ilisz, I. (2022). Enantiomeric separation of newly synthesized amino, thio, and oxy derivatives of monoterpene lactones, amides, and ester applying polysaccharide-based chiral stationary phases in normal-phase mode. *Journal of Chromatography A*, *1672*, 463050. https://doi.org/10.1016/j.chroma.2022.463050

Balli, D., Cecchi, L., Pieraccini, G., Venturi, M., Galli, V., Reggio, M., Di Gioia, D., Furlanetto, S., Orlandini, S. and Innocenti, M. 2023. Millet fermented by different combinations of yeasts and lactobacilli: effects on phenolic composition, starch, mineral content and prebiotic activity. Foods. 12(4):748. https://doi.org/10.3390/foods12040748

Balyatanda, S.B., Gowda, N.A.N., Subbiah, J., Chakraborty, S., Prasad, P.V.V. and Siliveru, K. 2024. Physiochemical, bio, thermal, and non-thermal processing of major and minor millets: a comprehensive review on antinutritional and antioxidant properties. Foods. 13(22):3684. https://doi.org/10.3390/foods13223684

Bao, X., Feng, H., Guo, G., Huo, W., Li, Q., Xu, Q., Liu, Q., Wang, C. and Chen, L. 2022. Effects of laccase and lactic acid bacteria on the fermentation quality, nutrient composition, enzymatic hydrolysis, and bacterial community of alfalfa silage. Front Microbiol. 13:1035942. https://doi.org/10.3389/fmicb.2022.1035942

Bi, H., Merchant, A., Gu, J., Li, X., Zhou, X. and Zhang, Q. 2022. CRISPR/Cas9-mediated mutagenesis of abdominal-A and ultrabithorax in the Asian corn borer *Ostrinia furnacalis*. Insects. 13(4):384. https://doi.org/10.3390/insects13040384

Boutet, S., Barreda, L., Perreau, F., Totozafy, J.C., Mauve, C., Gakiere, B., Delannoy, E., Martin-Magniette, M.L., Monti, A., Lepiniec, L. and Zanetti, F. 2022. Untargeted metabolomic analyses reveal the diversity and plasticity of the specialized metabolome in seeds of different *Camelina sativa* genotypes. Plant J. 110(1):147–165. https://doi.org/10.1111/tpj.15662

Brooks, H.B., Meier, T.I., Geeganage, S., Fales, K.R., Thrasher, K.J., Konicek, S.A., Spencer, C.D., Thibodeaux, S., Foreman, R.T. and Hui, Y.-H. 2018. Characterization of a novel AICARFT inhibitor which potently elevates ZMP and has anti-tumor activity in murine models. Sci Rep. 8(1):15458. https://doi.org/10.1038/s41598-018-33453-4

Caesar, L.K., Kellogg, J.J., Kvalheim, O.M. and Cech, N.B. 2019. Opportunities and limitations for untargeted mass spectrometry metabolomics to identify biologically active constituents in complex natural product mixtures. J Nat Prod. 82(3):469–484. https://doi.org/10.1021/acs.jnatprod.9b00176

Carciochi, R.A., Dimitrov, K. and Galván D´ Alessandro, L. 2016. Effect of malting conditions on phenolic content, Maillard reaction products formation, and antioxidant activity of quinoa seeds. J Food Sci Technol. 53(11):3978–3985. https://doi.org/10.1007/s13197-016-2393-7

Chambers, M.C., Maclean, B., Burke, R., Amodei, D., Ruderman, D.L., Neumann, S., Gatto, L., Fischer, B., Pratt, B. and Egertson, J. 2012. A cross-platform toolkit for mass spectrometry and proteomics. Nature Biotechnol. 30(10):918–920. https://doi.org/10.1038/nbt.2377

Cheenpracha, S., Park, E.-J., Rostama, B., Pezzuto, J.M. and Chang, L.C. 2010. Inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells by the norsesterterpene peroxide, epimuqubilin A. Marine Drugs. 8(3):429–437. https://doi.org/10.3390/md8030429

Chiodetti, M., Tuccio, M.G. and Carini, E. 2024. Effect of water content on gelatinization functionality of flour from sprouted sorghum. Curr Res Food Sci. 8:100780. https://doi.org/10.1016/j.crfs.2024.100780

Chu, K., Cui, X., Yang, Y., Wu, Q., Liu, R. and Xie, Y. 2025. Effects of pressure cooker treatment-induced protein oxidation of cereals on human gut microbiota using an *in vitro* fermentation model. Food Res Int. 220: 117123. https://doi.org/10.1016/j.foodres.2025.117123

Chuwech, M., Rakariyatham, N., Tinoi, J., Suwitchayanon, P. and Chandet, N. 2023. Effect of heat–moisture treatment on crystallinity, digestibility properties, bioactive compounds, and antioxidant activity of purple rice (*Oryza sativa* L. indica) flour. Processes. 11(3):969. https://doi.org/10.3390/pr11030969

Cui, Y., Wang, M., Zheng, Y., Miao, K. and Qu, X. 2021. The carbohydrate metabolism of *Lactiplantibacillus plantarum*. Int J Mol Sci. 22(24):13452. https://doi.org/10.3390/ijms222413452

Dangal, A., Tahergorabi, R., Acharya, D.R., Timsina, P., Rai, K., Dahal, S., Acharya, P. and Giuffrè, A.M. 2024. Review on deep-fat fried foods: physical and chemical attributes, and consequences of high consumption. Eur Food Res Technol. 250(6):1537–1550. https://doi.org/10.1007/s00217-024-04482-3

Das, R.S., Tiwari, B.K., Chemat, F. and Garcia-Vaquero, M. 2022. Impact of ultrasound processing on alternative protein systems: protein extraction, nutritional effects and associated challenges. Ultrason Sonochem. 91:106234. https://doi.org/10.1016/j.ultsonch.2022.106234

De Vuyst, L., Vrancken, G., Ravyts, F., Rimaux, T. and Weckx, S. 2009. Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. Food Microbiol. 26(7):666–675. https://doi.org/10.1016/j.fm.2009.07.012

Djorgbenoo, R., Hu, J., Hu, C. and Sang, S. 2023. Fermented oats as a novel functional food. Nutrients. 15(16):3521. https://doi.org/10.3390/nu15163521

Đorđević, T.M., Šiler-Marinković, S.S., Đurović-Pejčev, R.D., Dimitrijević-Branković, S. I., & Umiljendić, J. S. G. (2013). Efficiencies of Different Methods for Organophosphate Pesticide Residues Determination in Fermented Wheat Substrate. *Pesticides and Phytomedicine/Pesticidi i fitomedicina*, *28*(2). https://doi.org/10.2298/pif.v28i2.3963.

Downes, M., Verdecia, M.A., Roecker, A., Hughes, R., Hogenesch, J.B., Kast-Woelbern, H.R., Bowman, M.E., Ferrer, J.-L., Anisfeld, A.M. and Edwards, P.A. 2003. A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. Mol Cell. 11(4):1079–1092. https://doi.org/10.1016/s1097-2765(03)00104-7

Druart, P. and Wulf, O.D. 1993. Activated charcoal catalyses sucrose hydrolysis during autoclaving. Plant Cell Tissue Organ Cult. 32(1). https://doi.org/10.1007/BF00040122.

DrugBank. 2024. Bisibuthiamine. DrugBank accession number DB:13416. https://www.drugbank.ca/drugs/DB13416 (Accessed on: 30 October 2024).

El-Hashash, E.F., Al-Habeeb, A., Bakri, H. and Majjami, A.Y. 2023. A comprehensive review of pearl and small millets: taxonomy, production, breeding and future prospects in Saudi Arabia. Asian J Res Crop Sci. 8(4):151–166. https://doi.org/10.9734/AJRCS/2023/v8i4196

El-Shiekh, R.A., Meselhy, M.R., Elshimy, R., Ibrahim, M.A., Ali, M.E. and Hassanen, E.I. 2024. Plumieride as a novel anti-fungal and anti-inflammatory iridoid against superficial candidiasis in mice. BMC Complement Med Ther. 24(1):224. https://doi.org/10.1186/s12906-024-04508-z

Endalew, H.W., Atlabachew, M., Karavoltsos, S., Sakellari, A., Aslam, M.F., Allen, L., Griffiths, H., Zoumpoulakis, P., Kanellou, A. and Yehuala, T.F. 2024. Effect of fermentation on nutrient composition, antinutrients, and mineral bioaccessibility of finger millet based Injera: a traditional Ethiopian food. Food Res Int. 190:114635. https://doi.org/10.1016/j.foodres.2024.114635

ETHOS UP. (2024). High-performance microwave digestion system. www.milestone.sci.com (Accessed March 3, 2024).

