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Formulation and evaluation of probiotic starter culture: impact on Ethiopian cottage cheese "*Ayib*" safety, stability, sensory acceptability and antioxidant potential

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Abstract

Background *Ayib* is a traditionally processed dairy product in Ethiopia that demonstrates significant variability in shelf life, sensory attributes, and safety, primarily own to the spontaneous fermentation of milk and differing household practices. This study aimed to develop mixed probiotic starter cultures from top seven previously isolated lactic acid bacteria to achieve a synergistic effect on sensory qualities consistent, enhanced safety, extended storage stability, and antioxidant potential.

Methods Nine mixed starter cultures were formulated using seven lactic acid strains that are known for their superior fermentation and probiotic capabilities. Pasteurized milk was inoculated with 5% of each starter culture and incubated at 37 ± 2 °C for 8 h. Fermented milk was then defatted by shaking at 100 rpm for 1 h. Following fat removal, buttermilk was heated to 50–60 °C for 40–50 min to facilitate curd (*Ayib*) formation. After cooling, the curd was separated from whey. A 200-g portion of the curd was wrapped in sterile cheesecloth and immersed in pasteurized whey inoculated with 8 log CFU/mL of the formulated starter cultures for 30 min before being re-drained for 1 h.

Results The physicochemical properties, consumer acceptability, and storage stability of the resulting products were evaluated, revealing total solids ranging from 20.67 to 22.89%, pH values between 3.89 and 4.49, and titratable acidity ranging from 0.63 to 0.93%. Sensory evaluation, conducted using a five-point hedonic scale, showed overall acceptability scores ranging from 3.31 for *Ayib* treated with (F9) to 4.03 for Ayib treated with (F2). Remarkably, the storage stability of the treated *Ayib* was enhanced by 2–9 times compared to the control sample. The antioxidant analysis demonstrated that among the isolates, the *Lactobacillus curvatus* (NZ-44) exhibited the highest individual antioxidant activity of 57.77%. Furthermore, the formulated mixtures, particularly (F6), displayed synergistically enhanced antioxidant activity of 99.27%.

Conclusions These findings suggest that lactic acid bacteria strains can improve the nutritional value, safety, and storage stability of fermented dairy products, such as *Ayib*, with potential applications in both the food and pharmaceutical industries.

Keywords *Ayib*, Probiotic, Bioprotective, Formulation, Antioxidant potential

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Introduction

Fermentation is an essential process in dairy production, in which lactose is metabolized into lactic acid by lactic acid bacteria (LAB), profoundly influencing the texture, flavor, and nutritional profile of the resulting dairy products [1]. LAB strains such as *Lactobacillus delbrueckii* subsp. bulgaricus, *Lactobacillus acidophilus*, and *Streptococcus thermophilus* are pivotal in this process, they haverole of extending the shelf life through acidification and spoilage prevention, enhancing the bioavailability of nutrients, such as vitamin B12 and calcium [2, 3]. Moreover, these microorganisms can synthesize bioactive compounds, including exopolysaccharides, which are beneficial for gut health and immune functions [4, 5].

In Ethiopia, a country celebrated for its rich culinary diversity, fermented milk products, such as ergo and *Ayib*, are dietary staples, reflecting a unique cultural heritage. *Ayib*, an Ethiopian version of cottage cheese, is particularly valued across various ethnic groups for its nutritional benefits and versatility in local cuisine. It is traditionally made by churning fermented milk to remove fat, followed by heating buttermilk to form curds. Cheese is often seasoned with spices, salt, and herbs, catering to a wide array of taste preferences.

However, the microbiological safety and stability of traditional *Ayib* are concerns, especially when produced under unsanitary conditions, leading to high microbial loads and potential contamination and spoilage within 2–3 days of storage under ambient conditions [6–8]. Studies have indicated the presence of significant levels of bacteria and pathogens in *Ayib* from local markets, emphasizing the need for enhanced hygiene and quality control in *Ayib* production to ensure consumer safety.

