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Development of plant-based infant complementary food from *Cucumeropsis mannii*, *Dioscorea dumetorum*, *Musa paradisiaca*, and *Malus domestica* fermented with lactic acid bacteria

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Abstract

Plant-based complementary foods offer a promising alternative to conventional infant formulas; however, their nutritional limitations, anti-nutritional factors, and functional properties require optimization to ensure suitability for infant nutrition. In this study, a novel ready-to-eat plant-based infant food in paste form was developed from *Cucumeropsis mannii* (Melon seeds), *Dioscorea dumetorum* (sweet Yellow Yam tubers), banana, and apple through fermentation with lactic acid bacteria (LAB) to enhance its nutritional properties. After starter screening and fermentation optimization, the formulation was individually inoculated with 6 Log CFU/g of *Lactocaseibacillus paracasei* (SK8), *Leuconostoc mesenteroides* (GSL7), and *Lactiplantibacillus plantarum* (AFI5), and incubated at 30 °C for 24 h. Subsequently, the effect of fermentation on chemical composition, peptide profile, and antioxidant capacity was investigated. Compared to the control formulation (RAW-F3), fermentation induced distinct changes in sugar consumption, free amino acids content, peptides profiles, along with reductions in key anti-nutritional factors (particularly phytic acid and saponins). Proteolysis driven by enzymes produced by LAB contributed to a modified free amino acid profile, with the formulation inoculated with *L. paracasei* (SK8-F3) having the highest amounts of free amino acids (401.8 mg/100 g). Likewise, a notable change in peptide profile was observed, and the formulation was inoculated with *L. mesenteroides* (GSL7-F3) recorded the highest diverse number of peptides (3352), followed by the formulation inoculated with *L. plantarum* (3417), and SK8-F3 (2697). In SK8-F3, phytic acid and saponins were significantly reduced by 75% and 50%, respectively, compared to RAW-F3 ($P < 0.05$). Generally, fermentation enhanced total phenolic content and antioxidant activity, with the highest phenolics in GSL7-F3 (267 mg/L) and the strongest DPPH scavenging in AFI5-F3 (61.3%). Pro-cyanidin B2 and caffeic acid peaked in SK8-F3, while ferulic acid and phloridzin remained consistently high across all formulations. Metabolism by LAB also contributed to improved sensory properties, with the SK8-F3 and AFI5-F3 formulations receiving the highest sensory scores. Overall, our findings affirm the role of LAB in enhancing the nutritional quality, safety, and sensory characteristics of foods, supporting their application in infant food formulations.

Keywords Lactic acid fermentation, Plant-based complementary food, Anti-nutritional factors, Infant nutrition, Functional properties

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Introduction

Adequate nutrition during infancy is critical for optimal growth and development. At six months, breast milk alone cannot meet the increasing nutrient demands of infants, necessitating the introduction of complementary foods. However, in many developing countries, commercially complementary foods (CFs) are often imported from Northern countries and are inaccessible due to high costs and limited availability. This exposes infants to poor growth, malnutrition, including protein-energy malnutrition, and vitamin A and iron deficiency. These conditions are major contributors to childhood morbidity and mortality, particularly in sub-Saharan Africa [1]. Traditional complementary foods in Cameroon, such as corn pap (fermented maize porridge) and soya pap (corn-soy porridge), are widely used and are prepared using local cereals and legumes. These foods are predominantly cereal-based and often lack sufficient protein and essential micronutrients, which limits their ability to fully meet infant nutritional needs [2, 3]. To address these limitations, there is an opportunity to diversify the ingredients used in complementary foods by incorporating locally cultivated tubers, protein-rich seeds, and fruits. Such ingredients could serve as sustainable, nutritionally superior alternatives for developing infant complementary foods. This study explores the potential of *Dioscorea dumetorum*, *Cucumeropsis mannii*, *Musa paradisiaca*, and *Malus domestica*, combining their unique nutritional profiles to create a novel complementary food. *Dioscorea dumetorum* (commonly known as sweet yellow yam) is a nutrient-rich yam species that exhibits higher concentrations of carotenoids, iron, and zinc compared to other yam varieties and common root tubers like cassava (*Manihot esculenta*), making it nutritionally superior for food applications [4, 5, 6]. Similarly, *Cucumeropsis mannii* (commonly known as the melon seed) is a protein-rich oilseed containing essential amino acids, though it remains underutilized outside traditional recipes [7, 8]. *Musa paradisiaca* (banana) and *Malus domestica* (apple) provide vitamins, free sugars, and phytochemicals that simultaneously enhance both taste and nutritional value, making them particularly ideal for infant food fortification [9]. Despite their inherent nutritional value, the need to increase bioactive compound concentrations, alongside the persistent challenge of anti-nutritional factors that impair nutrient absorption and functional efficacy, remains a key driver of research for optimized solutions [10, 11]. In this context, processing methods (including cooking, soaking, and fermentation) serve as possible strategies to reduce anti-nutritional factors, enhance

nutrient bioavailability, and optimize sensory properties [12].

Among these methods, lactic acid fermentation stands out as an eco-sustainable biotechnology that enhances bio-functional properties, necessary for infant foods. Through their enzymatic activity and organic acid production during fermentation, lactic acid bacteria (LAB) lower pH levels to inhibit spoilage microorganisms while simultaneously degrading complex molecules into simpler forms. These actions reduce anti-nutritional factors while enhancing the bioavailability of proteins, bioactive compounds, and other nutrients essential for optimal health [13–15]. Among the vast diversity of LAB with varied enzymatic profiles, we selected *L. paracasei*, *L. plantarum*, and *L. mesenteroides* as starter cultures, due to their notable β -glucosidase, phytases, and proteolytic activities [16, 17], which are necessary for enhancing the nutritional and organoleptic properties of this complementary food. Unlike traditional complementary foods that require extensive preparation, this study focuses on developing a ready-to-eat infant food, addressing critical issues such as risk of contamination and the time constraints faced by caregivers. By leveraging the nutritional potential of melon seeds, yellow yams, banana, and apple, and employing fermentation to enhance their properties, this study aims to create a nutritious, sustainable, and plant-based alternative complementary food for infants.

Materials and methods

Raw materials, microorganisms, and culture conditions

Dioscorea dumetorum (yellow yam) tubers were sourced from local farmers in Mile 6 Nkwen, Bamenda, located in the North-West Region of Cameroon (5°58'29" N, 10°10'41" E). *Cucumeropsis mannii* (melon) seed kernels were obtained from farmers in the East Region of Cameroon, specifically around Bertoua (4°34'0" N, 13°41'0" E). *Musa paradisiaca* (banana) and *Malus domestica* (apple) were purchased from organic vendors at the Bozen local market in South Tyrol, Northern Italy (46°29'5" N, 11°21'13" E). Prior to use, the melon seeds were kept at room temperature, while the yellow yams, apples, and bananas were stored at 4 °C.

Three strains of LAB (*Leuconostoc mesenteroides*; GSL7, *Lactiplantibacillus plantarum* AFI5, and *Lacti-caseibacillus paracasei* SK8) belonging to the Micro-4Food culture collection from the Faculty of Agricultural, Environmental and Food Sciences at the University of Bolzano- Bozen were used as starters in this study. These strains were selected based on their previously demonstrated efficacy in enhancing fermentation efficiency (effective acidification and fermentation stability), reducing anti-nutritional factors, improving carbohydrate metabolism, enhancing phenolic metabolism, and

promoting protein hydrolysis with the release of bioactive peptides [18]. They were originally isolated from apple, apple by-products, and sauerkraut, respectively. The cultures were preserved as frozen stocks at -20°C in de Man Rogosa and Sharpe (MRS) broth medium (Oxoid, UK) with 20% glycerol. Prior to use, each strain underwent two successive subculturing steps in MRS broth at 30°C for activation.

Formulation development

The production of the different powders and pastes are shown in Fig. 1 (A-D). The yellow yam tubers were peeled, sliced, and pre-cooked at 85°C for 5 h, oven-dried at 40°C for 20 h, and subsequently blended using a Top Chef blender (model TC-01, Top Chef Appliances, USA). The resulting flour was then sieved using a stainless-steel sieve with a pore size of $250\ \mu\text{m}$. The Melon seeds were peeled, and the resulting seed kernels were blended using a Classic Blender 400 (PBI International) into flour. Apples and bananas were peeled and blended into puree using an IKA T25 easy clean homogenizer (IKA-Werke GmH & Co. HD, Staufen, Germany).

The ingredient ratios were optimized through preliminary trials to develop a baseline formulation that meets the nutritional requirements of infants during the complementary feeding period, while achieving desirable textural (paste-like consistency, comparable to commercial baby foods) and sensory properties. Initially, distinct ratios were proposed based on approximate nutrient contributions of each ingredient and the WHO's 2023 complementary feeding guidelines [19], with the melon seeds providing protein and fat, the yellow yam serving as a starchy energy source, and apple and banana enhancing palatability and micronutrient content. Based on previous considerations, three formulations were developed, each containing fixed proportions of melon seeds (15%) and yellow yam (5%), while varying the proportions of apple and banana (5%, 7.5%, or 10% each), with corresponding adjustments in water content (70%, 65%, or 60%) to generate Formulation 1 (F1), Formulation 2 (F2), and Formulation 3 (F3), respectively. The mixtures were blended using a Classic Blender 400 (PBI International) to ensure homogeneity, pasteurized at 85°C for 15 min, and then cooled. All formulations were subjected to pH measurements and sensory evaluation by the research team to identify the preferred formulation for subsequent fermentation. Sensory evaluation focused on appearance, mouthfeel, flavor, and taste, with a general sensory score for each formulation determined using a four-point liking scale (0 = the lowest, 4 = the highest).

Starters screening

Formulation 3 (F3) was used as the substrate to evaluate starter performance and to determine optimal fermentation conditions. LAB cells were harvested by centrifugation (10,000 rpm, 10 min, at 4°C), washed twice into sterile 0.9% (w/v) sodium chloride solution, and individually inoculated into F3 using two inoculum sizes (ca 6.0 or ca. 7.0 Log CFU/g) [20]. Following inoculation, starter F3 was incubated at 30°C for 48 h. To comprehensively monitor fermentation dynamics (microbial growth and pH reduction), formulations were collected at 0, 8, 24, 32, 40, and 48 h. F3 without inoculum but incubated at 30°C was used as a control. Bacterial growth was assessed by homogenizing one g of each formulation in 9 mL of 0.9% NaCl, then plating serial dilutions on MRS agar supplemented with 0.1% cycloheximide (Sigma-Aldrich, USA). Mesophilic LAB were counted after incubation for 48 h at 30°C . Starter acidification capacity was evaluated by measuring the pH values with a Foodtrode electrode (Hamilton, Bonaduz, Switzerland).