Fan, S., Zhang, C., Luo, T., Wang, J., Tang, Y., Chen, Z. and Yu, L. 2019. Limonin: a review of its pharmacology, toxicity, and pharmacokinetics. Molecules. 24(20):3679. https://doi.org/10.3390/molecules24203679

Faridah, D.N., Silitonga, R.F., Indrasti, D., Afandi, F.A., Jayanegara, A. and Anugerah, M.P. 2022. Verification of autoclaving-cooling treatment to increase the resistant starch contents in food starches based on meta-analysis result. Front Nutr. 9:904700. https://doi.org/10.3389/fnut.2022.904700

Fei, L. and Hao, L. 2024. *In vitro* and *ex vivo* antifungal activities of metconazole against the rice blast fungus *Pyricularia oryzae*. Molecules. 29(6):1353. https://doi.org/10.3390/molecules29061353

Food and Agriculture Organization (FAO). 2021. Production-yield quantities of millets in world + (total) 1994–2021. https://www.fao.org/faostat/en/#data/QCL/visualize (Accessed on: 23 March 2023).

Gabriele, M., Cavallero, A., Tomassi, E., Arouna, N., Árvay, J., Longo, V. and Pucci, L. 2024. Assessment of sourdough fermentation impact on the antioxidant and anti-inflammatory potential of pearl millet from Burkina Faso. Foods. 13(5):704. https://doi.org/10.3390/foods13050704

Giraud, E., Lelong, B. and Raimbault, M. 1991. Influence of pH and initial lactate concentration on the growth of *Lactobacillus plantarum*. Appl Microbiol Biotechnol. 36(1):96–99. https://doi.org/10.1007/BF00164706

Gleim, S. 2025. Health benefits of millet. https://www.webmd.com/diet/health-benefits-millet (Accessed on: 30 November 2025).

Goudar, G., Manne, M., Sathisha, G., Sharma, P., Mokalla, T.R., Kumar, S.B. and Ziouzenkova, O. 2023. Phenolic, nutritional and molecular interaction study among different millet varieties. Food Chem Adv. 2:100150. https://doi.org/10.1016/j.focha.2022.100150

Guo, L., Peng, Y., Zhao, C., Yang, X., Liu, L. and Wang, R. 2016. Pharmacokinetics of tecomin in rats after intragastric and intravenous administration. Biomed Chromatogr. 30(4):612–617. https://doi.org/10.1002/bmc.3604

Hameed, A., Al-Rashida, M. and Shah, M.R. 2021. α-Tertiary Amines En Route to Natural Products. Elsevier, Amsterdam, the Netherlands. https://doi.org/10.1016B978-0-12-822262-1.00001-9

Han, H., Dye, L. and Mackie, A. 2023. The impact of processing on the release and antioxidant capacity of ferulic acid from wheat: a systematic review. Food Res Int. 164:112371. https://doi.org/10.1016/j.foodres.2022.112371

Han, F.-Y., Song, X.-Y., Chen, J.-J., Yao, G.-D. and Song, S.-J. 2018. Timosaponin AIII: a novel potential anti-tumor compound from *Anemarrhena asphodeloides*. Steroids. 140:125–130. https://doi.org/10.1016/j.steroids.2018.09.014

Hanson, W., Strid, A., Hallman, A. and Jenkins, J. 2018. Cyfluthrin General Fact Sheet. National Pesticide Information Center, Oregon State University Extension Services, OR. npic.orst.edu/factsheets/cyfluthringen.html.

Hoffmann, J.J., Jolad, S.D., Torrance, S.J., Luzbetak, D.J., Wiedhopf, R.M. and Cole, J.R. 1978. Odoratin and paucin: cytotoxic sesquiterpene lactones from, *Baileya pauciradiata* (*Compositae*). J Pharm Sci. 67(11):1633–1634. https://doi.org/10.1002/jps.2600671136

Horvat, D., Šimić, G., Drezner, G., Lalić, A., Ledenčan, T., Tucak, M., Plavšić, H., Andrić, L. and Zdunić, Z. 2020. Phenolic acid profiles and antioxidant activity of major cereal crops. Antioxidants. 9(6):527. https://doi.org/10.3390/antiox9060527

Hussain, A., Arif, M.R., Ahmed, A., Fiaz, I., Zulfiqar, N., Ali, M.Q., Firdous, N., Fatima, H., Shehzad, A. and Elkhedir, A.E. 2024. Evaluation of leaves, flowers, and seeds of coriander (*Coriandrum sativum* L.) through microwave drying and ultrasonic-assisted extraction for biologically active components. J Food Proc Preserv. (1):2378604. https://doi.org/10.1155/2024/2378604

Hussain, A., Gorsi, F.I., Ali, M.Q., Yaqub, S., Asif, A., Bibi, B., Arshad, F., Cacciotti, I. and Korma, S.A. 2025. Exploration of underutilized chayote fractions following drying and extraction. Food Chem. 465:142129. https://doi.org/10.1016/j.foodchem.2024.142129

International Organization for Standardization (ISO). 2004. Microbiology of Food and Animal Feeding Stuffs: Horizontal Method for the Enumeration of Presumptive *Bacillus cereus*: Colony Count Technique at 30 Degrees C. ISO 7932:2004. ISO, Geneva, Switzerland.

International Organization for Standardization (ISO). 2016. ISO 17034:2016. https://www.iso.org/obp/ui/en/#iso:std:iso:17034:ed-1:v1:en (Accessed on: 25 November 2025).

International Organization for Standardization (ISO) and International Electrotechnical Commission (IEC). 2017. ISO/IEC 17025:2017. https://www.iso.org/obp/ui/en/#iso:std:iso-iec:17025:ed-3:v1:en (Accessed on: 25 November 2025).

Isembart, C., Zimmermann, B., Matić, J., Bolaño Losada, C., Afseth, N.K., Kohler, A., Horn, S.J., Eijsink, V., Chylenski, P. and Shapaval, V. 2025. Comparative analysis of pre-treatment strategies and bacterial strain efficiency for improvement of feather hydrolysis. Microb Cell Fact. 24(1):118. https://doi.org/10.1186/s12934-025-02743-8

Ismael, M., Huang, M. and Zhong, Q. 2024. The bacteriocins produced by lactic acid bacteria and the promising applications in promoting gastrointestinal health. Foods. 13(23):3887. https://doi.org/10.3390/foods13233887

Jan, S., Kumar, K., Yadav, A.N., Ahmed, N., Thakur, P., Chauhan, D., Rizvi, Q. and Dhaliwal, H.S. 2022. Effect of diverse fermentation treatments on nutritional composition, bioactive components, and anti-nutritional factors of finger millet (*Eleusine coracana* L.). J Appl Biol Biotechnol. 10(1):46–52. https://doi.org/10.7324/JABB.2022.10s107

Janiszewska-Turak, E., Rybak, K., Witrowa-Rajchert, D., Pobiega, K., Wierzbicka, A., Ossowski, S., Sękul, J., Kufel, A., Wiśniewska, A., Trych, U. and Szczepańska-Stolarczyk, J. 2024. Influence of heat treatment and lactic acid fermentation on the physical and chemical properties of pumpkin juice. Molecules. 29(19):4519. https://doi.org/10.3390/molecules29194519

Jayashree, H., Malashree, L., Devaraju, R., Arun Kumar, H., Pushpa, B. and Ramachandra, B. 2024. Sporicidal treatments to produce germinated finger millet. J Sci Res Rep. 30(6):586–592. https://doi.org/10.9734/jsrr/2024/v30i62076

Jeschke, P., Witschel, M., Krämer, W. and Schirmer, U. 2019. Modern Crop Protection Compounds. John Wiley, Hoboken, NJ.

Kalinowska, M., Gołębiewska, E., Zawadzka, M., Choińska, R., Koronkiewicz, K., Piasecka-Jóźwiak, K. and Bujak, M. 2023. Sustainable extraction of bioactive compound from apple pomace through lactic acid bacteria (LAB) fermentation. Sci Rep. 13(1):19310. https://doi.org/10.1038/s41598-023-46584-0

Karaçoban, İ., Bilgiçli, N. and Yaver, E. 2023. Impact of fermentation, autoclaving and phytase treatment on the antioxidant properties and quality of teff cookies. Food Technol Biotechnol. 61(3):328–338. https://doi.org/10.17113/ftb.61.03.23.8145

Kawai, Y., Kawai, M., Mackenzie, E.S., Dashti, Y., Kepplinger, B., Waldron, K.J. and Errington, J. 2023. On the mechanisms of lysis triggered by perturbations of bacterial cell wall biosynthesis. Nat Commun. 14(1):4123. https://doi.org/10.1038/s41467-023-39723-8

Kemal, M., Teka, T.A., Dibaba, K. and Urugo, M.M. 2025. Effects of processing methods on nutrient and antinutrient composition, techno-functional and antioxidant properties of mungbean (*Vigna radiata* L.) R. Wilczek varieties. Food Sci Technol (LWT). 222:117663. https://doi.org/10.1016/j.lwt.2025.117663

Khalil, I., Bashir, S., Saeed, K., Alsulami, T., Rafique, H. and Mukonzo, E.K.L. 2025. Phytochemical and structural portrayal of barley and pearl millet through FTIR and SEM. Food Sci Nutr. 13(5):e70120. https://doi.org/10.1002/fsn3.70120

Kim, C.-W. and Choi, K.-C. 2021. Potential roles of iridoid glycosides and their underlying mechanisms against diverse cancer growth and metastasis: do they have an inhibitory effect on cancer progression? Nutrients. 13(9):2974. https://doi.org/10.3390/nu13092974