In cheese production, the use of well-defined LAB starter cultures has become a cornerstone, enabling controlled fermentation and consistent cheese quality [9]. These cultures have replaced unpredictable natural fermentation to ensure uniformity in cheese production. LAB starter cultures produce various metabolites with antimicrobial properties, which not only prolong the shelf life of dairy products but also offer health benefits. Fermentation using defined microorganisms can offer a promising solution for enhancing stability, antioxidant properties, nutritional value, taste, and aroma [10, 11].

This study aimed to develop probiotic LAB starter cultures capable of improving the shelf life, sensory qualities, and safety of Ayib and confer health benefits to consumers.

Materials and methods

Probiotic lactic acid bacteria isolates

In this study, seven previously isolated lactic acid bacteria (LAB) strains with potential probiotic properties were utilized. The strains were originally isolated from traditionally fermented milk (ergo) collected from diverse agro-ecological zones of Ethiopia and characterized for their probiotic potential, fermentation capabilities, and antimicrobial activities. The lactic acid bacteria (LAB) strains utilized in the study included *Lacticaseibacillus rhamnosus* (GB-15), *Lacticaseibacillus paracasei* (SB-7), *Limosilactobacillus reuteri* (G-23), *Limosilactobacillus sakei* (BB-60), *Lactiplantibacillus curvatus* (NZ-44), *Lactiplantibacillus plantarum* (NN-33), and *Lacticaseibacillus casei* (BZ-26). These isolates were obtained from the Ethiopian National Agricultural Biotechnology Research Center (NABRC) and the Holeta Dairy Research Laboratory, where their probiotic attributes had been previously characterized [12].

Compatibility testing of the isolates

To establish bioprotective consortia, the compatibility of the individual probiotic isolates was evaluated. A cross-streaking method was employed in which each isolate was streaked against every other isolate on MRS agar plates. The plates were then incubated at 37 °C for 48 h and the growth patterns of the isolates were observed after 48 h of incubation [13–16]. Isolates exhibiting the best compatibility were selected for inclusion in mixed starter culture formulations.

Starter culture formulation and standardization

Each LAB species was cultured on MRS agar plates and incubated under anaerobic conditions at 37 °C for 24 h. The mass spectrometer was calibrated to zero using a sterile liquid whey blank filtered through a 0.45 µm filter. Loopfuls of individual colonies were inoculated into 500 mL of sterile liquid whey following the species composition outlined in Table 1. The resulting mixtures were manually combined. Subsequently, these formulations were standardized to a concentration of 6×10^8 colony-forming units per mL (CFU/mL) using a 2 McFarland standard, which corresponds to an absorbance of 0.31 at 600 nm [12, 17]. Nine different starter culture combinations were prepared, each with varying proportions of the individual LAB species (Table 1).

Preparation of *Ayib*

Ayib was produced following the method described by [8, 18]. Two liters of pasteurized milk were inoculated with 5% of each starter culture and incubated at 37 ± 2 °C for 8 h. The fermented milk was then defatted by shaking at 100 rpm for 1 h. The floating fat was removed using a sterile spoon. The defatted fermented milk was heated in a water bath at 50 °C for 55 min. The resulting curd (*Ayib*) was separated from the whey after cooling to room temperature and was filtered through a sterile cheesecloth.