Fermentation set-up

Based on starter screening, optimal fermentation conditions were established at an inoculum size (ca. 6 log CFU/g) with a 24-h incubation period. To comprehensively evaluate nutritional and functional modifications attributable to microbial fermentation, three control were implemented: (i) RAW-F3 (pasteurized F3 formulation without microbial inoculation and incubation), serving as a baseline; (ii) UNST-F3 (pasteurized F3 formulation incubated at 30°C for 24 h without starter culture), to account for the effects of spontaneous fermentation; and (iii) CA-F3 (pasteurized F3 formulation chemically acidified to pH ca. 4 with food-grade lactic acid, without starter culture), designed to exclude the effect of acidification, which might interfere with the activity of endogenous plant enzymes. Except for formulations used for sensory analysis, all other formulations were freeze-dried (Epsilon 2-6D LSC plus freeze-drier, Martin Christ, Osterode am Harz, Germany) before being analyzed as described below.

Chemical characterization of fermented formulations

To assess sugar and organic acids metabolism during fermentation, water-soluble extracts (WSE-1) were prepared by extracting 1 g of each formulation with 5 mL of a 95:5 (v/v) water/perchloric acid solution. The mixture was ice-cooled, sonicated (60% amplitude, 1 min, two cycles with 5-min intervals), stirred at room temperature for 1 h, incubated at 4°C overnight, and centrifuged at 10,000 rpm for 10 min. The supernatant was filtered through $0.2\ \mu\text{m}$ membrane filters and stored at -20°C . High-performance liquid chromatography (HPLC) was

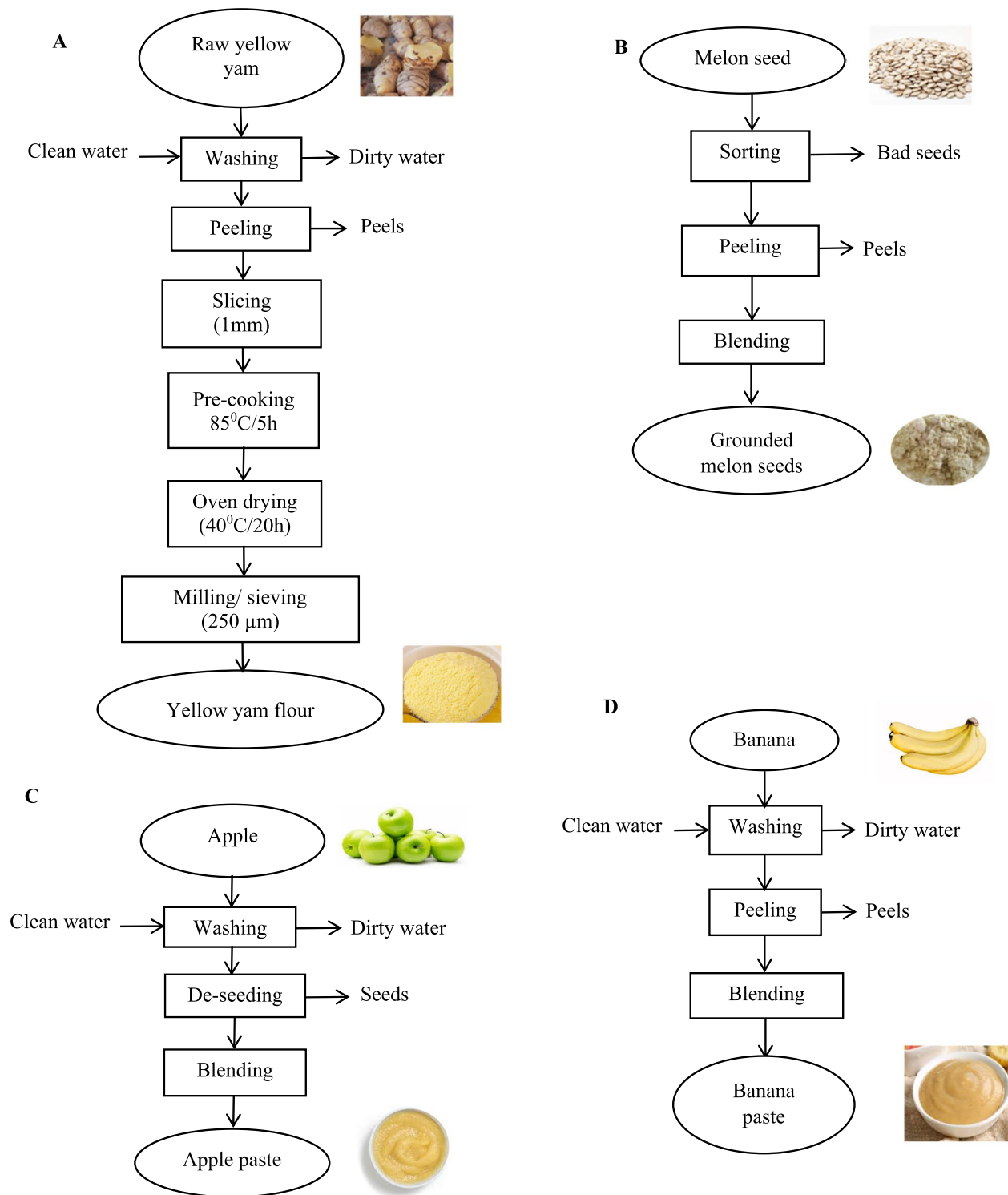


Fig. 1 Production of Yellow Yam Powder, Melon Seed powder, Apple, and Banana Paste

employed to quantify glucose, fructose, mannitol, lactic acid, and acetic acid in the WSE. The HPLC system consisted of an Aminex HPX-87H column (Bio-Rad,

CA, USA), a Perkin Elmer 200a refractive index detector (IDEX Health & Science, Rohnert Park, CA, USA), and a UV detector set at 210 nm. Analytical standards were

purchased from Sigma-Aldrich (Steinheim, Germany) for calibration and quantification.

Total protein content was measured in freeze-dried formulation powders using NDA 701 Dumas nitrogen analyzer (Velp Scientifica, Italy) based on the AOAC Method 992.15 [21]. Carbohydrates were determined by difference according to Merrill and Watt [22]. Dietary fiber content was determined using the enzymatic–gravimetric method as described by AOAC Method 985.29 [23], which involves sequential enzymatic digestion of starch and protein followed by gravimetric measurement of the fiber residue. Fat content was determined using the Soxhlet extraction method with petroleum ether as the extraction solvent [24], and ash content was determined using the dry ashing method [24].

Potassium and sodium contents in raw and fermented formulations were determined through flame spectrophotometry as described by ANFOR [25]. Calcium and magnesium contents were quantified using the titrimetric method [26], and iron was estimated by the ortho-phenanthroline colorimetry using the method of Rodier [27].

Free amino acid profiling

Total and individual amino acids were determined in water-soluble extracts (WSE-2), which were prepared by extracting one gram of formulation with 15 mL of Tris–HCl 50 mM (pH 8.8). The suspension was vortexed and centrifuged at 10,000 rpm for 10 min, then filtered using a 0.45 µm filter (Millipore Corporation, Bedford, MA, USA). Proteins and peptides were precipitated by adding 5% (v/v) cold solid sulfosalicylic acid, kept at 4 °C for 1 h, and centrifuged at 12,000 rpm for 15 min. The supernatant was filtered again through a 0.22 µm pore size filter. The resulting extract was analyzed using a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd., Cambridge, UK) equipped with a lithium cation exchange column, as described by Tlais et al. [18].

Low-molecular-weight peptide profiling

The low-molecular-weight peptide profile was determined using WSE-2. To separate the active peptide fractions, ultrafiltration (molecular weight cut-off < 3 kDa) was carried out following the method described by Tagliazucchi [28] with a few modifications. Specifically, 2 mL of the extract was loaded into a Vivaspın®20 column, 3000 MWCO- PES (Sartorius, Italy), and centrifuged at 4000×g for 40 min. The resulting low molecular weight water-soluble extracts (LMW-WSE) were identified by UHPLC/HR-MS2 (UHPLC Ultimate 3000 with Q Exactive Hybrid Quadrupole–Orbitrap Mass Spectrometer, Thermo Scientific, San Jose, CA, USA) equipped with a C18 column (Acquity UPLC-C18 Reverse-phase,

2.1×100 mm, 1.8 µm particle size, Waters Corporation, Milford, MA, USA). The MS data were directly processed with the Proteome Discoverer 2.3 (Thermo Fisher Scientific, Dreieich, Germany) coupled with Matrix software (Matrix Science, Boston, MA, USA) for peptide sequencing and identification. The main parameters used for the identification process were: enzyme, no-enzyme; peptide mass tolerance, ± 5 ppm; fragment mass tolerance, ± 0.1 Da; variable modification, Demethylation (NQ), oxidation (M), and phosphorylation (ST); and the maximal number of post-translational modifications permitted in a single peptide. Additionally, peptides identified in the formulations were examined for homology with bioactive peptides identified in the literature by using the Bioactive Peptide Database BIOPEP UWM [29].

Total and free phenolic compounds characterization

Total free phenolic compounds were estimated using the Folin–Ciocalteu method [30], with analysis performed on methanol/water-soluble extract (MWSE) following the protocol described by Tlais et al. [18]. Free phenolic compounds in the MWSE were profiled using an HPLC system (Ultimate 3000, Dionex, Germany) with a Kinetex C18 column, photodiode array detection, and a gradient elution of solvents A (water + 0.1% trifluoroacetic acid) and B (acetonitrile + 0.1% trifluoroacetic acid). Detection occurred at 280, 310, and 320 nm. Phenolic compounds were identified by comparison with commercial standards, and calibration curves were generated for quantification [31].

In vitro antioxidant assays

The in vitro antioxidant activity of LMW-WSE and MWSE obtained from all the formulations was estimated through the determination of the radical scavenging capacity on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) as previously described by Blois [32].