Kong, C.S., Kim, K.H., Choi, J.S., Kim, J.E., Park, C. and Jeong, J.W. 2014. Salicin, an extract from white willow bark, inhibits angiogenesis by blocking the ROS-ERK pathways. Phytother Res. 28(8):1246–1251. https://doi.org/10.1002/ptr.5126

Krahn, I., Bonder, D., Torregrosa-Barragán, L., Stoppel, D., Krause, J.P., Rosenfeldt, N., Meiswinkel, T.M., Seibold, G.M., Wendisch, V.F. and Lindner, S.N. 2021. Evolving a new efficient mode of fructose utilization for improved bioproduction in *Corynebacterium glutamicum*. Front Bioeng Biotechnol. 9:669093. https://doi.org/10.3389/fbioe.2021.669093

Krul, E.S. 2019. Calculation of nitrogen-to-protein conversion factors: A Review with a focus on soy protein. J Am Oil Chem Soc. 96(4):339–364. https://doi.org/10.1002/aocs.12196

Kumar, R., Khan, M. and Prasad, M. 2019. Solasodine: a perspective on their roles in health and disease. Res J Pharm Technol. 12(5):2571–2576. https://doi.org/10.5958/0974-360X.2019.00432.3

Li, W., Yin, X., Yan, Y., Liu, C. and Li, G. 2023. Kurarinone attenuates hydrogen peroxide-induced oxidative stress and apoptosis through activating the PI3K/Akt signaling by upregulating IGF1 expression in human ovarian granulosa cells. Environ Toxicol. 38(1):28–38. https://doi.org/10.1002/tox.23659

Liangyu, L.I., Yang, L.I.N.G., Tianfeng, H.E., Chaoyang, L.I. and Rong-an, C.A.O. 2023. Analysis of polyphenols in millet and processing effects on antioxidant activity. Food Sci Technol.Vol.43:1-8.ISSN.1678-457X. https://doi.org/10.5327/fst.129722

Maidana, L., de Souza, M., & Bracarense, A.P.F. (2022). Lactobacillus plantarum and deoxynivalenol detoxification: A concise review. *Journal of Food Protection*, *85*(12), 1815-1823. https://doi.org/10.4315/JFP-22-077

Maidana, L., Gerez, J., Hohmann, M., Verri Jr, W., & Bracarense, A. (2021). Lactobacillus plantarum metabolites reduce deoxynivalenol toxicity on jejunal explants of piglets. *Toxicon*, *203*, 12-21. https://doi.org/10.1016/j.toxicon.2021.09.023.

Mann, A., Kiefer, M. and Leuenberger, H. 2001. Thermal sterilization of heat-sensitive products using high-temperature short-time sterilization. J Pharm Sci. 90(3):275–287. https://doi.org/10.1002/1520-6017(200103)90:3<275::aid-jps3>3.0.co;2-i

Martinez, K.A., Kitko, R.D., Mershon, J.P., Adcox, H.E., Malek, K.A., Berkmen, M.B. and Slonczewski, J.L. 2012. Cytoplasmic pH response to acid stress in individual cells of Escherichia coli and Bacillus subtilis observed by fluorescence ratio imaging microscopy. Appl Environ Microbiol. 78(10):3706–3714. https://doi.org/10.1128/AEM.00354-12

McNulty, J., Krishnamoorthy, V., Amoroso, D. and Moser, M. 2015. Tris(3-hydroxypropyl) phosphine (THPP): a mild, air-stable reagent for the rapid, reductive cleavage of small-molecule disulfides. Bioorg Med Chemy Lett. 25(19):4114–4117. https://doi.org/10.1016/j.bmcl.2015.08.027

MedChemExpress. 2024. Bisibuthiamine. Accession number: MCE:HY-B2229.CAS No:(3286-46-2). 03-01-2024 https://www.medchemexpress.com/Sulbutiamine.htmLsrsltid=AfmBOooMm7cvr5vII0uictLfB6f_aEDaWFmjW4KOcZVthULcT4ff6Ed

McNulty, J., Krishnamoorthy, V., Amoroso, D. and Moser, M. 2015. Tris(3-hydroxypropyl) phosphine (THPP): a mild, air-stable reagent for the rapid, reductive cleavage of small-molecule disulfides. Bioorg Med Chemy Lett. 25(19):4114–4117. https://doi.org/10.1016/j.bmcl.2015.08.027

Melini, F., Melini, V., Luziatelli, F., Ficca, A.G. and Ruzzi, M. 2019. Health-promoting components in fermented foods: an up-to-date systematic review. Nutrients. 11(5):1189. https://doi.org/10.3390/nu11051189

Ming, G.-x., Liu, J.-y., Wu, Y.-h., Li, L.-y., Ma, X.-y., Liu, P., Pan, Y.-p., He, X.-n. and Li, Y.-h. 2024. Strictosamide promotes wound healing through activation of the PI3K/AKT pathway. Heliyon. 10(9):1–17. https://doi.org/10.1016/j.heliyon.2024.e30169

Mititelu, M., Neacșu, S.M., Busnatu, Ș.S., Scafa-Udriște, A., Andronic, O., Lăcraru, A.E., Ioniță-Mîndrican, C.B., Lupuliasa, D., Negrei, C. and Olteanu, G. 2025. Assessing heavy metal contamination in food: implications for human health and environmental safety. Toxics. 13(5):333. https://doi.org/10.3390/toxics13050333

Mohammed, R.S.H. 2025. A Comparative study to evaluate the results of skin pigmentation resulting from thigh friction (meta-analysis). Cent Asian J Med Nat Sci. 6(4):1503–1511. https://doi.org/10.17605/cajmns.v6i4.2869

Mohapatra, D., Nickhil, C., Kar, A., Sharma, Y., Deshpande, S.S., Tripathi, M.K. and Haromuchadi, S.R. 2024. Impact of LAB fermentation on the nutrient content, amino acid profile, and estimated glycemic index of sorghum, pearl millet, and kodo millet. Front Biosci Elite. 16(2):18. https://doi.org/10.31083/j.fbe1602018

Mohiuddin, S.G., Ghosh, S., Ngo, H.G., Sensenbach, S., Karki, P., Dewangan, N.K., Angardi, V. and Orman, M.A. 2021. Cellular self-digestion and persistence in bacteria. Microorganisms. 9(11):2269. https://doi.org/10.3390/microorganisms9112269

Moiseenko, K.V., Glazunova, O.A. and Fedorova, T.V. 2024. Fermentation of rice, oat, and wheat flour by pure cultures of common starter lactic acid bacteria: growth dynamics, sensory evaluation, and functional properties. Foods. 13(15):2414. https://doi.org/10.3390/foods13152414

Mouhoubi, K., Boulekbache-Makhlouf, L., Madani, K., Freidja, M.L., Silva, A.M. and Cardoso, S.M. 2023. Microwave-assisted extraction optimization and conventional extraction of phenolic compounds from coriander leaves: UHPLC characterization and antioxidant activity. Nor Afr J Food Nutr Res. 7(15):69–83. https://doi.org/10.51745/najfnr.7.15.69-83

Mudau, M. and Adebo, O.A. 2025. Effect of traditional and novel processing technologies on the thermo-pasting, microstructural, nutritional, and antioxidant properties of finger millet and Bambara groundnut flours. Int J Food Sci Technol. 60(1):vvae037. https://doi.org/10.1093/ijfood/vvae037

Mutshinyani, M., Mashau, M.E. and Jideani, A.I.O. 2020. Bioactive compounds, antioxidant activity and consumer acceptability of porridges of finger millet (*Eleusine coracana*) flours: effects of spontaneous fermentation. Int J Food Prop. 23(1):1692–1710. https://doi.org/10.1080/10942912.2020.1825485

Ntsamo, T.M.B., Mohammadou, B.A., Sokamte, A.T., Njintang, N.Y. and Tatsadjieu, L.N. 2020. Effect of fermentation using *Lactobacillus plantarum* A6 on the physicochemical and functional properties of precooked *Sorghum bicolor* and *Voandzeia subterranea* blended flour. Int J Food Sci. 2020(1):9234083. https://doi.org/10.1155/2020/9234083

Ogodo, A.C., Ugbogu, O.C., Onyeagba, R.A. and Okereke, H.C. 2019. Microbiological quality, proximate composition and *in vitro* starch/protein digestibility of Sorghum bicolor flour fermented with lactic acid bacteria consortia. Chem Biol Technol Agric. 6(1):1–9. https://doi.org/10.1186/s40538-019-0145-4

Olmeda, I., Casino, P., Collins, R.E., Sendra, R., Callejon, S., Huesa, J., Soares, A.S., Ferrer, S. and Pardo, I. 2021. Structural analysis and biochemical properties of laccase enzymes from two *Pediococcus* species. Microb Biotechnol. 14(3):1026–1043. https://doi.org/10.1111/1751-7915.13751

Omeje, K.O., Ezema, B.O., Okonkwo, F., Onyishi, N.C., Ozioko, J., Rasaq, W.A., Sardo, G. and Okpala, C.O.R. 2021. Quantification of heavy metals and pesticide residues in widely consumed Nigerian food crops using atomic absorption spectroscopy (AAS) and gas chromatography (GC). Toxins. 13(12):870. https://doi.org/10.3390/toxins13120870