Table 1 Composition of LAB isolates and total percent of inoculant

Formulates	LAB Isolates							
	GB-15	SB-7	G-23	BB-60	NZ-44	BB-33	BZ-26	Total inoculum (%) (v/w)
F 1	-	1.00	1.50	1.00	-	-	1.50	5
F 2	1.50	-	1.00	-	1.00	1.50	-	5
F 3	0.75	0.75	-	2.00	-	1.50	-	5
F 4	0.75	0.75	1.50	-	0.50	0.50	1.00	5
F 5	0.50	0.50	1.50	0.50	-	0.50	1.5	5
F 6	1.5	-	-	1.00	2.00	-	0.5	5
F 7	-	2. 50	0.50	1.50	-	0.50	-	5
F 8	0.5	0.50	1.00	-	0.50	2.5	-	5
F 9	1.00	1.00	1.50	-	-	-	1.50	5

Where: *F1* = (SB-7, G-23, BB-60, BZ-26); *F2* = (GB-15, G-23, NZ-44, BB-33); *F3* = (GB-15, SB-7, BB-60, BB-33); *F4* = (GB-15, SB-7, G-23, NZ-44); *F5* = (GB-15, SB-7, G-23, BB-60, BB-33, BZ-26); *F6* = (GB-15, BB-60, NZ-44, BZ-26); *F7* = (SB-7, G-23, BB-60, BB-33); *F8* = (GB-15, SB-7, G-23, NZ-44, BB-33); *F9* = (GB-15, SB-7, G-23, BZ-26); and GB-15 = *L. rhamnosus (GG);*SB-7 = *L. paracasei;* G-23 = *L. reuteri;* BB-60 = *L. sakei*(23K); NZ-44 = *L. curvatus;* BB-33 = *L. plantarum;*BZ-26 = *L. casei*

Treatment of *Ayib* with starter culture formulations

Two hundred grams of *Ayib* were wrapped in sterile cheesecloth and immersed in pasteurized whey inoculated with standardized starter cultures for 30 min to recover the inactivated culture during heating and the final count of the culture is 3×10^6 CFU/g. Finally, the *Ayib* was re-drained for 1 h to minimize the moisture content.

Organoleptic evaluation of *Ayib*

Nine *Ayib* products treated with different starter culture formulations and one control (untreated *Ayib*) were subjected to sensory evaluation. The samples were randomized, coded with three-digit numbers, and served at 7–10 °C in individual plastic cups. A sensory evaluation form with five attributes (color, flavor, odor, texture, and overall acceptability) was provided to each panelist.

Ten semi-trained adult panelists (male and female) participated in the sensory assessments. The panelists were instructed to rinse their mouths with water between samples [19]. A five-point hedonic scale was used for evaluation, with 1 representing "dislike extremely" and 5 representing "like extremely."

Storage stability estimation

Ayib products were stored at ambient temperature (18–24 °C) and periodically evaluated for sensory attributes. The overall acceptability cut-off point was set at 3.5, as suggested by Mahendradatta et al. [20].

Sensory storage stability was predicted using a linear regression analysis of overall acceptability versus storage time and a first-order kinetic reaction model. The natural logarithm of the overall acceptability was plotted against storage time to determine the rate constant (K). Using K, the initial overall sensory acceptability (A0), and the quality limit (At), the storage stability was calculated based on the Arrhenius first-order reaction equation, as described by Mahendradatta et al. [20].

$$\text{Storage stability}\left(\text{in days}\right) = \frac{\text{InA0/At}}{\text{K}}$$

Where: Ao = natural log of initial (1st day overall sensory mean score).

A_t = quality limit (Cutoff point = 3.5), K = rate constant.

Proximate composition and physicochemical analysis

Determination of crude protein content

The crude protein content was determined using the macro Kjeldahl method, as outlined in [21]. Two grams of each sample were weighed into a digestion flask. Ten grams of copper sulfate and sodium sulfate (5:1 ratio) and 25 mL of concentrated sulfuric acid were added to the flask. The flask was placed in a digestion block in a fume hood and heated until frothing ceased, and a clear, light-blue color was obtained. The mixture was then cooled and diluted with distilled water to a final volume of 25 mL in a volumetric flask. Ten milliliters of the diluted mixture was transferred to a distillation apparatus and 10 mL of 40% sodium hydroxide was added. The released ammonia was then trapped in a boric acid solution. The boric acid solution was titrated with 0.02 M hydrochloric acid until the color changed from green to purple. The percentage of nitrogen in the sample was determined and the protein content was calculated using the conversion factor described by Eshetu and Asresie [22].