Anti-nutrient profiling

Phytic acid and raffinose-series oligosaccharides (RFOs) were assessed using the Phytic Acid (Phytate)/Total Phosphorus (K-PHYT 05/19) and Raffinose/D-galactose (K-RAFGA) assay kits (Megazyme International, Ireland), following the manufacturer's instructions. Phytate content was determined based on AOAC Official Method 986.11 [33]. Fructan content was determined using the Fructo-oligosaccharides assay kit (K-FRUC 11/22; Megazyme International, Ireland), following the standard procedure based on AOAC Official Method 999.03 [34] and AOAC 32.32.01 [35]. This method involves the enzymatic hydrolysis of fructans to glucose and fructose, followed by spectrophotometric quantification at 340 nm. Saponin

content was determined using the foam measurement method as described by Koziol [36].

Sensory evaluation of fermented formulations

Sensory evaluation was conducted on all formulations except RAW-F3 and CA-F3, with 20 non-trained panelists performing Quantitative Descriptive Analysis (QDA) according to the method described by Satora [37].

Statistical analysis

All experiments were performed in triplicate. Data normality was confirmed using the Shapiro–Wilk test. One-way ANOVA, followed by Duncan's Multiple Range Test, was conducted using Statgraphics Centurion XVII (2014) to assess treatment group differences ($P < 0.05$). Two-way ANOVA, with Tukey's HSD test for post hoc comparisons, evaluated the effects of strain type and inoculum concentration and their interactions ($P < 0.05$).

Results and discussion

Formulation design and optimization of fermentation parameters

Considering the nutritional requirements of infants during the complementary feeding period, while achieving desirable textural properties, three formulations were developed. Following pasteurization, sensory evaluation revealed formulation 3 (F3) as the preferred formulation in terms of texture, flavor, and taste, composed of 15% melon seed flour, 5% yam flour, 10% banana puree, 10% apple puree, and 60% water (Fig. 2), with an initial pH of

6.14. This formulation was subsequently selected for further screening.

After obtaining the formulation of choice, a screening phase was carried out over 48 h as previously described in Sect. "Starters screening". A two-way ANOVA revealed significant effects of strain and inoculum level on pH and presumptive LAB counts throughout the incubation. At 8 h, the pH of inoculated formulations was lower than UNST-F3 ($F(3,14) = 25.12$, $P < 0.001$), with GSL7-F3 (7 Log) reaching the lowest value (4.32 ± 0.07). Inoculum level significantly influenced pH ($F(2,14) = 7.03$, $P = 0.008$), with a notable strain-inoculum interaction ($F(2,14) = 4.50$, $P = 0.031$). AFI5-F3 (7 Log) had the highest viable counts (8.51 ± 0.04), significantly exceeding UNST-F3 (7.10 ± 0.02 , $P < 0.01$). At 16 h, pH remained highest for UNST-F3 (6.14 ± 0.01 , $F(3,14) = 20.32$, $P < 0.001$), with inoculum level affecting pH reduction ($F(2,14) = 5.45$, $P = 0.017$), especially for SK8-F3 and GSL7-F3 (7 Log). Viable counts increased sharply for SK8-F3 and AFI5-F3 (7 Log) (~ 10.14 – 10.15 Log CFU/g). After 24 h, UNST-F3 still maintained the highest pH (5.17 ± 0.57), while SK8-F3 and AFI5-F3 (7 Log) dropped within the range of 3.94–4.02 ($F(3,14) = 22.45$, $P < 0.001$). The effect of the inoculum level ($F(2,14) = 6.18$, $P = 0.012$) and strain-inoculum interaction ($F(2,14) = 4.02$, $P = 0.038$) were significant. Viable counts for SK8-F3 and AFI5-F3 (7 Log) were highest (~ 10.20 – 10.31), significantly exceeding GSL7-F3 (8.26) and UNST-F3 (7.80, $P < 0.001$). At 32 h, AFI5-F3 (7 Log) had the lowest pH (3.76 ± 0.08 , $F(3,14) = 20.45$, $P < 0.001$), with inoculum ($F(2,14) = 6.01$, $P = 0.013$) and interaction

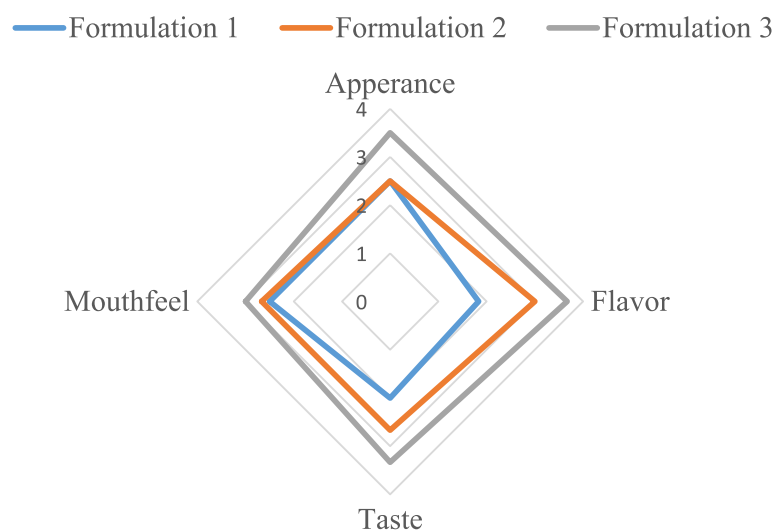


Fig. 2 Web plot of sensory evaluation results conducted by the research team for three formulation variants containing different proportions of water and plant ingredients during the development phase. Formulation 1 (70% water, 5% banana, 5% apple, 5% yellow yam, 15% melon seeds), Formulation 2 (65% water, 7.5% banana, 7.5% apple, 5% yellow yam, 15% melon seeds), and Formulation 3 (60% water, 10% banana, 10% apple, 5% yellow yam, 15% melon seeds)

effects ($F(2,14)=3.80$, $P=0.048$) being significant. SK8-F3 (7 Log) achieved the highest viable counts (~ 10.66 Log CFU/g), with the inoculum effect being significant ($F(2,14)=10.05$, $P=0.002$). At 40 h, SK8-F3 (7 Log) had the lowest pH (3.72 ± 0.06 , $F(3,14)=18.12$, $P<0.001$). At 48 h, pH ranged from 3.60 to 4.52 ($F(3,14)=15.23$, $P<0.001$), with SK8-F3 (7 Log) being the lowest. Viable counts for SK8-F3 and AFI5-F3 (7 Log) declined (7.07–8.01), while formulations inoculated at 6 Log remained higher (8.95 , $F(3,14)=10.45$, $P<0.001$).

Rapid acidification (Fig. 3) and microbial growth (Fig. 4) were observed during the fermentation screening phase, reflecting the distinct metabolic traits of the individual strains and the effect of inoculum size. By 8 h, GSL7-F3 (7 Log CFU/g) achieved the lowest pH (4.32 ± 0.07), indicating early acidification. Likely due to its adaptation to the carbohydrate-rich substrate enhanced by apple and banana inclusion. However, by 16–24 h, SK8-F3 and AFI5-F3 showed lower pH values (4.14 – 4.57) and higher viable counts (9.14 – 10.31 Log CFU/g), underscoring the superior acidification efficiency of homo-fermentative *Lactobacillus* strains. In contrast, the heterofermentative strain, *Leuconostoc*

mesenteroides, produced neutral byproducts such as ethanol and mannitol [38], limiting its acidification and cell growth, and thus its suitability for meeting safety and probiotic standards in infant foods.

The non-inoculated control (UNST-F3) showed slower acidification (pH 4.52–6.31) with microbial growth up to 8.50 Log CFU/g, driven by native flora in the substrate. This highlights the importance of starter cultures to ensure consistent acidification and pathogen control, key for infant food safety [39]. The inclusion of fruits enhanced fermentability due to their simple sugars, supporting acid production and microbial proliferation, a favorable trait for developing probiotic-rich complementary foods. At 32–48 h, SK8-F3 and AFI5-F3 reached intense acidification (pH 3.60–3.76), but their counts declined at 7 Log CFU/g (7.07–8.01 Log CFU/g), likely due to nutrient exhaustion and lactic acid accumulation [40]. Conversely, the 6 Log inoculated formulations retained higher viability (8.95 ± 0.07 Log CFU/g), suggesting greater resilience during prolonged fermentation. This emphasizes the need to balance acidification and cell survival for probiotic efficacy.

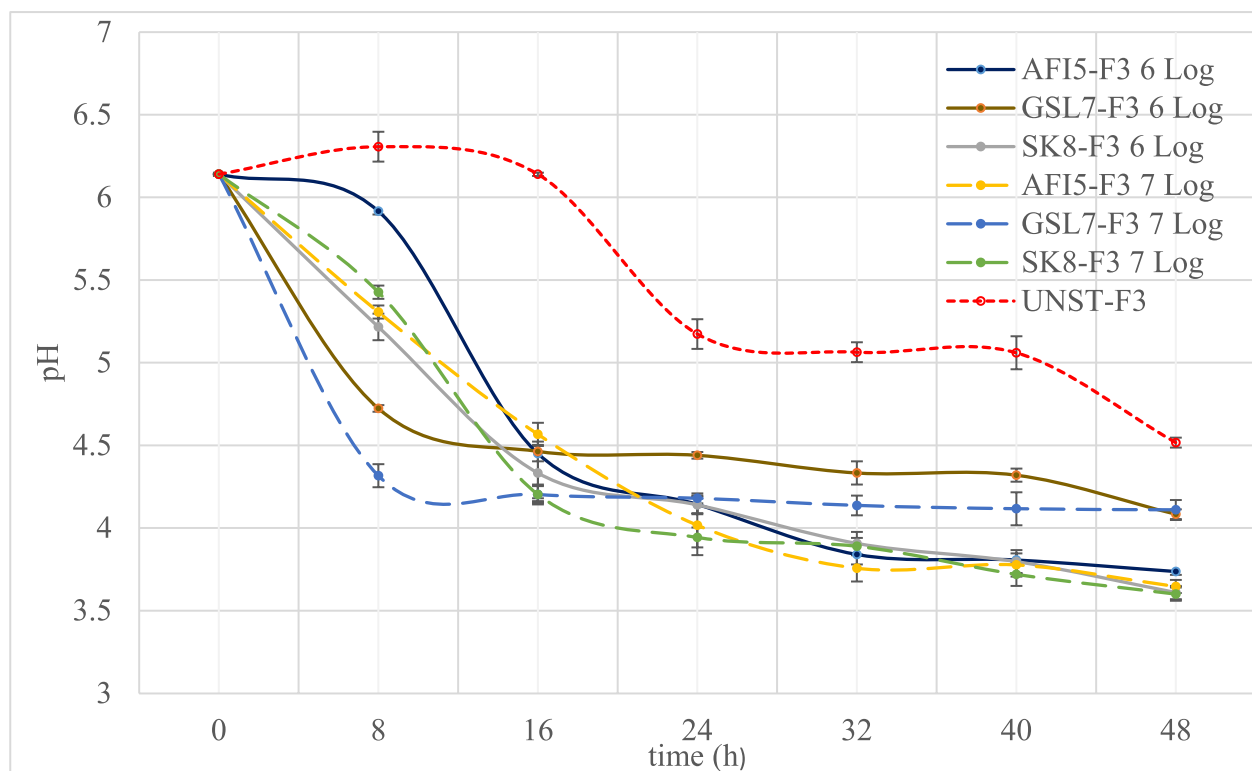


Fig. 3 Acidification kinetics during fermentation at 30 °C for 48 h with starter cultures inoculated at 6 and 7 Log CFU/g into pasteurized formulation (F3). UNST-F3: incubated, non-inoculated control; *Lactiplantibacillus plantarum*; GSL7-F3 with *Leuconostoc mesenteroides*; and SK8-F3 with *Lactocaseibacillus paracasei*. Data represent mean $\pm$ standard deviation of 3 replicates. Statistical analysis across sample groups was performed using two-way ANOVA ($P<0.05$)