Paramithiotis, S. 2025. *Lactiplantibacillus plantarum*, the integral member of vegetable fermentations. Appl Biosci. 4(1):7. https://doi.org/10.3390/applbiosci4010007

Paventi, G., Di Martino, C., Coppola, F. and Iorizzo, M. 2025. β-glucosidase activity of *Lactiplantibacillus plantarum*: a key player in food fermentation and human health. Foods. 14(9):1451. https://doi.org/10.3390/foods14091451

Paventi, G., Di Martino, C., Crawford Jr, T.W. and Iorizzo, M. 2024. Enzymatic activities of *Lactiplantibacillus plantarum*: technological and functional role in food processing and human nutrition. Food Biosci. 61:104944. https://doi.org/10.1016/j.fbio.2024.104944

Pehrsson, P., Patterson, K., Haytowitz, D. and Phillips, K. 2015. Total carbohydrate determinations in USDA's National Nutrient Database for standard reference. FASEB J. 29(S1): 740-6.https://doi.org/10.1096/fasebj.29.1_supplement.740.6

Pontonio, E., Dingeo, C., Di Cagno, R., Blandino, M., Gobbetti, M. and Rizzello, C.G. 2020. Brans from hull-less barley, emmer and pigmented wheat varieties: from by-products to bread nutritional improvers using selected lactic acid bacteria and xylanase. Int J Food Microbiol. 313:108384. https://doi.org/10.1016/j.ijfoodmicro.2019.108384

Popoola, O.O. 2022. Phenolic compounds composition and *in vitro* antioxidant activity of Nigerian *Amaranthus viridis* seed as affected by autoclaving and germination. Measure Food. 6:100028. https://doi.org/10.1016/j.meafoo.2022.100028

Popova-Krumova, P., Danova, S., Atanasova, N. and Yankov, D. 2024. Lactic acid production by *Lactiplantibacillus plantarum* AC 11S—kinetics and modeling. Microorganisms. 2024(12):739. https://doi.org/10.3390/microorganisms12040739

PubChem National Center for Biotechnology Information (NCBI). 2004. PubChem compound summary for CID 440649, 3-Carboxy-1-hydroxypropylthiamine diphosphate. https://pubchem.ncbi.nlm.nih.gov/compound/3-Carboxy-1-hydroxypropylthiamine-diphosphate (Accessed on: 21 March 2025).

PubChem National Center for Biotechnology Information (NCBI). 2005. PubChem compound summary for CID 441107, 1,3,10,11,12-Pentahydroxy-6-methyltetracene-2-carboxamide. https://pubchem.ncbi.nlm.nih.gov/compound/441107 (Accessed on: 21 February 2025).

PubChem National Center for Biotechnology Information (NCBI). 2012. PubChem compound summary for CID 56927995, 7beta-Hydroxytaxusin. https://pubchem.ncbi.nlm.nih.gov/compound/7beta-Hydroxytaxusin (Accessed on: 2 March 2025).

PubChem National Center for Biotechnology Information (NCBI). 2015. PubChem compound summary for CID 102232250, beta-Geranylfarnesene. https://pubchem.ncbi.nlm.nih.gov/compound/beta-Geranylfarnesene (Accessed on: 3 March 2025).

Pulido-Mateos, E.C., Lessard-Lord, J., Desjardins, Y. and Roy, D. 2024. *Lactiplantibacillus plantarum* interstrain variability in the production of bioactive phenolic metabolites from Flavan-3-ols. J Agric Food Chem. 72(39):21677–21689. https://doi.org/10.1021/acs.jafc.4c07890

Quan, Q., Liu, W., Guo, J., Ye, M., & Zhang, J. (2022). Effect of six lactic acid bacteria strains on physicochemical characteristics, antioxidant activities and sensory properties of fermented orange juices. *Foods*, *11*(13), 1920. https://doi.org/10.3390/foods11131920.

Rahman, M.J., Malunga, L.N., Eskin, M., Eck, P., Thandapilly, S.J. and Thiyam-Hollander, U. 2021. Valorization of heat-treated brewers' spent grain through the identification of bioactive phenolics by UPLC-PDA and evaluation of their antioxidant activities. Front Nutr. 8:634519. https://doi.org/10.3389/fnut.2021.634519

Rico, D., Villaverde, A., Martinez-Villaluenga, C., Gutierrez, A.L., Caballero, P.A., Ronda, F., Peñas, E., Frias, J. and Martin Diana, A.B. 2020. Application of autoclave treatment for development of a natural wheat bran antioxidant ingredient. Foods. 9(6):781. https://doi.org/10.3390/foods9060781

Rodrigues, C.I., Marta, L., Maia, R., Miranda, M., Ribeirinho, M. and Máguas, C. 2007. Application of solid-phase extraction to brewed coffee caffeine and organic acid determination by UV/HPLC. J Food Comp Anal. 20(5):440–448. https://doi.org/10.1016/j.jfca.2006.08.005

Sakhare, P.S. 2018. A Study of Efficacy and Safety of Methotrexate versus Azathioprine in Psoriasis. Rajiv Gandhi University of Health Sciences, Bengaluru, Karnataka, India.

Șerban, L.R., Păucean, A., Chiș, M.S., Pop, C.R., Man, S.M., Pușcaș, A., Ranga, F., Socaci, S.A., Alexa, E. and Berbecea, A. 2023. Metabolic profile of einkorn, spelt, emmer ancient wheat species sourdough fermented with strain of *Lactiplantibacillus plantarum* ATCC 8014. Foods. 12(5):1096. https://doi.org/10.3390/foods12051096

Șerban, L.R., Păucean, A., Man, S.M., Chiș, M.S. and Mureșan, V. 2021. Ancient wheat species: biochemical profile and impact on sourdough bread characteristics—a review. Processes. 9(11):2008. https://doi.org/10.3390/pr9112008

Shashank, D., Rajendra, S. and Mistry, A. 2017. An overview of phytoconstituents and pharmacological activities of celastrus paniculatus willd. J Pharm Res. 16(4):307–313. https://doi.org/10.18579/jpcrkc/2017/16/4/119582

Sidari, R., Martorana, A., Zappia, C., Mincione, A. and Giuffrè, A.M. 2020. Persistence and effect of a multistrain starter culture on antioxidant and rheological properties of novel wheat sourdoughs and bread. Foods. 9(9):1258. https://doi.org/10.3390/foods9091258

Sionek, B., Szydłowska, A., Trząskowska, M. and Kołożyn-Krajewska, D. 2024. The impact of physicochemical conditions on lactic acid bacteria survival in food products. Fermentation. 10(6):298. https://doi.org/10.3390/fermentation10060298

Śliżewska, K. and Chlebicz-Wójcik, A. 2020. Growth kinetics of probiotic Lactobacillus strains in the alternative, cost-efficient semi-solid fermentation medium. Biology. 9(12):423. https://doi.org/10.3390/biology9120423

Solmaz, B., Levent, H. and Şahin, N. 2025. The influence of various cereal brans, stabilized through hot air, microwave, and autoclave methods, on the physicochemical properties of cookies. Food Sci Nutr. 13(6):e70328. https://doi.org/10.1002/fsn3.70328

Srivastava, U., Saini, P. and Singh, A. 2024. Synergistic enhancement of iron, folate, and antioxidant properties in pearl millet via RSM-optimized probiotic fermentation with *Lactiplantibacillus plantarum*. Measure Food. 13:100137. https://doi.org/10.1016/j.meafoo.2024.100137

Srivastava, U., Saini, P., Singh, A., Singh, Z., Ahmed, M. and Iqbal, U. 2021. Enhancement in iron and folate by optimizing fermentation of barnyard millet by *Lactobacillus plantarum* using response surface methodology (RSM). Plant Arch. 21(1):09725210. https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.138

Sultana, N. and Ata, A. 2008. Oleanolic acid and related derivatives as medicinally important compounds. J Enzy Inhib Med Chem. 23(6):739–756. https://doi.org/10.1080/14756360701633187

Suma, F. and Urooj, A. 2017. Impact of household processing methods on the nutritional characteristics of pearl millet (*Pennisetum typhoideum*): a review. J Food Proc Technol. 4(1):28–32. https://doi.org/10.15406/mojfpt.2017.04.00082

Sun, X., Cheng, H., Li, L. and Jin, W. 2017. X500R QTOF system with SWATH® acquisition for pesticide residue screening in fruits and vegetables. Food Environ. Document number: RUO-MKT-02-5390-A.Page No: P1-P5.