$$\%N = \frac{14.007 \times V \times N}{W} \times 100$$

Where: % N = % of nitrogen by weight, V = the volume of HCl used for titration, N = normality of HCl used, W = weight of sample used, 14.007 = atomic weight of nitrogen

$$\text{Crude Proteien} = \%\,\text{Nitrogen} * 6.38$$

Where: 6.38 is the nitrogen-to-protein conversion factor.

Fat content determination

The fat content of the samples was determined using a modified Soxhlet extraction method, as described previously [23]. Two grams of the sample were accurately weighed and placed in a Soxhlet thimble. The thimble was then inserted into the extraction apparatus. Diethyl ether was used as the extraction solvent, and the extraction process was performed in the temperature range of 40–60 °C for 8 h to ensure complete fat extraction. Following the extraction, the solvent was removed by evaporation. The remaining lipid residue was dried in an oven at 80 °C for 30 min to remove residual solvent. The dried flask was cooled in a desiccator and weighed to determine the weight of extracted lipids. The percentage of fat in the original sample was calculated using the following standard gravimetric formula.

$$\%\text{Fat} = \frac{\text{Weight of Extracted fat}}{\text{Weight of sample used}} \times 100$$

Determination of total solid contents

The total solid content of the samples was determined according to the previously described method [24]. A 3-gram sample was accurately weighed into a pre-dried tapered crucible. The crucible and the sample were then placed in an oven at 100 ± 2 °C and dried to a constant weight. The drying process was repeated at 30-minute intervals until no significant change in weight was observed. Once a constant weight was attained, the total solids in the sample were calculated using the following equation.

$$\text{Total Solid } \% = \frac{W2 - W1}{W} \times 100$$

Where: W_1 is the weight of the empty crucible, W is the initial weight of the sample, and W_2 is the final weight of the crucible + dried sample.

Determination of ash contents

The ash content was determined following Nielsen and Ismail [25], specifically using the direct heating method, as outlined in the literature. In this procedure, 3 g of each sample was accurately weighed and placed in a preweighed crucible. The samples were subsequently incinerated in a muffle furnace at 550 °C for 5 ± 1 h to ensure complete conversion of the samples to ash. After incineration, the crucibles containing the resultant ash were allowed to cool in a desiccator to prevent atmospheric moisture absorption. After cooling, the weight of the crucible containing the ash was recorded. The ash contents of the samples were calculated using the following formula:

$$\text{Ash } \% = \frac{W2 - W1}{W} \times 100$$

Where: W1 is the weight of the crucible, W is the initial weight of the sample, and W_2 is the weight of the crucible + dried sample.

Determination of the pH

The pH content was determined in accordance with the method of Karastogianni et al. [26], employing the potentiometric method for accurate measurements. This method relies on the detection of the potential difference between the sample and the electrolyte solution contained within the electrode of the pH meter. A digital pH meter (HI 2483; Hanna Instruments, Italy) was used for analysis. Prior to measurement, the pH meter was calibrated using fresh standard buffer solutions of pH 4.0 and 7.0, to ensure measurement accuracy. Following calibration, the electrode of the pH meter was directly immersed in the *Ayib* sample, which was prepared for consumption. pH readings were recorded to provide a precise indication of the acidity or alkalinity of the sample. This method adheres to established standards, ensuring the reliability and reproducibility of the pH measurements.