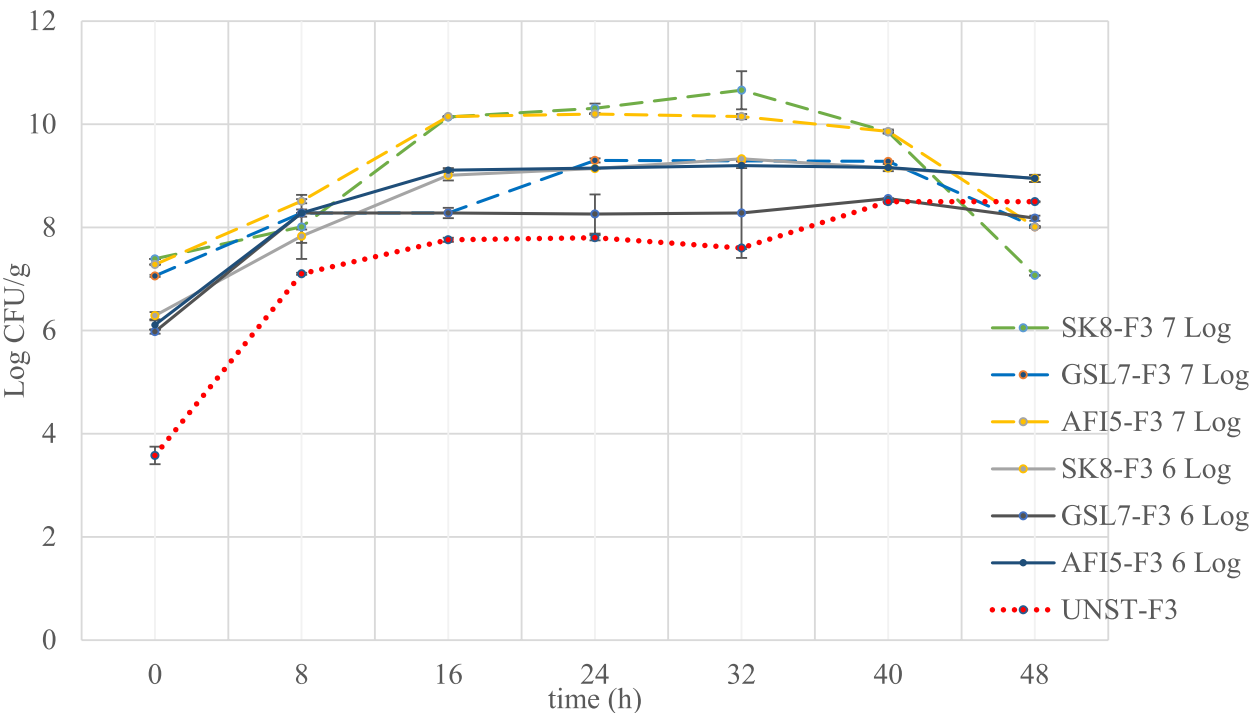


Fig. 4 Microbial plate count dynamics during fermentation screening at 30 °C for 48 h using starter cultures inoculated at 6 and 7 Log CFU/g into the pasteurized formulation (F3). UNST-F3 represents the incubated but non-inoculated control; AFI5-F3 is inoculated with *Lactiplantibacillus plantarum*; GSL7-F3 with *Leuconostoc mesenteroides*; and SK8-F3 with *Lacticaseibacillus paracasei*. Data represent mean $\pm$ standard deviation of three replicates. Statistical analysis to compare differences across sample groups was performed using two-way ANOVA ($P < 0.05$)

Ultimately, 6 Log CFU/g at 24 h was selected as optimal, since the pH of inoculated formulations ranged between 4.14 to 4.4 (Fig. 3) which is below the pathogen inhibition threshold of 4.6 [41], and they had a viable count of ≥ 9.14 CFU/g, which surpassed the 10^9 CFU/g threshold for probiotic benefits [42]. While 7 Log inoculum led to slightly lower pH (3.94–4.02) and higher counts (10.20–10.31), the 6 Log concentration balanced safety and cell viability, supporting its use in the fermentation of the infant food.

Analysis of free sugars, organic acids, and proximate composition of formulations

Analysis of sugars, organic acids (Table 1) and proximate composition (Table 2), revealed significant ($P < 0.05$) variations among formulations inoculated with different LAB strains and control formulations. Glucose content ranged from 1.9 to 7.1 mg/g, with RAW-F3 (7.1 ± 0.1 mg g⁻¹) showing significantly higher levels than all other formulations, while GSL7-F3 (1.9 ± 0.0 mg g⁻¹) had the lowest values. Fructose concentrations varied between 2.0 and 9.2 mg/g, with RAW-F3 (9.2 ± 0.0 mg g⁻¹) and UNST-F3 (8.7 ± 0.0 mg g⁻¹) exhibiting significantly higher levels than the remaining formulations, Mannitol was notably elevated in GSL7-F3 (10.1 ± 0.1 mg g⁻¹), significantly

Table 1 Sugars and organic acids quantification					
Parameter (mg g ⁻¹)	RAW-F3	UNST-F3	AFI5-F3	GSL7-F3	SK8-F3
Glucose	7.1 ± 0.1 ^d	4.6 ± 0.0 ^c	3.2 ± 0.2 ^b	1.9 ± 0.0 ^a	3.3 ± 0.0 ^b
Fructose	9.2 ± 0.0 ^d	8.7 ± 0.0 ^d	6.8 ± 0.1 ^c	2.0 ± 0.0 ^a	4.3 ± 0.1 ^b
Mannitol	1.7 ± 0.0 ^a	1.7 ± 0.0 ^a	1.8 ± 0.0 ^a	10.1 ± 0.1 ^b	1.7 ± 0.0 ^a
Lactic acid	0.3 ± 0.0 ^a	1.5 ± 0.0 ^b	6.6 ± 0.3 ^c	5.5 ± 0.0 ^c	5.8 ± 0.0 ^c
Acetic acid	0.7 ± 0.0 ^a	0.9 ± 0.1 ^a	1.5 ± 0.9 ^{ab}	2.2 ± 0.0 ^b	0.9 ± 0.0 ^a

Values represent the mean $\pm$ standard deviation of three measurements. Values within the same row with different superscripts (a–c) are significantly different ($P < 0.05$), while those sharing the same superscript are not significantly different. RAW-F3: pasteurized control; UNST-F3: incubated but non-inoculated control; AFI5-F3, GSL7-F3, and SK8-F3 were individually inoculated with 6 Log CFU/g of *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides*, and *Lacticaseibacillus paracasei*, respectively. The incubation was carried out at 30 °C for 24 h

exceeding the low and similar levels (~ 1.7 – 1.8 mg g⁻¹) found in other formulations. Lactic acid content was lowest in RAW-F3 (0.3 ± 0.0 mg g⁻¹) and significantly increased in the inoculated formulations. Acetic acid was highest in GSL7-F3 (2.2 ± 0.0 mg g⁻¹).

Protein content varied significantly ($P < 0.05$), with AFI5-F3 ($12.3 \pm 0.5\%$) and GSL7-F3 ($12.2 \pm 0.4\%$) showing significantly higher values than CA-F3 ($11.7 \pm 0.0\%$).

Table 2 Proximate Composition

Parameters (%)	RAW-F3	UNST-F3	CA-F3	AFI5-F3	GSL7-F3	SK8-F3
Protein	12.0 ± 0.2 ^{ab}	12.1 ± 0.3 ^{ab}	11.7 ± 0.0 ^a	12.3 ± 0.5 ^b	12.2 ± 0.4 ^b	12.2 ± 0.3 ^b
Carbohydrate	72.6 ± 0.3 ^{ab}	72.6 ± 0.1 ^c	73.9 ± 0.2 ^c	74.2 ± 0.0 ^a	73.2 ± 0.0 ^a	73.3 ± 0.8 ^b
Fats	7.5 ± 0.0 ^a	7.4 ± 0.4 ^{ab}	7.5 ± 0.0 ^a	7.3 ± 0.9 ^b	7.3 ± 0.0 ^b	7.3 ± 0.0 ^b
Fiber	5.1 ± 0.1 ^{ab}	5.1 ± 0.1 ^{ab}	5.1 ± 0.1 ^{ab}	4.4 ± 0.4 ^a	4.5 ± 0.5 ^a	4.4 ± 0.4 ^a
Ash	2.8 ± 0.1 ^a	2.8 ± 0.1 ^a	2.8 ± 0.0 ^a	2.8 ± 0.0 ^a	2.8 ± 0.1 ^a	2.8 ± 0.0 ^a

Values represent the mean ± standard deviation of three measurements. Values within the same row with different superscripts (a–c) are significantly different ($P < 0.05$), while those sharing the same superscript are not significantly different. RAW-F3: pasteurized control; UNST-F3: incubated but non-inoculated control; CA-F3: acidified control; AFI5-F3, GSL7-F3, and SK8-F3 were individually inoculated with 6 Log CFU/g of *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides*, and *Lactocaseibacillus paracasei*, respectively. The incubation was carried out at 30 °C for 24 h

Carbohydrate content was significantly lower in RAW-F3 (72.6 ± 0.3%) compared to the inoculated formulations, GSL7-F3 (73.2 ± 0.0%), SK8-F3 (73.3 ± 0.8%), and AFI5-F3 (74.2 ± 0.0%). Fiber content was significantly higher ($P < 0.05$) in RAW-F3 (5.1 ± 0.1%), UNST-F3, and CA-F3 (approximately 5.1 ± 0.1%) compared to the inoculated fermented formulations, which showed lower fiber levels averaging 4.4 ± 0.42 (Table 2).