Sun, J., Niu, H., Li, B., Wang, Y., Chen, X. and Han, X. 2025. Calcium and magnesium improve *Lactobacillus bulgaricus* survival via membrane and metabolic protection. Food Sci Technol (LWT). 234:118584. https://doi.org/10.1016/j.lwt.2025.118584

Talmon, M., Bosso, L., Quaregna, M., Lopatriello, A., Rossi, S., Gavioli, D., Marotta, P., Caprioglio, D., Boldorini, R. and Miggiano, R. 2020. Anti-inflammatory activity of absinthin and derivatives in human bronchoepithelial cells. J Nat Prod. 83(6):1740–1750. https://doi.org/10.1021/acs.jnatprod.9b00685

Tomar, T., Sachdeva, A., Dutta, J., Al Tawaha, A.R.M., Karnwal, A., Malik, T. and Selvaraj, M. 2025. Fermentation dynamics of millet beverages: microbial interactions, nutritional enhancements, and health implications. Food Chem. X:102199. https://doi.org/10.1016/j.fochx.2025.102199

Üçok, G. and Sert, D. 2020. Growth kinetics and biomass characteristics of *Lactobacillus plantarum* L14 isolated from sourdough: effect of fermentation time on dough machinability. Food Sci Technol (LWT). 129:109516. https://doi.org/10.1016/j.lwt.2020.109516

Udensi, E., Arisa, N. and Ikpa, E. 2010. Effects of soaking and boiling and autoclaving on the nutritional quality of *Mucuna flagellipes* ("ukpo"). Afr J Biochem Res. 4(2):47–50. http://www.academicjournals.org/AJBR

Uwaegbute, A., Iroegbu, C. and Eke, O. 2000. Chemical and sensory evaluation of germinated cowpeas (*Vigna unguiculata*) and their products. Food Chem. 68(2):141–146. https://doi.org/10.1016/S0308-8146(99)00134-X

Verni, M., De Mastro, G., De Cillis, F., Gobbetti, M. and Rizzello, C.G. 2019. Lactic acid bacteria fermentation to exploit the nutritional potential of Mediterranean faba bean local biotypes. Food Res Int. 125:108571. https://doi.org/10.1016/j.foodres.2019.108571

Wang, H., Fu, Y., Zhao, Q., Hou, D., Yang, X., Bai, S., Diao, X., Xue, Y. and Shen, Q. 2022. Effect of different processing methods on the millet polyphenols and their anti-diabetic potential. Front Nutr. 9:780499. https://doi.org/10.3389/fnut.2022.780499

Wei, C., Zhang, C., Gao, Y., Yu, L., Zhao, J., Zhang, H., Chen, W. and Tian, F. 2022. Insights into the metabolic response of *Lactiplantibacillus plantarum* CCFM1287 upon patulin exposure. Int J Mol Sci. 23(19):11652. https://doi.org/10.3390/ijms231911652

Weiß, K. and Alt, M. 2017. Determination of single sugars, including inulin, in plants and feed materials by high-performance liquid chromatography and refraction index detection. Fermentation. 3(3):36. https://doi.org/10.3390/fermentation3030036

Woo, S.-H., Shin, Y.-J., Jeong, H.-M., Kim, J.-S., Ko, D.-S., Hong, J. S., Choi, H.-D. and Shim, J.-H. 2020. Effects of maltogenic amylase from *Lactobacillus plantarum* on retrogradation of bread. J Cereal Sci. 93:102976. https://doi.org/10.1016/j.jcs.2020.102976

Xiang, H., Sun-Waterhouse, D., Waterhouse, G.I., Cui, C. and Ruan, Z. 2019. Fermentation-enabled wellness foods: a fresh perspective. Food Sci Human Wellness. 8(3):203–243. https://doi.org/10.1016/j.fshw.2019.08.003

Yadav, J.S.S., Yan, S., Ajila, C.M., Bezawada, J., Tyagi, R.D. and Surampalli, R.Y. 2016. Food-grade single-cell protein production, characterization and ultrafiltration recovery of residual fermented whey proteins from whey. Food Bioprod Proc. 99:156–165. https://doi.org/10.1016/j.fbp.2016.04.012

Yan, J., Cai, M., Zang, C., Li, W., Liu, Z., Li, X., Gao, Y. and Qi, Y. 2024. The natural sesquiterpene lactone inulicin suppresses the production of pro-inflammatory mediators via inhibiting NF-κB and AP-1 pathways in LPS-activated macrophages. Immunopharmacol Immunotoxicol. 46(5):583–593. https://doi.org/10.1080/08923973.2024.2384899

Yang, F., Chen, C., Ni, D., Yang, Y., Tian, J., Li, Y., Chen, S., Ye, X. and Wang, L. 2023. Effects of fermentation on bioactivity and the composition of polyphenols contained in polyphenol-rich foods: a review. Foods. 12(17):3315. https://doi.org/10.3390/foods12173315

Yang, X., Hong, J., Wang, L., Cai, C., Mo, H., Wang, J., Fang, X. and Liao, Z. 2024. Effect of lactic acid bacteria fermentation on plant-based products. Fermentation. 10(1):48. https://doi.org/10.3390/fermentation10010048

Yehuala, T.F., Atlabachew, M., Aslam, M.F., Allen, L., Griffith, H., Ward, J.L., Shewry, P.R., Kanellou, A., Gichohi-Wainaina, W., Endalew, H.W. and Abera, M.K. 2025. Fermentation kinetics and changes in levels of antinutrients in Pearl millet and Pearl millet–maize composite dough recipes used to prepare injera. Food Sci Nutr. 13(7):e70598. https://doi.org/10.1002/fsn3.70598

Yépez, A., Russo, P., Spano, G., Khomenko, I., Biasioli, F., Capozzi, V. and Aznar, R. 2019. *In situ* riboflavin fortification of different kefir-like cereal-based beverages using selected *Andean* LAB strains. Food Microbiol. 77:61–68. https://doi.org/10.1016/j.fm.2018.08.008

Yoon, J.H., Oh, M.S. and Lee, S.Y. 2024. Effectiveness of organic acids for inactivating pathogenic bacteria inoculated in laboratory media and foods: an updated minireview. Food Sci Biotechnol. 33(12):2715–2728. https://doi.org/10.1007/s10068-024-01618-9

Yousaf, L., Hou, D., Liaqat, H. and Shen, Q. 2021. Millet: a review of its nutritional and functional changes during processing. Food Res Int. 142:110197. https://doi.org/10.1016/j.foodres.2021.110197

Yuca, H. 2022. *Capsicum annuum* L. in Novel Drug Targets with Traditional Herbal Medicines: Scientific and Clinical Evidence. Springer, New York, NY, pp. 95–108. https://doi.org/10.1007/978-3-031-07753-1_7

Zdziobek, P., Jodłowski, G.S. and Strzelec, E.A. 2023. Biopreservation and bioactivation juice from waste broccoli with *Lactiplantibacillus plantarum*. Molecules. 28(12):4594. https://doi.org/10.3390/molecules28124594

Zhang, R., Khan, S.A., Chi, J., Wei, Z., Zhang, Y., Deng, Y., Liu, L. and Zhang, M. 2017. Different effects of extrusion on the phenolic profiles and antioxidant activity in milled fractions of brown rice. Food Sci Technol (LWT). 88:64–70. https://doi.org/10.1016/j.lwt.2017.09.042

Zhao, X., Liang, Q., Song, X. and Zhang, Y. 2023. Whole genome sequence of *Lactiplantibacillus plantarum* MC5 and comparative analysis of *EPS* gene clusters. Front Microbiol. 14:1146566. https://doi.org/10.3389/fmicb.2023.1146566

Zhao, C., Shen, J., Cai, X., Zhao, Y., Meng, X., Liu, Z. and Qian, Y. 2025. A novel bacteriostatic system for seafood preservation: impact of plantaricin Bac-329 on bacterial cell wall integrity driven by lactic acid levels. Food Cont. 176:111331. https://doi.org/10.1016/j.foodcont.2025.111331

Zhao, Z.-M., Wang, L. and Chen, H.-Z. 2015. A novel steam explosion sterilization improving solid-state fermentation performance. Biores Technol. 192:547–555. https://doi.org/10.1016/j.biortech.2015.05.099

Zheng, R. and Pan, F. 2019. On-line tendency control of dissolved oxygen concentration during aerobic fed-batch fermentations. Appl Sci. 9:5232. https://doi.org/10.3390/app9235232

Zheng, H., Sun, Y., Zheng, T., Zeng, Y., Fu, L., Zhou, T., Jia, F., Xu, Y., He, K. and Yang, Y. 2023. Effects of shear emulsifying/ball milling/autoclave modification on structure, physicochemical properties, phenolic compounds, and antioxidant capacity of lotus (Nelumbo) leaves dietary fiber. Front Nutr. 10:1064662. https://doi.org/10.3389/fnut.2023.1064662

Zhou, M., Fakayode, O.A., Ren, M., Li, H., Liang, J., Yagoub, A.E.A., Fan, Z. and Zhou, C. 2023. Laccase-catalyzed lignin depolymerization in deep eutectic solvents: challenges and prospects. Biores Bioproc. 10(1):21. https://doi.org/10.1186/s40643-023-00640-9