Determination of the titratable acidity

Titratable acidity was measured using the titration method described in [27]. This method determines the lactic acid content of the product, by titrating a sample with a 0.1 N sodium hydroxide (NaOH) solution. For this assay, a 1:9 (m/v) mixture of Ayib (10 g of sample) and distilled water (90 mL) was prepared to ensure proper homogenization for accurate titration. This mixture was then titrated with standardized NaOH (0.1 N), using three drops of 0.1% phenolphthalein as an indicator. Titration was continued until a faint pink color persisted,

indicating an endpoint at pH 8.2 [28]. The titratable acidity produced during fermentation was calculated using the following question.

$$\text{Titratableacidity}(\%) = \frac{(\text{Volume of NaOH used}(ml) \times \text{Normality of NaOH} \times 90\ \text{g/mol})}{\text{Weight of sample}\ (\text{g})} \times 100$$

Where: 90 g/mol is the equivalent weight of lactic acid.

Antagonistic activity of lactic acid bacteria (LAB)

The antagonistic activity of lactic acid bacteria (LAB) was assessed using the well-diffusion method on Mueller-Hinton agar (MHA) as described in [29, 30]. This method allows for the evaluation of the inhibitory effects of LAB against common foodborne pathogens and spoilage bacteria.

Test organisms

The following standard strains of food-borne pathogens were used to evaluate the antagonistic potential of formulates.

Escherichia coli ATCC 43,895.
Salmonella Typhimurium ATCC 14,028.
Listeria monocytogenes ATCC 15,313.
Staphylococcus aureus ATCC 25,923.

These organisms are major foodborne pathogens and spoilage bacteria.

Preparation of cell-free supernatants

All seven probiotic species were streaked on MRS agar plates and incubated at 37 °C for 24 h. From fresh cultures, a loopful of each LAB species was inoculated into 150 mL MRS broth and incubated at 37 °C for 72 h. Following incubation, the broth cultures were centrifuged at 12,000 rpm for 15 min to separate the cells, and the supernatants were collected in screw-cap tubes. The resulting cell-free supernatants were sterilized via membrane filtration using filters with a pore size of 0.45 µm [31, 32].

Well preparation and standardization of test organisms

The test organisms were cultured on Brain Heart Infusion (BHI) agar for 24 h at 37 °C. A loopful of the cultured colonies was transferred to 0.85% saline solution and standardized to a concentration of 10^6 CFU/mL using a 0.5 McFarland standard of 600 nm [33, 34].

Using a sterilized cotton swab, the standardized test organism suspension was evenly spread across the surface of pre-prepared and solidified Mueller-Hinton agar plates. After allowing the plates to dry, a sterile cork borer (6 mm diameter) was used to create uniform wells in the agar [31, 35].

Each well was filled with 100 µL cell-free supernatant from each LAB species. The plates were incubated aerobically at 37 °C for 48 h. The zones of inhibition surrounding each well were measured in millimeters using calipers to assess the antagonistic activity of the LAB species against the test organisms.

Antioxidant activity

DPPH radical scavenging activity

To assess DPPH radical scavenging activity, each isolate and formulation was inoculated into sterile MRS broth separately and incubated for 24 h at 37 °C anaerobically. MRS broth without the inoculum was used as the control. For each inoculated and control broth sample, 500 µL of the supernatant was combined with 500 µL of DPPH solution (100 µmol/L). The reaction mixture was then incubated at 25 °C in the dark for 30 min. Subsequently, the mixture was centrifuged at 6000 × g for 10 min. The absorbance of the supernatant was measured at 517 nm using a UV-visible spectrophotometer following the methodology described in [36]. DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity}\ (\%) = 1 - \frac{A - B}{C} \times 100$$

Where: A = absorbance of sample; B = absorbance of the blank group; C = absorbance of the control group.

Statistical analysis

The data were analyzed using R statistical software. Normality of continuous variables was assessed using the Shapiro-Wilk test and significant differences among treatments were analyzed using one-way ANOVA, followed by Duncan's Multiple Range Test at a significance level of $p \leq 0.05$ to identify specific differences between treatment means.