Glucose and fructose concentrations significantly decreased in the inoculated formulations compared to the pasteurized and non-inoculated controls, reflecting their utilization as primary energy substrates by the LAB during fermentation. The effective metabolism of these sugars is essential for the production of organic acids and other bioactive metabolites that contribute to product safety and preservation. Furthermore, the reduction of simple sugars like glucose and fructose can be particularly beneficial in infant foods by potentially reducing sweetness and lowering the risk of early childhood dental caries and metabolic disorders associated with high sugar intake [46, 47]. Additionally, controlled sugar metabolism during fermentation may enhance nutrient bioavailability and improve sensory properties, supporting better infant acceptance and promoting gut health through prebiotic effects [48]. Formulations inoculated with *L. mesenteroides* showed a notable increase in mannitol, attributed to the ability of *L. mesenteroides* to convert fructose via mannitol dehydrogenase [49]. Mannitol, a prebiotic sugar alcohol, may support infant gut health by acting as a mild laxative and promoting the growth of beneficial microbiota [50].

Organic acid profile varied across formulations, with lactic acid as the dominant metabolite. Fermentation with *L. plantarum* produced the highest amount of lactic acid, consistent with the homofermentative nature of *L. plantarum* [43]. Higher acetic acid levels in GSL7-F3 confirmed the heterofermentative pathway of *L. mesenteroides*, which produces acetic acid as a key secondary metabolite [44]. These organic acids play a vital role in

infant food formulations by enhancing safety through antimicrobial effects, improving mineral bioavailability, and supporting gut health via modulation of the microbiota [45].

The proximate composition of the infant food formulations showed notable nutritional modifications following LAB fermentation. The higher protein content found in inoculated formulations aligns with reports that LAB can enhance protein quality through amino acid synthesis and enzymatic hydrolysis, improving overall protein fractions' bioavailability in fermented foods [51]. Carbohydrate content was also significantly higher in the inoculated fermented formulations compared to the pasteurized control, a trend that may result from the breakdown of complex carbohydrates or concentration effects during microbial activity, thereby enhancing energy availability and digestibility in infant nutrition [52]. In contrast, fiber content was significantly reduced ($P < 0.05$) in the inoculated formulations relative to the non-inoculated controls, likely due to microbial enzymatic degradation of complex polysaccharides into more accessible compounds. This fiber reduction can be beneficial in infant formulations by reducing bulk and improving the absorption of other nutrients [53]. Fat and ash contents, however, remained relatively unchanged across formulations ($P > 0.05$), indicating that fermentation selectively impacted macronutrients while maintaining the stability of lipid and mineral fractions.

Quantification of mineral elements

The mineral profiles of the formulations are presented in Table 3. Iron contents ranged from 1.0–5.1 mg/100 g, with CA-F3 recording the highest level (5.1 ± 0.0 mg/100 g), followed by AFI5-F3 (3.2 ± 0.01 mg/100 g) and PAST-F3 (1.30 ± 0.0 mg/100 g), while the lowest iron content was observed in UNST-F3 (1.0 ± 0.0 mg/100 g). Calcium varied from 10.5–14.2 mg/100 g, with CA-F3 yielding the highest value, followed by UNST-F3 (13.5 ± 0.3 mg/100 g) and AFI5-F3 (12.5 ± 0.5 mg/100 g). Zinc ranged from

Table 3 Mineral Profile of Raw and Fermented Formulations

Mineral Element	RAW-F3	UNST-F3	CA-F3	AFI5-F3	GSL7-F3	SK8-F3
Iron (mg/100 g)	1.3 ± 0.0 ^a	1.0 ± 0.0 ^a	5.1 ± 0.0 ^c	3.2 ± 0.01 ^b	1.7 ± 0.0 ^a	1.6 ± 0.0 ^a
Calcium (mg/100 g)	10.5 ± 0.1 ^a	13.5 ± 0.3 ^d	14.2 ± 0.0 ^e	12.5 ± 0.5 ^c	11.8 ± 0.1 ^b	11.3 ± 0.1 ^b
Zinc (mg/100 g)	Nd	nd	2.9 ± 0.3 ^c	0.3 ± 0.0 ^a	1.2 ± 0.8 ^b	1.5 ± 0.1 ^b
Potassium (mg/100 g)	167 ± 0.0 ^d	81 ± 0.2 ^b	182 ± 0.4 ^f	73 ± 0.2 ^a	147 ± 1.2 ^c	170 ± 0.9 ^e
Sodium (mg/100 g)	0.7 ± 1.0 ^a	1.0 ± 1.4 ^a	1.3 ± 1.4 ^b	0.8 ± 0.4 ^a	0.9 ± 0.6 ^a	1.1 ± 0.3 ^b

Values represent the mean ± standard deviation of three measurements. nd; not determined. Values within the row bearing the same superscripts are statistically insignificant at $P < 0.05$. RAW-F3: pasteurized formulation; UNST-F3: pasteurized but non-inoculated formulation; CA-F3: acidified formulation; AFI5-F3, GSL7-F3, SK8-F3: Formulations individually inoculated with 6 Log CFU/g of *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides*, and *Lacticaseibacillus paracasei*. Formulations were incubated at 30 °C for 24 h

0.3–2.9 mg/100 g, with CA-F3 exhibiting the highest content (2.9 ± 0.3 mg/100 g), followed by SK8-F3 (1.5 ± 0.1 mg/100 g), GSL7-F3 (1.2 ± 0.8 mg/100 g), and AFI5-F3 (0.3 ± 0.0 mg/100 g). Potassium levels varied between 73.0–182.0 mg/100 g across formulations, with AC-F3 yielding the highest concentration, followed by SK8-F3 (170.0 ± 0.9 mg/100 g). Sodium contents ranged from 0.7–1.3 mg/100 g, with AC-F3 presenting the highest value.

The mineral composition of the formulations reflects the potential of plant-derived complementary foods to support infant micronutrient requirements. Iron levels ranged from 1.0–5.1 mg/100 g across formulations, aligning with the range (1–6 mg/100 g) typically reported for plant-based complementary foods [54]. Iron is vital for infant neurodevelopment, supporting the synthesis of hemoglobin and facilitating early cognitive and psychomotor milestones [55]. Though not very high, its presence is significant for addressing iron-deficiency anemia, a common risk in early Life. Calcium contents varied between 10.5–14.2 mg/100 g, which is within expected ranges for cereal–legume–fruit formulations [54]. Even at these relatively modest levels, calcium contributes to bone mineralization, tooth development, and muscular function, making its inclusion in complementary foods critical for supporting infant growth. Zinc contents ranged from 0.3–2.9 mg/100 g, aligning well with the range generally observed in plant-derived complementary foods [54]. Zinc plays an essential role in immune function, cellular growth, and DNA synthesis, making it a priority micronutrient for infants [56]. Potassium levels (73–182 mg/100 g) were within the range commonly observed in fruits, most especially banana and tuber-based formulations [57]. As an electrolyte, potassium is vital for fluid balance, cardiovascular health, and neuromuscular activity in infants, supporting physiological stability and growth. Meanwhile, sodium levels remained low (0.7–1.3 mg/100 g), aligning with EFSA recommendations for infant nutrition [58], making the

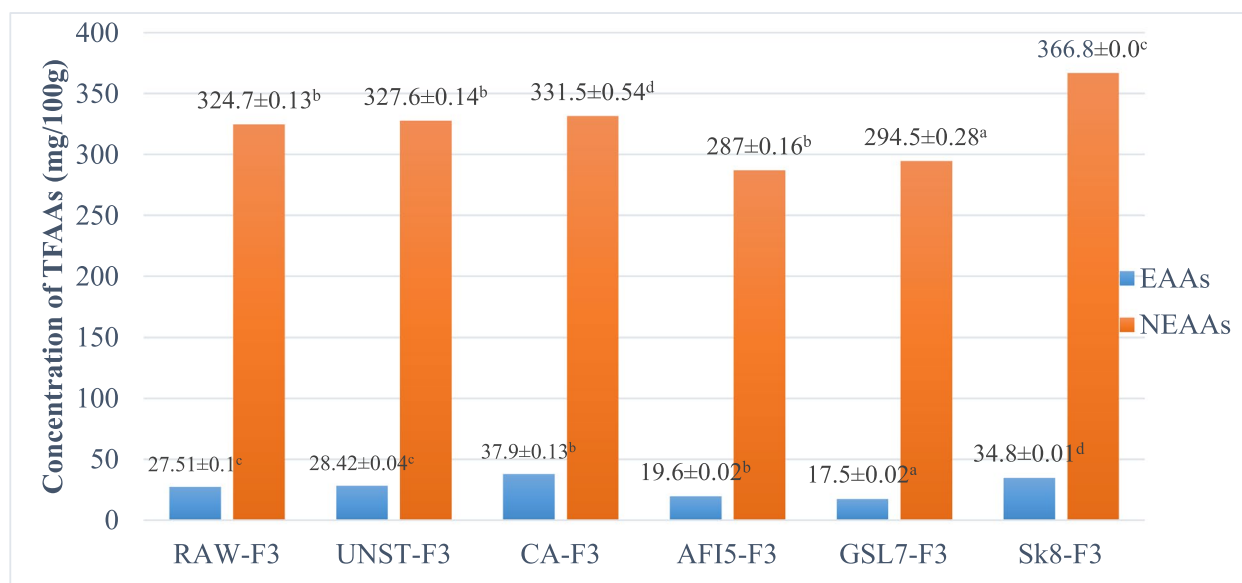
formulations suitable for early complementary feeding. Overall, while the mineral levels observed are modest, as is typical of plant-derived formulations, they can still make a valuable contribution to an infant's micronutrient intake. When combined with breastmilk or other complementary meals within a diverse feeding regimen, this formulation can help support the infant's mineral requirements.