Supplementary

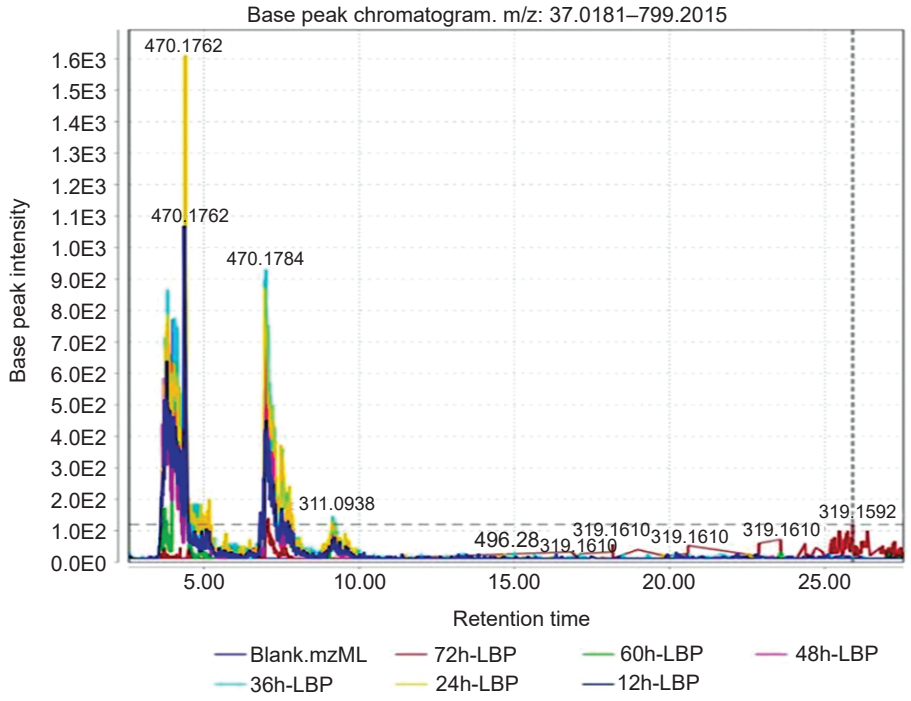

Figure S1. The TIC of a 95% methanol extract from millet flour subjected to single-mode autoclaving (SMA), followed by fermentation with *L. plantarum* ATCC 8014.

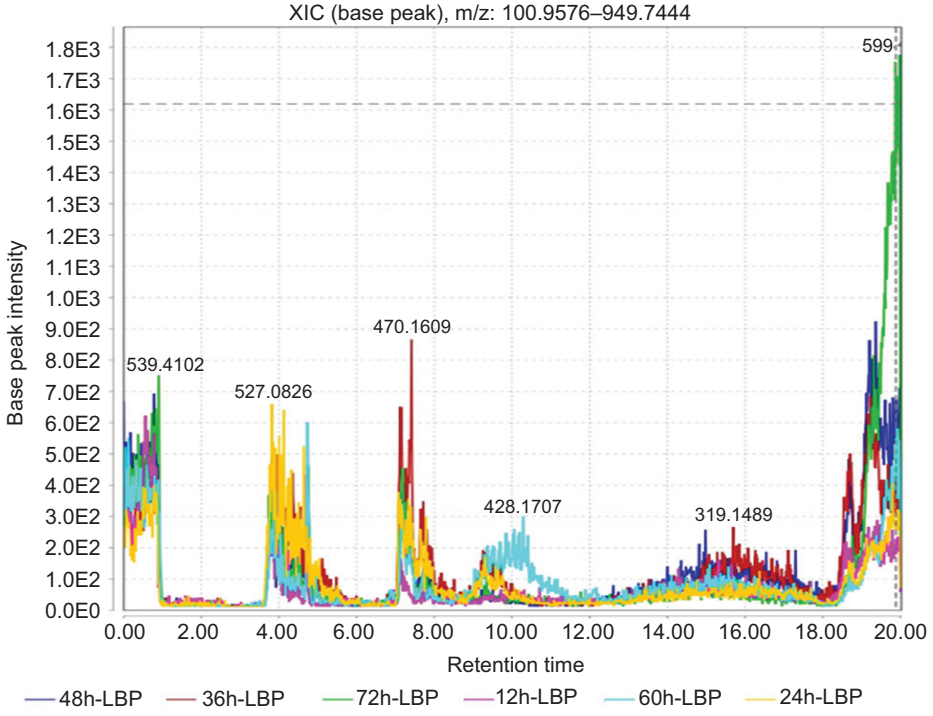

Figure S2. The TIC of a 95% methanol extract from millet flour subjected to mixed-mode autoclaving (MMA), followed by fermentation with *L. plantarum* ATCC 8014.

Table S1. Nutrient contents (g/100 g, dw) in autoclave-treated pearl millet, followed by fermentation with *L. plantarum* ATCC 8014.

Millet flour	Fermentation time	Dry matter	Ash	Fat	Protein	Carbohydrates
0r	0	97.29±0.083^{a}	1.89±0.03^{f}	7.76±0.23^{a}	6.19±0.11^{c}	84.16±0.24^{e}
0MMA	0	96.11±0.056^{a,b}	2.25±0.07^{e}	7.00±0.19^{b}	5.68±0.28^{c}	85.07±0.20^{d,e}
SMA	12	93.39±0.001^{d}	2.57±0.11^{c,d}	6.45±0.09^{b,c}	7.06±0.33^{a,b}	83.92±0.27^{e}
	24	95.30±0.064^{b,c}	2.52±0.05^{d}	6.07±0.58^{c,d}	7.61±0.18^{a}	83.80±0.29^{e}
	36	94.76±0.066^{c}	2.25±0.13^{e}	6.50±0.30^{b,c}	7.03±0.10^{a,b}	84.22±0.10^{e}
	48	92.77±0.050^{e}	2.65±0.13^{c,d}	4.69±0.08^{d,e}	7.43±0.43^{a}	85.23±0.29^{e}
	60	93.75±0.048^{d}	2.61±0.05^{c,d}	4.50±0.17^{g}	7.44±0.19^{a}	85.45±0.02^{c}
	72	95.76±0.066^{b,c}	2.50±0.08^{g}	4.70±0.08^{g}	7.12±0.50^{a,b}	85.68±0.04^{b}
MMA	12	93.66±0.509^{d}	2.82±0.11^{b,c}	6.35±0.16^{c,d}	6.45±0.14^{b,c}	84.38±0.23^{e}
	24	94.05±0.261^{c}	3.03±0.09^{a,b}	4.92±0.08^{f,g}	7.46±0.28^{a}	84.59±0.31^{d,e}
	36	94.54±0.066^{c}	3.03±0.14^{a,b}	5.46±0.14^{e,f}	7.41±0.76^{a}	84.10±0.91^{e}
	48	96.37±0.053^{a,b}	3.12±0.10^{a}	4.69±0.08^{g}	6.22±0.14^{c}	85.97±0.12^{b,c}
	60	95.06±0.124^{b,c}	2.49±0.10^{d}	3.54±0.16^{h}	6.19±0.19^{c}	87.78±0.15^{a}
	72	93.45±0.018^{d}	2.00±0.09^{f}	3.60±0.08^{h}	6.11±0.19^{c}	88.29±0.21^{a}

Notes: Means ± SD (n = 3) were statistically analyzed using one-way ANOVA. According to Tukey's test ($p < 0.05$), the mean values accompanied by different superscript alphabets differ significantly within a column.
0r: raw millet flour; 0MMA: raw millet flour sterilized with MMA; MMA: mixed-mode autoclaving: sterilizing flour and water together; control); SMA: single-mode autoclaving (sterilizing each flour and water separately); 12–72 h: fermentation time; dw: dry weight.

Table S2. Levels of glucose and fructose in autoclave-treated pearl millet, followed by fermentation with *L. plantarum* ATCC 8014.

Fermentation time (h)	Glucose (mg/kg, dw)			Fructose (mg/kg, dw)		
	SMA	MMA	Total mean (n = 6)	SMA	MMA	Total mean
0r	94.11±17.35	94.11±17.35	**94.11±17.35**^{d}	59.92±2.84	59.92±2.84	**59.92±2.84**^{b}
0MMA	86.81±5.94	86.81±5.94	**86.81±5.94**^{d}	73.18±1.14	73.18±1.14	**73.18±1.14**^{a}
12	181.58±6.71	71.72±0.77	**126.65±60.33**^{c}	25.27±1.31	55.10±0.95	**40.19±16.37**^{c}
24	369.30±29.24	70.67±1.97	**219.98±64.61**^{b}	0.00±0.00	27.43±0.43	**13.71±15.03**^{e}
36	388.25±22.11	107.01±3.73	**247.63±54.7**^{a}	0.00±0.00	33.28±0.95	**16.64±18.24**^{d}
48	154.61±2.91	0.00±0.00	**77.31±84.74**^{d}	0.00±0.00	18.13±2.30	**9.06±10.03**^{f}
60	152.88±1.94	0.00±0.00	**76.44±83.75**^{d}	0.00±0.00	4.56±0.06	**2.28±2.50**^{g}
72	112.99±1.37	0.00±0.00	**56.50±61.90**^{e}	0.00±0.00	0.00±0.00	**0.00±0.00**^{h}
Total mean (n = 24)	**192.57±115.04**^{a}	**53.79±44.18**^{b}		**19.80±12.90**^{b}	**33.95±25.12**^{a}	
Grand mean (n = 48)			**123.18 (SE = 1.239)**			**26.873 (SE = 0.177)**

Notes: Statistical differences among factor mean values were analyzed using Type III two-way ANOVA. According to pairwise comparisons, different superscript alphabets indicate that total mean values differ significantly ($p < 0.05$) within a row or a column.
0r: raw millet flour; 0MMA: raw millet flour sterilized with MMA; MMA: mixed-mode autoclaving: sterilizing flour and water together; control); SMA: single-mode autoclaving (sterilizing each flour and water separately); 12–72 h: fermentation time; dw: dry weight.