Results and discussion

Compatibility test

All seven lactic acid bacteria (LAB) strains GB-15, SB-7, G-23, BB-60, NZ-44, BB-33, and NZ-26 were exhibited compatibility with one another. Notably, there was no evidence of antagonistic activity among the isolates, indicating that none of them inhibited the growth of other isolates. This suggests that none of the LAB strains inhibited the growth of their counterparts during the experimental procedures. This compatibility is significant for potential applications in fermentation processes, as it

Table 2 Sensory characteristics of *Ayib* treated with the formulated starter culture

The score for sensory characteristics (Mean score ± SD)					
Product	**Color**	**Flavor**	**Odor**	**Texture**	**Overall acceptability**
AY1	3.58 ± 0.37^{b}	3.13 ± 0.38^{d}	3.41 ± 0.29^{cb}	3.72 ± 0.23^{cab}	3.47 ± 0.32^{cb}
AY2	3.89 ± 0.53^{ab}	3.91 ± 0.36^{ab}	4.02 ± 0.24^{a}	4.15 ± 0.39^{a}	4.03 ± 0.35^{a}
AY3	3.65 ± 0.41^{ab}	3.69 ± 0.33^{cab}	3.58 ± 0.41^{ab}	3.85 ± 0.32^{cab}	3.69 ± 0.36^{cab}
AY4	3.60 ± 0.41^{ab}	3.44 ± 0.37^{cd}	3.69 ± 0.37^{ab}	3.55 ± 0.31^{cdb}	3.57 ± 0.36^{cb}
AY5	3.80 ± 0.42^{ab}	3.79 ± 0.48^{cab}	3.82 ± 0.44^{ab}	3.55 ± 0.34^{cdb}	3.62 ± 0.38^{cab}
AY6	4.13 ± 0.42^{a}	3.97 ± 0.28^{a}	3.39 ± 0.29^{cb}	3.66 ± 0.24^{cb}	3.79 ± 0.3^{ab}
AY7	3.76 ± 0.46^{ab}	3.76 ± 0.4^{cab}	3.63 ± 0.41^{ab}	3.92 ± 0.60^{ab}	3.77 ± 0.42^{ab}
AY8	3.78 ± 0.40^{ab}	3.67 ± 0.44^{cab}	3.38 ± 0.25^{cb}	3.67 ± 0.60^{cb}	3.63 ± 0.41^{cab}
AY9	2.86 ± 0.32^{c}	3.35 ± 0.35^{cd}	3.63 ± 0.45^{ab}	3.38 ± 0.29^{cd}	3.31 ± 0.34^{c}
AYB	3.66 ± 0.78^{ab}	3.47 ± 0.36^{cdb}	3.05 ± 0.69^{c}	3.10 ± 0.56^{d}	3.35 ± 0.54^{cb}

Where; AY1-AY9 = *Ayib* products made and treated with formulate F1-F9 and FYB = control; *Ayib* without a starter culture formulate treatment

$^{a, ab, b, c, cab, cb, cd, cdb, d}$ Products that share the same superscript letter do not exhibit a significant difference

indicates a favorable environment for co-culturing these strains without the risk of competitive inhibition.

Sensory characteristics of *Ayib* treated with a mixed starter culture

The sensory evaluation of Ayib revealed significant differences ($P < 0.05$) among the products for attributes such as color, flavor, odor, and texture (Table 2). Notably, the overall sensory acceptability scores were highest for products AY2 and AY6, which achieved scores of 4.03 and 3.79, respectively. Table 2 provides the mean scores and standard deviations for the sensory attributes of the nine experimental treatments (AY1–AY9) and the control sample (AYB).

Product AY9 exhibited significantly lower scores for color, indicating a less desirable appearance. Conversely, AY6 scored highest for flavor, reflecting a more pronounced and appealing flavor profile. Similarly, AY2 received relatively high scores for odor and texture, suggesting a more intense and attractive scent and a desirable mouthfeel (Table 2).