Total free amino acid profile

Total free amino acids (TFAA) varied significantly ($P < 0.05$) across formulations, ranging from 306.8 ± 0.07 mg/100 g in GSL7-F3 to 401.6 ± 0.0 mg/100 g in SK8-F3. Among fermented formulations, SK8-F3 and AFI5-F3 retained higher TFAA levels compared to UNST-F3 (352.6 ± 0.1 mg/100 g) and CA-F3 (369.4 ± 0.3 mg/100 g) (Fig. 5). RAW-F3 specifically, essential amino acids (EAAs) such as histidine were consistently high in all formulations (15.1–17.1 mg/100 g), with SK8-F3 and UNST-F3 showing the highest concentrations. Methionine was absent in most formulations, but detectable in SK8-F3 (2.4 ± 0.0 mg/100 g) and CA-F3 (1.2 ± 0.0 mg/100 g). Lysine was only detected in RAW-F3 and CA-F3 (0.9 and 1.9 mg/100 g, respectively). Notably, GABA and ornithine, bioactive amino acids, were markedly higher in SK8-F3 (32.7 ± 0.0 and 32.2 ± 0.0 mg/100 g). Glutamic acid was detected only in RAW-F3 and CA-F3.

Observed variation in total free amino acids (TFAA) and individual amino acid profiles reflects the impact of fermentation and processing on protein hydrolysis and amino acid release. Fermented formulations, especially SK8-F3 and AFI5-F3, showed the highest TFAA levels, suggesting effective proteolytic activity by *L. paracasei* and *L. plantarum*, respectively. This enzymatic hydrolysis enhances protein digestibility and supports optimal growth and development in infants, particularly where plant-based complementary foods are used [59, 60]. Although reductions or undetectable levels of essential amino acids (EAAs) such as lysine and methionine

(a)



(b)

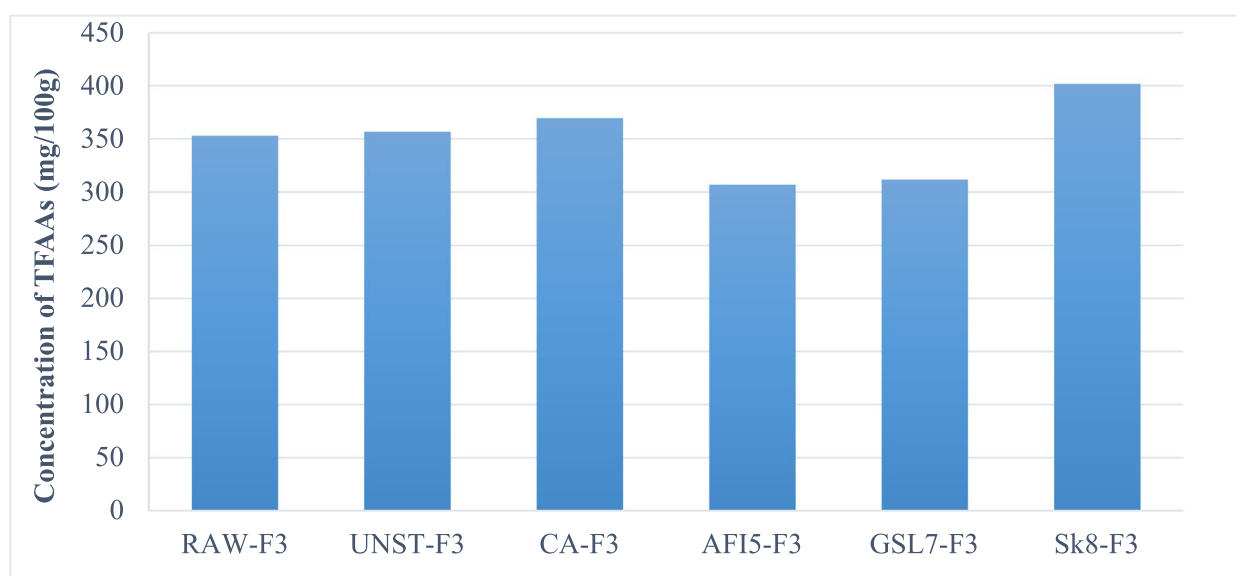


Fig. 5 (a) Quantification of essential amino acids (EAAs) and non-essential amino acids (NEAAs) found in each formulation. (b) Total free amino acids found in each formulation. Values represent the mean ± standard deviation of three measurements. Values bearing the same superscripts are statistically insignificant at $P < 0.05$: RAW-F3: pasteurized control; UNST-F3: incubated but non-inoculated control; the acidified control (CA-F3); and the formulations individually inoculated with 6 Log CFU/g of *Lactiplantibacillus plantarum* (AFI5-F3), *Leuconostoc mesenteroides* (GSL7-F3) and *Lactocaseibacillus paracasei* (SK8-F3), all incubated at 30 °C for 24 h

were observed in several inoculated formulations, the presence of these amino acids in CA-F3 and the notably elevated methionine levels in SK8-F3 suggest that both acidification and specific microbial metabolism may contribute to their retention. The decline in lysine content is likely attributable to Maillard-type reactions during heat

treatment or microbial utilization during fermentation [61]. In contrast, the significant accumulation of methionine in SK8-F3 may indicate strain-specific biosynthesis or resistance to degradation pathways [62]. Importantly, the elevated methionine concentration in SK8-F3 is of particular nutritional relevance for infant complementary

foods. Methionine is not only a critical sulfur-containing EAA required for protein synthesis and growth, but also a precursor for key metabolites involved in methylation reactions (e.g., S-adenosylmethionine), antioxidant defense (via cysteine and glutathione synthesis), and immune system development [62]. Its intake is vital during infancy, a period characterized by rapid tissue development and heightened vulnerability to oxidative stress. The significant increase in γ -aminobutyric acid (GABA) in inoculated formulations, particularly SK8-F3, indicates successful decarboxylation of glutamic acid, which was undetectable in those same formulations. GABA has known neuroprotective, hypotensive, and calming effects, and its presence enhances the functional profile of fermented infant foods [63]. The presence of ornithine, another bioactive compound involved in ammonia detoxification and gut health, further supports the value of fermentation in improving the functional quality of food, especially that meant for infants with developing digestive systems [64]. Overall, the free amino acid profile demonstrated that fermentation with specific LAB improves the nutritional and functional properties of complementary foods by increasing TFAA, enhancing bioactive amino acid production, and preserving some EAAs. This highlights the role of strain selection and fermentation optimization in designing safe, nutritionally enhanced, plant-based infant foods [65].

Low molecular weight peptide profile

Peptide sequences were identified and quantified using Proteome Discoverer 2.3 coupled with Matrix software

(version 6.0-SP4) to assess the impact of fermentation on protein hydrolysis, based on detectable ion signals. A total of 16,236 peptides, ranging from 5 to 33 amino acids in length, were identified across all formulations. Inoculated formulations exhibited the highest total peptide numbers (TPN), with GSL7-F3 yielding 3,352 peptides, AFI5-F3 yielding 3,417 peptides, and SK8-F3 yielding 2,697 peptides (Fig. 6). In contrast, non-inoculated formulations showed lower TPN: RAW-F3 (1,748), UNST-F3 (2,425), and CA-F3 (2,597) formulations. LAB-fermented formulations, particularly GSL7-F3 and AFI5-F3, were enriched in short-chain peptides (5–13 amino acids), with peak peptide numbers at 8AA (297 for GSL7-F3, 264 for SK8-F3) and 9AA (274 for AFI5-F3). The non-inoculated formulation had higher cumulative sum abundance (SA) values, particularly for longer peptides ($2.81\text{E}+09$ for RAW-F3 and $2.72\text{E}+09$ for UNST-F3 at 7AA), reflecting stronger ion signals from less hydrolyzed sequences.

The peptide analysis revealed that LAB fermentation significantly enhances protein hydrolysis, as evidenced by the higher TPN in inoculated compared to non-inoculated formulations. The predominance of short-chain peptides (5–13 amino acids) in LAB-fermented formulations, particularly GSL7-F3 and AFI5-F3, suggests that LAB strains possess robust proteolytic activity, likely driven by cell-envelope proteinases and intracellular peptidases that cleave proteins at specific sites [66, 67]. These enzymes facilitate the breakdown of complex proteins into smaller peptides, increasing peptide diversity and favoring shorter sequences, which were less

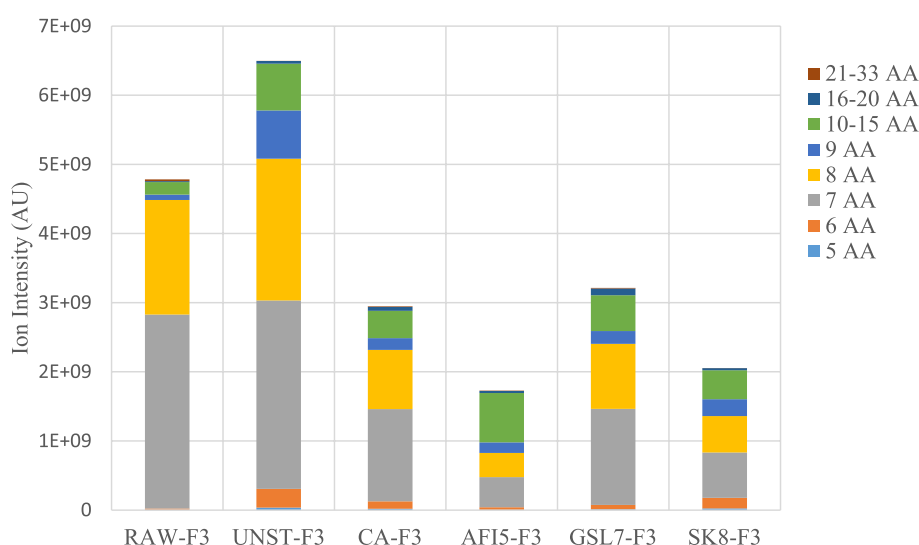


Fig. 6 Total ion intensities of peptides detected in low-molecular-weight water-soluble extracts of the pasteurized control (RAW-F3), non-inoculated control (UNST-F3), acidified control (CA-F3), and samples inoculated with 6 Log CFU/g of *Lactiplantibacillus plantarum* (AFI5-F3), *Leuconostoc mesenteroides* (GSL7-F3), and *Lactocaseibacillus paracasei* (SK8-F3), after incubation at 30 °C for 24 h

prevalent in the raw and unstarted formulations. The higher cumulative SA in non-inoculated formulations, particularly for longer peptides, is attributed to stronger ion signals from less hydrolyzed proteins, which may mask the detection of shorter peptides during mass spectrometry [68]. The enrichment of short-chain peptides in LAB-fermented formulations is particularly relevant for infant nutrition, where peptides in the 5–10 amino acid range are associated with enhanced bioactivity. Previous studies have demonstrated that such peptides improve mineral absorption (e.g., calcium and iron) by acting as chelators, thereby increasing bioavailability [69, 70]. Additionally, short peptides exhibit immunomodulatory properties, such as stimulating cytokine production, and possess antioxidant activity, which may support infant immune development and reduce oxidative stress [71, 72]. The increased peptide diversity in AFI5-F3 and GSL7-F3 suggests that these LAB strains could be leveraged to develop infant formulas with improved digestibility and reduced allergenicity, as smaller peptides are less likely to trigger immune responses compared to intact proteins [73]. This is particularly critical for infants with sensitivities to milk proteins, where hydrolyzed formulations can enhance tolerance and nutritional uptake [74]. The observed differences in peptide profiles highlight the potential of LAB fermentation to tailor protein hydrolysates for specific health benefits. Fermentation, particularly with *L. plantarum* and *L. mesenteroides*, significantly enhanced the production of short-chain peptides, which may enhance digestibility, reduce allergenicity, and promote beneficial gut microbiota in infants [73].