Table S3. Nutritional element analysis (mg/kg, dw) in autoclave-treated pearl millet, followed by fermentation with *L. plantarum* ATCC 8014.

Millet flour	Fermentation time	Cr	Cu	Fe	Ni	Mn	Zn	Ca	K	Mg	Na
0r	0	1.23±0.01^{a}	6.30±0.71$^{d–f}$	110.40±1.30^{d,e}	3.58±0.02^{a,b}	19.8±0.12$^{a–d}$	46.2±0.20^{a,b}	201.8±0.10^{e}	3432.5±12.1^{i}	1011.1±1.3$^{d–f}$	127.4±1.0^{h}
0MMA	0	1.19±0.04^{a}	6.20±0.012$^{d–g}$	111.6±1.31^{d,e}	3.61±0.54^{a,b}	18.9±0.09$^{b–d}$	45.8±0.20^{a,b}	201.7±0.10^{e}	3772.8±11.2^{h}	1039.0±2.9^{d}	115.9±0.4^{i}
SMA	12	1.14±0.13$^{a–c}$	5.40±1.85^{g}	114.2±0.87^{d}	3.61±0.43^{a,b}	20.4±0.10$^{a–d}$	45.9±0.20^{a,b}	202.1±0.2^{e}	4422.4±7.6^{f}	1199.8±1.8^{c}	146.3±0.3^{f,g}
	24	1.07±0.12^{b,c}	7.3±0.83^{b,c}	104.7±0.29^{f,g}	3.62±0.72^{a,b}	20.5±0.22$^{a–c}$	46.3±0.70^{a}	204.0±0.10^{d,e}	3942.4±7.6^{g}	1022.9±5.8^{d,e}	151.1±0.6^{f}
	36	1.04±0.03^{c}	8.70±1.22^{a}	143.0±0.42^{a}	3.60±0.30^{a,b}	17.9±0.13^{d}	46.2±0.60^{a,b}	203.5±0.10^{d,e}	3935.2±14.8^{g}	958.9±3.32^{g}	142.6±0.2^{g}
	48	1.13±0.04$^{a–c}$	7.60±0.70^{b}	130.0±0.55^{b}	3.41±0.14^{a,b}	18.3±0.05^{c,d}	46.1±0.30^{a,b}	204.8±0.30^{d}	3770.5±29.5^{h}	1004.2±4.7^{e,f}	157.5±0.4^{e}
	60	1.16±0.02^{a,b}	6.90±0.024$^{b–d}$	127.3±0.84^{b}	3.31±0.54^{b}	17.9±0.14^{d}	45.7±0.30$^{a–c}$	207.7±0.10^{b,c}	3756.6±7.8^{h}	812.9±2.3^{j}	119.7±0.2
	72	1.06±0.02^{b,c}	7.30±0.60^{b,c}	144.3±1.13^{a}	3.40±0.06^{a,b}	18.9±0.09$^{b–d}$	45.8±0.30^{a,b}	209.3±0.20^{a,b}	3709.7±13.6	861.8±4.2^{i}	122.0±0.5^{h,i}
MMA	12	1.22±0.11^{a}	6.50±0.24$^{c–e}$	122.0±0.030^{c}	3.60±0.07^{a,b}	20.3±0.13$^{a–d}$	46.0±0.30^{a,b}	202.2±0.10^{e}	4153.4±16.6^{c}	1286.1±1.3^{a}	145.4±0.3^{f,g}
	24	1.14±0.23$^{a–c}$	5.50±1.69^{f,g}	108.5±0.53^{e,f}	0.362±0.022^{a,b}	21.7±0.11^{a}	45.8±1.10^{b}	205.5±0.10^{c,d}	4287.6±12.4^{a}	1254.7±2.0^{b}	180.9±0.4^{b}
	36	1.14±0.01$^{a–c}$	5.30±0.411^{g}	12.70±0.113^{b}	3.74±0.08^{a}	1.85±0.014$^{b–d}$	46.4±0.30^{a}	211.2±0.10^{a}	4243.7±13.9^{b}	1226.2±4.2^{c}	173.2±0.6^{c}
	48	0.91±0.03^{d}	5.80±1.76$^{e–g}$	105.4±0.120^{f,g}	3.50±0.09^{a,b}	18.7±0.09$^{b–d}$	46.3±0.50^{a}	211.6±0.13^{a}	4521.4±18.6^{e}	991.1±1.9^{f}	165.3±0.2^{d}
	60	0.93±0.09^{d}	5.80±2.85$^{e–g}$	111.8±0.59^{d,e}	3.57±0.43^{a,b}	20.9±0.07^{a,b}	45.8±0.20^{a,b}	211.6±0.30^{a}	4741.1±21.1^{d}	912.9±2.2^{h}	190.1±0.4^{a}
	72	0.87±0.10^{d}	5.60±2.40^{f,g}	103.4±1.23^{g}	3.29±0.25^{b}	18.2±0.05^{c,d}	45.5±0.20^{b,c}	21120±0.02^{a}	4718.1±21.9^{d}	832.4±2.8^{j}	178.4±0.7^{b,c}
RDA*/AI# for adults (mg/day)		0.025–0.035#	0.90*	8.0-18.0*	0.07-0.40*	1.8–2.3#	8.0–11.0*	1,000*	2,600–3,400#	310–400*	1,500#

Notes: Means ± SD (n = 3) were statistically analyzed using one-way ANOVA. According to Tukey's test ($p < 0.05$), the mean values accompanied by different superscript alphabets differ significantly within a column.
0r: raw millet flour; 0MMA: raw millet flour sterilized with MMA; MMA: mix-mode autoclaving: sterilizing flour and water together; control); SMA: single-mode autoclaving (sterilizing each flour and water separately); dw: dry weight; RDA: recommended dietary allowance; AI: adequate intake.

Table S4. Heavy metal contents (µg/kg, dw) in autoclave-treated pearl millet, followed by fermentation with *L. plantarum* ATCC 8014.

Millet flour	Fermentation time	As	Cd	Hg	Pb	Sb
0r	0	6.62±0.31^{d}	66.87±1.57^{f}	33.74±1.22^{b,c}	16.59±0.23^{c}	1256.62±33.88^{b,c}
0MMA	0	6.67±0.04^{d}	67.18±0.42^{f}	32.76±2.93^{c,d}	17.31±0.36^{b,c}	1247.49±34.42^{b,c}
SMA	12	6.80±0.20^{b,c}	67.67±0.13^{f}	31.42±0.68^{d,e}	17.37±0.23^{b,c}	1246.37±33.54^{b,c}
	24	6.83±0.11^{a,b}	69.23±0.90^{e,f}	34.20±0.50^{a,b}	17.34±0.12^{b,c}	1317.21±52.13^{a,b}
	36	6.79±0.07^{b,c}	76.26±0.44^{cd}	33.88±0.18^{b}	17.57±0.23$^{a–c}$	1352.05±50.41^{a,b}
	48	6.81±0.19^{b,c}	76.25±0.35^{c,d}	34.94±0.06^{a}	18.02±0.34$^{a–c}$	1345.49±83.95^{a,b}
	60	6.83±0.09^{a,b}	75.65±0.35^{d}	31.34±0.06^{d}	18.32±0.38^{a,b}	1325.51±10.30^{a,b}
	72	6.77±0.06	75.38±0.62^{d}	33.09±0.91^{c}	18.12±0.12$^{a–c}$	1370.36±53.86^{a,b}
MMA	12	6.83±0.15^{a,b}	66.92±0.52	32.99±0.31^{c}	17.47±0.13$^{a–c}$	1253.86±34.61^{b,c}
	24	6.85±0.46^{a,b}	76.93±0.27^{a,b}	33.61±0.12^{b,c}	17.56±0.44$^{a–c}$	1300.48±70.01^{a,b}
	36	6.87±0.13^{a}	77.34±0.84^{a}	30.92±1.08^{d}	18.31±0.50^{a,b}	1263.19±31.84$^{a–c}$
	48	6.78±1.10^{b,c}	76.49±0.21^{b}	32.50±0.50^{c,d}	17.15±0.32^{b,c}	1300.78±15.49^{a,b}
	60	6.85±0.04^{a,b}	75.89±0.35^{d}	33.43±0.63^{b,c}	17.19±0.11^{b,c}	1356.59±37.36^{a,b}
	72	6.81±0.12^{b,c}	76.19±0.11^{c,d}	32.59±0.41^{d}	17.77±0.23^{a,b}	1388.09±12.08^{a}
MPL (mg/kg)		0.20 (inorganic)	0.10	0.01	0.02	0.02 (inorganic)

Notes: Means ± SD (n = 3) were statistically analyzed using one-way ANOVA. According to Tukey's test ($p < 0.05$), the mean values accompanied by different superscript alphabets differ significantly within a column.
0r: raw millet flour; 0MMA: raw millet flour sterilized with MMA; MMA: mixed-mode autoclaving: sterilizing flour and water together; control; SMA: single-mode autoclaving (sterilizing each flour and water separately); dw: dry weight; MPL: maximum permissible limit (Mititelu *et al.*, 2025).