These findings align with previous research by Bekele et al. [37], which demonstrated that starter cultures significantly influence the sensory attributes of cheese, including appearance, aroma, taste, and overall acceptability. The observed variations in sensory characteristics of *Ayib* across treatments can be attributed to differences in starter culture formulations. Comparable results have been reported for cheeses made from camel milk, where lactic acid bacteria starter cultures significantly affected sensory properties such as aroma, texture, and overall acceptability [37].

The differences in flavor and odor among the *Ayib* samples may be linked to the metabolic activity of lactic acid bacteria, particularly their ability to produce volatile compounds such as acetaldehyde, diacetyl, and acetoin, which are known to enhance aroma and flavor [37–39]. Additionally, as highlighted by Falkeisen et al. [40], the choice of starter culture plays a critical role in modifying sensory attributes and influencing consumer acceptance. The distinct sensory profiles observed in this study, particularly for color, odor, flavor, and texture, likely stem from the specific properties of the starter cultures employed, which contribute to the development of compounds such as carbon dioxide (CO_2), diacetyl, and acetaldehyde that influence cheese texture and flavor [41, 42].

Previous studies have also reported comparable findings. Eshetu and Asresie [22] observed overall sensory acceptability scores of 3.48–3.53 in cottage cheese, while Regu et al. [43] reported higher scores of 4.91. These differences in sensory acceptability are likely due to variations in production processes and starter culture formulations, both of which significantly impact the organoleptic properties and consumer perception of the product.

The findings of this study underscore the pivotal role of starter culture formulations in determining the sensory attributes and consumer acceptability of Ayib. By tailoring starter cultures, it is possible to enhance specific sensory characteristics, thereby optimizing product quality and market appeal.

Storage stability Estimates of Ayib

The storage stability of *Ayib* products treated with various starter cultures and stored at ambient temperatures between 18 and 25 °C, varied from 4 to 18 days. Notably,

Table 3 Storage stability of *Ayib* treated with the formulates

Product	Quality criteria	Storage temperature	Quality limit	R^2	Estimated storage stability (in days)
AY1	Overall acceptance	18–24 °C	3.5	0.94	10
AY2			3.5	0.92	7
AY3			3.5	0.92	5
AY4			3.5	0.99	17
AY5			3.5	0.94	12
AY6			3.5	0.89	18
AY7			3.5	0.95	17
AY8			3.5	0.93	4
AY9			3.5	0.87	11
AYB			3.5	0.88	2

Where; AY1-AY9 = *Ayib* products made and treated with formulate F-F9 and FYB = control; *Ayib* made traditionally without a starter culture

Table 4 Proximate composition and physicochemical properties of *Ayib* treated with mixed starter culture

Product	Total solid	Fat	Protein	Ash	pH	TA
AY1	20.67 ± 0.67^{cb}	2.02 ± 0.06^{a}	15.77 ± 0.49^{dce}	1.13 ± 0.18^{a}	4.45 ± 0.11^{a}	0.73 ± 0.02^{cd}
AY2	22.52 ± 0.49^{ab}	2.01 ± 0.13^{a}	18.247 ± 0.06^{ab}	1.33 ± 0.09^{a}	3.95 ± 0.14^{c}	0.66 ± 0.08^{cd}
AY3	21.25 ± 0.83^{cb}	1.90 ± 0.03^{a}	16.68 ± 0.48^{cb}	1.22 ± 0.19^{a}	4.37 ± 0.33^{ab}	0.63 ± 0.13^{c}
AY4	21.41 ± 0.88^{cb}	1.90 ± 0.07^{a}	18.30 ± 0.30^{ab}	1.22 ± 0.22^{a}	4.03 ± 0.04^{cb}	0.91 ± 0.03^{a}
AY5	21.09 ± 0.31^{c}	1.93 ± 0.06^{a}	16.64 ± 0.78^{cb}	1.24 ± 0.19^{a}	4.49 ± 0.05^{a}	0.86 ± 0.03^{ab}
AY6	22.89 ± 0.72^{a}	2.01 ± 0.14^{a}	19.20 ± 0.50^{a}	1.20 ± 0.17^{a}	3.89 ± 0.11^{c}	0.93 ± 0.08^{a}
AY7	21.30 ± 1.40^{cb}	2.01 ± 0.08^{a}	17.46 ± 0.40^{bc}	1.23 ± 0.16^{a}	4.14 ± 0.06^{b}	0.89 ± 0.17^{ab}
AY8	21.26 ± 0.42^{cb}	2.02 ± 0.08^{a}	15.31 ± 0.23^{fe}	1.17 ± 0.21^{a}	4.40 ± 0.14^{a}	0.58 ± 0.05^{ce}
AY9	21.14 ± 0.45^{cb}	1.96 ± 0.12^{a}	16.34 ± 0.70^{cb}	1.22 ± 0.17^{a}	4.42 ± 0.07^{a}	0.83 ± 0.11^{bc}
AYB	20.09 ± 0.05^{c}	2.01 ± 0.07^{a}	14.44 ± 0.06^{fe}	1.13 ± 0.17^{a}	4.33 ± 0.05^{ab}	0.57 ± 0.03^{ce}