Free phenolic compounds profiling

High-performance liquid chromatography (HPLC) identified six free phenolic compounds across the test formulations: protocatechuic acid, procyanidin B2, caffeic acid, ferulic acid, resveratrol, and phloridzin. Their concentrations varied significantly ($P < 0.05$) among formulations. Fermented formulations (AFI5-F3, GSL7-F3, and SK8-F3) showed markedly higher levels of procyanidin B2, with the highest levels observed in SK8-F3 (69.4 ± 0.9 mg/kg) and AFI5-F3 (61.9 ± 1.8 mg/kg), compared to the pasteurized control (RAW-F3, 4.1 ± 0.0 mg/kg). Caffeic acid concentrations remained relatively stable (46.5–53 mg/kg) across all formulations. Ferulic acid peaked in AFI5-F3 (42.3 ± 0.1 mg/kg), significantly higher than UNST-F3 and CA-F3. Phloridzin was consistently retained across formulations, ranging from 38.2 to 40.2 mg/kg. Protocatechuic acid was only detected in UNST-F3, AFI5-F3, and SK8-F3 (~ 38.5 – 39.1 mg/kg), and resveratrol concentrations did not vary significantly (24.7–26.7 mg/kg), indicating a stable profile after fermentation.

Phenolic compounds, recognized for their antioxidant, anti-inflammatory, and neuroprotective potential, play a vital role in improving the functional quality of infant foods [75]. In this study, fermentation markedly enhanced the concentration and diversity of free phenolics, particularly in LAB-inoculated formulations, suggesting enzymatic hydrolysis by microbial tannase, esterases, and β -glucosidases that may increase the release of bound phenolics from the plant matrix [43]. Procyanidin B2 was significantly elevated in AFI5-F3 and SK8-F3 formulations, indicating the efficient depolymerization of apple-derived proanthocyanidins. This compound has been linked to gut barrier protection, immune modulation, and prebiotic effects in early life, contributing to improved gastrointestinal and systemic health in infants [76, 77]. Protocatechuic acid, which appeared only in fermented formulations, especially AFI5-F3 and SK8-F3, is known for its anti-inflammatory and neuroprotective properties and may help mitigate oxidative stress during infancy [78]. Ferulic acid, most pronounced in AFI5-F3, is another notable bioactive associated with neurodevelopmental support and cognitive protection through its antioxidant mechanisms [79, 80]. Although caffeic acid levels remained relatively stable across formulations, its known anti-inflammatory properties and capacity to modulate gut microbiota still offer potential benefits for gastrointestinal and immune system development in infants [81]. Resveratrol, a polyphenol with anti-aging and cardioprotective functions, showed no significant changes across formulations, suggesting its thermostability and preservation through fermentation [82]. While most evidence for its benefits comes from adult studies, its immunomodulatory and antioxidant effects may also support gut and immune maturation in infants. Phloridzin, a flavonoid glycoside abundant in apples, was consistently retained across all formulations and may help modulate glucose uptake and reduce metabolic stress in infants during growth [83]. Overall, the observed enrichment of procyanidin B2, ferulic acid, and protocatechuic acid in fermented formulations, particularly AFI5-F3 and SK8-F3, underscores the ability of lactic acid fermentation to enhance the functional properties of complementary infant foods.

Total polyphenol content and dpph scavenging activity

The total polyphenol content (TPC) of the formulations ranged from 157.2 ± 52.9 mg/100 g in CA-F3 to 267 ± 52.5 mg/100 g in GSL7-F3. Among the formulations, GSL7-F3 exhibited the highest TPC, significantly ($P < 0.05$) exceeding values recorded in RAW-F3 (177.3 ± 45.7 mg/100 g), UNST-F3 (197 ± 61.3 mg/100 g), and the inoculated formulations (Table 4). For antioxidant activity, measured as DPPH radical scavenging

Table 4 Free Phenolic Compounds Quantification

Compound (mg Kg ⁻¹)	RAW-F3	UNST-F3	CA-F3	AFI5-F3	GSL7-F3	SK8-F3
Protocatechuic acid	nd	39.0 ± 0.1 ^a	nd	39.2 ± 0.1 ^a	nd	38.5 ± 0.0 ^a
Procyanidin B2	4.1 ± 0.0 ^a	56.3 ± 0.9 ^c	28.8 ± 0.1 ^b	61.9 ± 1.8 ^c	33.2 ± 0.9 ^b	69.4 ± 0.9 ^c
Caffeic acid	51.7 ± 0.1 ^a	52.7 ± 0.0 ^a	46.5 ± 1.1 ^a	50.7 ± 0.3 ^a	52.3 ± 0.1 ^a	53.0 ± 0.1 ^a
Ferulic acid	40.0 ± 0.0 ^{ab}	38.6 ± 0.1 ^a	38.7 ± 0.1 ^a	42.3 ± 0.1 ^b	39.9 ± 0.0 ^{ab}	41.4 ± 0.0 ^{ab}
Resveratrol	26.7 ± 0.0 ^a	24.8 ± 0.3 ^a	24.7 ± 0.2 ^a	26.5 ± 0.1 ^a	26.5 ± 0.0 ^a	26.6 ± 0.0 ^a
Phloridzin	39.8 ± 0.0 ^{ab}	40.1 ± 0.0 ^b	40.1 ± 0.0 ^b	38.2 ± 0.2 ^a	39.4 ± 0.0 ^{ab}	40.2 ± 0.0 ^b

Values represent the mean ± standard deviation of three measurements. Values within the row bearing the same superscripts are statistically insignificant at $P < 0.05$, nd: not determined. RAW-F3: pasteurized formulation; UNST-F3: pasteurized but non-inoculated formulation; CA-F3: acidified formulation; AFI5-F3, GSL7-F3, SK8-F3: Formulations individually inoculated with 6 Log CFU/g of *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides*, and *Lacticaseibacillus paracasei*. The incubation was carried out at 30 °C for 24 h

activity, values ranged from 40.32% in RAW-F3 to 61.3% in AFI5-F3. Among all the formulations, AFI5-F3 and SK8-F3 (59.7%) demonstrated the highest DPPH scavenging capacities, while RAW-F3 exhibited the lowest DPPH scavenging activity.

The assessment of total polyphenol content (TPC) and DPPH radical scavenging activity in the formulated infant foods provides valuable insights into the antioxidant properties of these formulations. Phenolic compounds are well-recognized for their role in neutralizing free radicals and mitigating oxidative stress, a consideration of utmost importance for infants due to the vulnerability of their developing physiological and immunological systems [84]. In this study, the inoculated formulations exhibited higher TPC compared to the controls, with GSL7-F3 recording the highest concentration, followed closely by SK8-F3. This enhancement is attributed to the enzymatic activity of LAB, which can hydrolyze bound phenolic compounds from plant cell walls, releasing them into more bioavailable forms [84]. Similar findings have been reported in other studies, which highlight the ability of fermentation to liberate and increase the concentration of phenolic compounds and other bioactive metabolites [85]. Interestingly, despite GSL7-F3 exhibiting the highest TPC, its DPPH radical scavenging activity was lower than that of some other inoculated formulations. This discrepancy suggests that while fermentation increased the total quantity of phenolic compounds, the structural characteristics of these compounds, such as

degree of polymerization and specific functional groups, play a pivotal role in determining their antioxidant efficacy [86]. The structure–activity relationship of polyphenols is well established, wherein low-molecular-weight phenolic acids often display higher radical scavenging activity compared to more complex polymeric forms [87]. LAB fermentation not only increased the overall polyphenolic content but also enhanced the bioavailability of specific phenolic acids, including caffeic acid, ferulic acid, protocatechuic acid, and phloridzin, which were detected at higher levels in inoculated formulations (Table 5). These compounds have been linked with a range of biological benefits beyond antioxidant activity, including anti-inflammatory, immunomodulatory, and neuroprotective effects, making their presence in infant foods particularly significant [75]. Considering that oxidative stress has been implicated in adverse infant outcomes such as impaired immunity, chronic inflammation, and neurodevelopmental disturbances [88], the inclusion of antioxidant-rich ingredients in infant complementary foods may offer long-term benefits. Moreover, the positive correlation observed between TPC and DPPH activity across the LAB-inoculated formulations highlights the efficacy of fermentation as a means of both enriching the quantity of bioactive phenolic compounds and preserving their functional properties. This combined improvement in polyphenolic content and antioxidant activity underscores the value of utilizing plant-based ingredients and LAB fermentation in the formulation of infant

Table 5 Total Polyphenol Content and DPPH Scavenging Activity of Formulations

	RAW-F3	UNST-F3	CA-F3	AFI5-F3	GSL7-F3	SK8-F3
Total phenols (mg/100 g)	177.3 ± 46 ^b	197.0 ± 61 ^c	157.2 ± 53 ^a	166.0 ± 66 ^{ab}	267.0 ± 53 ^d	195.0 ± 37 ^c
DPPH SA. (%)	40.3 ± 0.0 ^a	50.0 ± 0.0 ^c	45.2 ± 0.0 ^b	61.3 ± 0.1 ^d	46.8 ± 0.0 ^b	59.7 ± 0.8 ^c

Values represent the mean ± standard deviation of three measurements (n = 3). Values within the row bearing the same superscripts are statistically insignificant at $P < 0.05$. RAW-F3: pasteurized formulation; UNST-F3: pasteurized but non-inoculated formulation; CA-F3: acidified formulation; AFI5-F3, GSL7-F3, SK8-F3: Formulations individually inoculated with 6 Log CFU/g of *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides*, and *Lacticaseibacillus paracasei*. The incubation was carried out at 30 °C for 24 h

complementary foods, aligning with global efforts to optimize nutrition and health during early life.