Table S5. Contents of total polyphenols (TPC), total flavonoids (TFC), and total tannins content (TTC) in autoclave-treated pearl millet, followed by fermentation with *L. plantarum* ATCC 8014.

Millet flour	Fermentation time (h)	TPC (mg GAE/g dw)	TFC (mg QE/g dw)	TTC (mg TAE/g dw)	DPPH scavenging rate (%)
0r	0	2.81±0.02^{f}	2.20±0.18^{g}	2.04±0.12^{a}	76.82±0.74^{e}
0MMA	0	2.73±0.03^{f,g}	2.67±0.03^{f}	2.00±0.07^{a}	63.40±0.80^{f}
SMA	12	2.83±0.08^{f}	2.59±0.02^{f,g}	1.79±0.08^{b}	77.02±1.07^{e}
	24	3.47±0.17$^{a–c}$	2.53±0.01^{g}	1.78±0.09^{b}	79.39±0.89^{d}
	36	3.24±0.12^{d}	3.36±0.08^{d}	1.51±0.05^{d,e}	83.14±0.49^{b}
	48	3.55±0.06^{a}	3.30±0.03^{d,e}	1.46±0.04^{e}	81.76±0.68^{c}
	60	3.35±0.05^{c,d}	4.66±0.20^{a}	1.46±0.02^{e}	81.78±0.71^{c}
	72	3.01±0.02^{e}	3.79±0.35^{c}	1.45±0.03^{e}	76.78±0.75^{e}
MMA	12	3.01±0.05^{e}	2.66±0.10^{f,g}	1.68±0.02^{b,c}	81.15±1.36^{c}
	24	3.22±0.05^{d}	3.36±0.03^{d}	1.62±0.06^{c,d}	80.35±0.30^{c,d}
	36	3.51±0.01^{a,b}	4.37±0.15^{b}	1.61±0.03^{c,d}	85.84±0.38^{a}
	48	3.32±0.04^{c,d}	3.24±0.10^{d,e}	1.55±0.02$^{c–e}$	81.91±0.39^{c}
	60	2.43±0.02^{g}	3.07±0.03^{e,f}	1.56±0.03$^{c–e}$	79.62±0.19^{d}
	72	2.50±0.04^{g}	3.96±0.52^{b,c}	1.57±0.01$^{c–e}$	77.43±0.57^{e}

Notes: Means ± SD (n = 3) were statistically analyzed using one-way ANOVA. According to Tukey's test ($p < 0.05$), the mean values accompanied by different superscript alphabets differ significantly within a column.
GAE: gallic acid equivalents; QE: quercetin equivalents; TAE: tannic acid equivalents; 0r: raw millet flour; 0MMA: raw millet flour sterilized with MMA; MMA: mixed-mode autoclaving: sterilizing flour and water together; control); SMA: single-mode autoclaving (sterilizing each flour and water separately); dw: dry weight.

Untargeted LC-MS/MS profiles of processed pearl millet samples

Although untargeted LC-MS/MS is an effective method for identifying active ingredients in complex natural mixtures, it has several limitations. Because of the issues such as ionization bias and combined effects, results depend heavily on data processing. They can be affected by false positives (detecting inert chemicals) and false negatives (missing potent and low-concentration compounds) (Caesar *et al.*, 2019). Nevertheless, despite possible quantitative errors from using percentage total area, we used these data to preliminarily indicate that our fermented samples contain bioactive compounds, possibly from millets or microbes, which may contribute to their DPPH radical scavenging activity (Section 3.6) as well as some contaminants that could be impacted by autoclaving/fermentation processes.

A total of 19 bioactive compounds and contaminants were identified in SMA-pretreated fermented pearl millet samples after 72 h, compared to 15 in MMA-pretreated samples under the same conditions. These compounds mainly originate from millet, microbial sources, and contaminants. The SMA- and MMA-fermented samples share several compounds, such as indoxacarb, 3-carboxy-1-hydroxypropyl-thpp, methotrexate, absinthin, and metconazole (Tables 5 and 6; Supplementary Figures S1 and S2).

In Table 5, salicin 6-phosphate, a bacterial metabolite with anti-inflammatory properties that inhibits tumor growth and angiogenesis (Kong *et al.*, 2014), is detected in 12-h SMA-fermented millet, with a total peak area of 1.07%. Its level decreases with increasing fermentation period, ultimately reaching a minimum at 72 h. *L. plantarum* utilizes salicin-6-phosphate as a carbon source through its 6-phospho-β-glucosidase enzyme (Acebrón *et al.*, 2017). Some phytochemicals, such as 6-methylpretetramide, paucin, and absinthin, which are sesquiterpene lactones with antibiotic and anti-inflammatory properties (PubChem National Center for Biotechnology Information (NCBI), 2005; Talmon *et al.*, 2020), are present in SMA and MMA samples fermented for 12–60 h but disappear by 72 h. This is potentially due to *L. plantarum*'s ability to metabolize these compounds. It has been reported that *L. plantarum* CCFM1287 can metabolize certain sesquiterpene lactones (Wei *et al.*, 2022). Other phytochemicals, such as inulicin, a terpene lactone possessing anti-inflammatory activities (Yan *et al.*, 2024), are discovered in pearl millet flour after 48 h of fermentation. This phenomenon may stem from the activity of hydrolytic enzymes, such as saccharases and proteases, which may release inulicin from conjugated molecules such as proteins and polysaccharides, as some terpene lactones can associate with these molecules (Bajtai *et al.*, 2022). The levels of certain compounds, such as plumieride, which has antifungal, anti-virulence, and anti-inflammatory properties (El-Shiekh *et al.*, 2024), are unaffected by fermentation. Meanwhile, other substances such as plant-based obacunone, a limonoid, decrease during fermentation and are undetectable by 72 h. However, limonin, a natural tetracyclic triterpenoid belonging to limonoids, can be metabolized by *L. plantarum*, resulting in a reduction of its content in orange juice (Quan *et al.*, 2022).

Regarding contaminants, indoxacarb is an insecticide present at 0.26–0.39% of the total peak area in both 12–48 h SMA- and MMA-fermented samples, while cyfluthrin, another insecticide, is only detected in SMA-fermented samples at levels of 0.29–0.38% (Table S5). These compounds disappear after 60–72 h of fermentation. It has been reported that *L. plantarum* fermentation can reduce insecticide levels in cereal flours by breaking down pesticide residues through enzymatic activity, especially organophosphorus insecticides (Armenova *et al.*, 2023; Đorđević *et al.*, 2013). *L. plantarum* ATCC 8014 is shown to reduce pesticide levels in bread and wheat flour, notably lowering levels of pirimiphos-methyl, phorate, chlorpyrifos, and bifenthrin (Armenova *et al.*, 2023). Furthermore, metconazole, a fungicide, is detected in both SMA- and MMA-fermented samples at levels ranging from 0.94% to 0.94%. It gradually diminishes with extended fermentation, reaching its lowest point at 72 h. Some strains of *L. plantarum* can act as binding agents, adsorbing or trapping fungicides within their cell structure and metabolizing them, breaking down these into less toxic compounds (Li *et al.*, 2023; Maidana *et al.*, 2022). 1-(5'-Phosphoribosyl)-5-formamido-4-imidazolecarboxamide (faicar), commonly known as faicar, playing a key role in nucleotide synthesis in living organisms like humans and bacteria and exhibits anti-inflammatory and anti-cancer activities (Brooks *et al.*, 2018). Maidana *et al.* (2021) reported that *L. plantarum* aids in detoxifying E. coli metabolites by producing antimicrobial compounds.

Some phytochemicals, such as solasodine—an alkaloid compound with antioxidant, hepatoprotective, immunomodulatory, cytotoxic, antinociceptive, anti-inflammatory, antiatherosclerotic, antimicrobial, and anti-obesity properties (Kumar *et al.*, 2019)—are only present in the 72-h MMA-fermented sample at a level of 8.32%. They are not found in samples fermented for less than 72 h (Table 6). This may be because solasodine binds to other molecules, such as glycosides, preventing its detection during 12–60 h of fermentation. Since *L. plantarum* releases glycoside hydrolases (Cui *et al.*, 2021), it could facilitate the release of conjugated solasodine. Additionally, solasodine can bind to other components, such as rhamnose molecules, forming

solasodine rhamnosyl glycosides (Cham, 2013). Beta-geranylfarnesene, timosaponin A-III, and celapanine—triterpenoids with biological activities—are detected in MMA samples fermented for 48–72 h (Table 6). These terpenoids may bind to proteins and polysaccharides and are released by glycolytic hydrolase enzymes (Bajtai *et al.*, 2022).

In conclusion, regarding contaminants, *L. plantarum* has been shown to reduce pesticide levels in millet flour, with these levels decreasing progressively during fermentation, reaching a minimum level, after which they disappear. It has been reported that fermenting millet flour with LAB can yield probiotic-rich products and enhance bioactive compounds (Tomar *et al.*, 2025).