Anti-nutrient profiling

The levels of phytic acid, saponins and raffinose are presented in Table 6. Phytic acid levels ranged from 0.40 to 1.90 g/100 g across formulations, with the lowest value observed in SK8-F3 (0.40 ± 0.3 g/100 g), which was significantly lower than the other formulations ($P < 0.05$). Saponins ranged from 0.05 to 0.14 g/100 g, with CA-F3 recording the lowest value (0.05 ± 0.0 g/100 g), followed by SK8-F3 (0.07 ± 0.0 g/100 g), AFI5-F3 and GSL7-F3 (0.08 g/100 g), and UNST-F3 (0.12 g/100 g). Fructans ranged from 0.05 to 0.41% w/w, with AC-F3 exhibiting the lowest content (0.05%), followed by AFI5-F3 (0.26%), SK8-F3 (0.25%), GSL7-F3 (0.33%), UNST-F3 (0.36%), and RAW-F3 (0.41%). Raffinose contents were relatively low across formulations, ranging from 0.02 to 0.03 g/100 g, with SK8-F3 presenting the lowest value (0.02 g/100 g) and the rest of the formulations not differing significantly ($P > 0.05$).

Phytic acid, saponins, and fermentable oligosaccharides (FODMAPs) are common anti-nutritional factors in plant-derived complementary foods that can limit mineral bioavailability, protein digestibility, and gastrointestinal tolerance in infants. In this study, fermentation with selected LAB strains effectively reduced these anti-nutritional compounds across the formulations, yielding a formulation more suitable for infant consumption. Phytic acid, a strong mineral chelator, was significantly reduced in the SK8-F3, reaching 0.40 g/100 g; a marked decrease compared to the pasteurized and non-inoculated formulation. This is consistent with earlier findings that *L. paracasei* strains produce robust phytase activity, aiding in the breakdown of phytic acid and releasing bound micronutrients [89]. Similar studies have reported up to an 80% reduction in phytate after LAB fermentation, enhancing the bioavailability of essential micronutrients such as iron, zinc, and calcium, which are critical for infant growth and cognitive development [90]. Saponin

levels, while generally low across the formulations, were significantly decreased by acidification and LAB fermentation, with the acidified formulation yielding the lowest saponin content (0.05 g/100 g). This finding supports evidence that acid and lactic acid fermentation can hydrolyze the complex glycosidic linkages in saponins, thereby reducing their potential to cause gastrointestinal discomfort and bitter taste [91]. Reduced saponin levels have been associated with improved protein digestibility and palatability, making the formulations more acceptable for infant feeding. Similarly, the levels of fructans and raffinose, both of which are fermentable oligosaccharides implicated in bloating and discomfort in infants, were significantly decreased following fermentation. The acidified formulation (0.05%) and the inoculated formulations (0.25–0.33%) demonstrated the most notable reductions in fructan content. The low raffinose content observed across formulations, especially in the SK8-F3 (0.02 g/100 g), suggests that this strain may release α -galactosidase, an enzyme responsible for hydrolyzing raffinose and related oligosaccharides [92]. Reduced FODMAP levels have been linked to improved gut comfort and a lower risk of colic and bloating in infants, making the fermented formulations especially advantageous for this sensitive demographic [93].

Sensory evaluation

As shown in Fig. 7, sensory evaluation revealed that SK8-F3 had the most acceptable sensory profile, with high scores for flavor (8.0), sweetness (7.2), and texture (8.3). It was followed by AFI5-F3, which received the second-highest score.

Fermentation significantly influenced key sensory attributes such as flavor, sweetness, sourness, texture, astringency, aftertaste, and overall acceptability. Among the LAB-fermented formulations, SK8-F3 consistently received the highest scores. The superior sensory performance of the SK8 (*L. paracasei*) may be attributed to its elevated proteolytic activity, which facilitates the release of bound glycosides, free amino acids, and

Table 6 Quantification of Anti-nutritional Factors

	RAW-F3	UNST-F3	CA-F3	AFI5-F3	GSL7-F3	SK8-F3
Phytic A (g/100 g)	1.63 ± 0.0^b	1.71 ± 0.0^{bc}	1.90 ± 0.1^{bc}	1.75 ± 0.1^{bc}	1.76 ± 0.3^{bc}	0.40 ± 0.3^a
Saponins (g/100 g)	0.14 ± 0.0^e	0.12 ± 0.0^d	0.05 ± 0.0^a	0.08 ± 0.0^c	0.08 ± 0.0^c	0.07 ± 0.0^b
Fructans (%w/w)	0.41 ± 0.1^b	0.36 ± 0.2^b	0.05 ± 0.0^a	0.26 ± 0.0^{ab}	0.33 ± 0.1^b	0.25 ± 0.1^{ab}
Raffinose (g/100 g)	0.03 ± 0.0^b	0.03 ± 0.0^b	0.03 ± 0.0^b	0.03 ± 0.0^b	0.03 ± 0.0^b	0.02 ± 0.0^a

Values represent the mean $\pm$ standard deviation of three measurements. Values within the row bearing the same superscripts are statistically insignificant at $P < 0.05$. RAW-F3: pasteurized formulation; UNST-F3: pasteurized but non-inoculated formulation; CA-F3: acidified formulation; AFI5-F3, GSL7-F3, SK8-F3: Formulations individually inoculated with 6 log CFU/g of *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides*, and *Lacticaseibacillus paracasei*. The incubation was carried out at 30 °C for 24 h

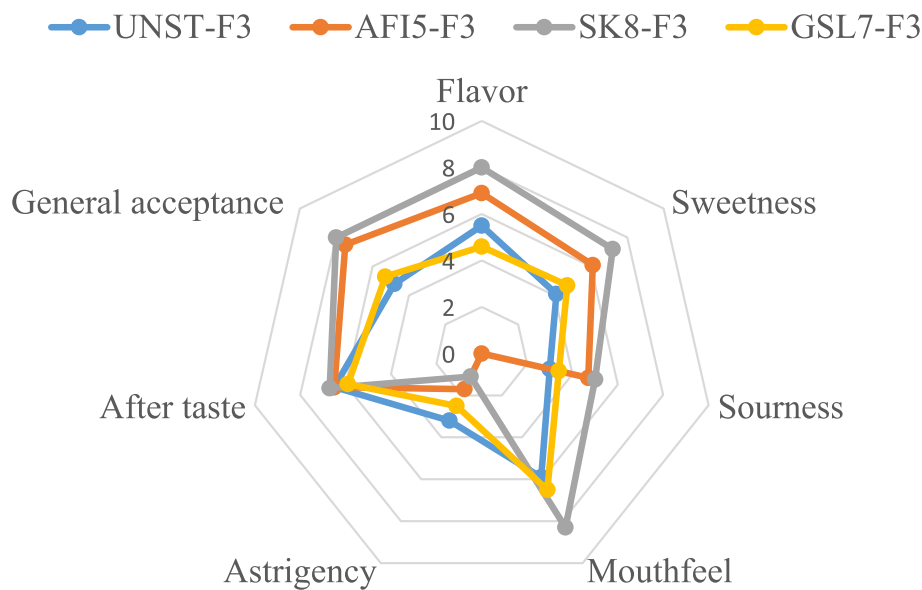


Fig. 7 Web plot of means of sensory evaluation scores of formulations incubated at 30 °C for 24 h. UNST-F3 (pasteurised but non-inoculated formulation) and GSL7-F3, AFI5-F3, and SK8-F3 formulations inoculated with 6 Log CFU/g of *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides*, and *Lacticaseibacillus paracasei*

peptides, which are compounds known to enhance flavor complexity, umami notes, and mild sweetness [67]. Fermentation with LAB under controlled conditions is well-documented for improving the organoleptic qualities of fermented foods by generating bioactive metabolites and modifying texture and taste profiles [94], which generally improves organoleptic properties.

Conclusion

This study developed a nutrient-dense, plant-based complementary food using melon seeds, yellow yams, banana, and apple, with enhanced nutritional and functional properties achieved through lactic acid fermentation. Generally, fermentation with *Lacticaseibacillus paracasei* (SK8), *Leuconostoc mesenteroides* (GSL7), and *Lactiplantibacillus plantarum* (AFI5) enriched the formulations with essential amino acids, potential bioactive peptides, and antioxidants, while reducing anti-nutritional factors. In specific, SK8-F3 fermented with *Lacticaseibacillus paracasei* showed the most favorable nutritional and functional profile, demonstrating the potential of controlled fermentation to produce high-quality, locally sourced complementary foods for infant nutrition, especially in low-resource or dairy-limited settings.

Authors' contributions

Tata Tatiana Munghang performed experiments, collected data, and drafted the manuscript. Noumo Ngangmou Thierry developed the processing methods for *Dioscorea dumetorum* flour, contributed to the infant formula framework, and reviewed the manuscript. Chiara Virretto: Supported laboratory analysis. Ali Zein Alabiden Tlais: Oversaw laboratory procedures and reviewed

the manuscript. Andrea Polo: Contributed to the conceptual development of the infant formula and reviewed the manuscript. Marco Gobetti: Facilitated the research visit at the Free University of Bozen-Bolzano, offered institutional support and scientific insights, and reviewed the final manuscript. Ejoh Aba Richard: Acted as principal academic supervisor, guided the research development, and provided critical revisions of the manuscript.

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Data availability

All relevant data generated or analyzed during this study can be obtained from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted in both Cameroon and Italy, following the ethical principles and research standards applicable in each country. Although formal ethical clearance was not sought, all research activities were carried out responsibly, with careful attention to safety, confidentiality, and scientific integrity. For the sensory evaluation involving adult participants, informed consent was obtained following a clear explanation of the study's purpose. Participation was entirely voluntary, and anonymity and confidentiality were ensured throughout the process.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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