Where: *TA* Titratable acidity, Ay1 to Ay9 = *Ayib* products made and treated with formulate F1-F9 and AYB = control; *Ayib* made traditionally without a starter culture

$^{a, ab, bc, c, cb, cd, ce, dce, fe}$ Products that share the same superscript letter do not exhibit a significant difference

products AY6, AY7, and AY4 demonstrated the longest storage stability, lasting 18, 17, and 17 days, respectively, under specified conditions (Table 3). In contrast, products AY8 and AY3 exhibited considerably shorter storage stability lives of only 4 and 5 days, respectively.

The *Ayib* products AY5 and AY9 exhibited storage stability of 12 days and 11 days, respectively. In comparison, the control sample AYB demonstrated a significantly shorter storage stability of only 2 days. The storage stability of *Ayib* products treated with formulated starter cultures in this study surpassed the previously reported durations of 3 to 4 days for ambient temperature storage [44] and 5 to 6 days for storage at 18 °C [45].

The variation in the storage stability of the product depends on the overall sensory properties of *Ayib*, which are significantly influenced by the formation of flavor-enhancing aromatic compounds and biogenic amines (BAs), compounds like tyramine and putrescine potentially imparting undesirable flavors and off-odors that negatively impact consumer acceptance [46]. Certain LAB strains can produce enzymes that degrade these biogenic amines, thus reducing their concentration and mitigating sensory defects. The choice of starter culture is critical, as it affects the accumulation of specific BAs during the ripening process; strains that produce amine oxidase enzymes can lower BA levels, preserving the sensory quality of the cheese [47]. Additionally, factors such as ripening time and storage conditions further influence BA formation and degradation [48]. LAB strains that enhance fermentation while minimizing harmful BAs is essential for enhancing the sensory shelf life of *Ayib.*

Proximate composition and physicochemical properties of *Ayib*

Table 4 presents the proximate composition and physicochemical properties of *Ayib* products prepared with various mixed starter culture formulations. An analysis of variance revealed significant differences ($P < 0.05$) in protein content across the different formulations. Notably, product AY6 exhibited the highest protein content

of 19.23%, ($p < 0.05$), whereas product AY8 had the lowest protein content of 15.31%. The protein content of the control sample (AYB) was 14.44% (Table 4). The observed variation in protein concentration may be due to the protein coagulation, curd formation efficiency, and whey loss reduction in the final product by the LAB strain in the consortium.

Certain strains of LAB exhibit improved fermentation efficiency, which facilitates more effective coagulation of milk proteins and subsequent curd formation. This enhanced coagulation process significantly increases protein retention within the curd, consequently elevating the overall protein content of cottage cheese, while also reducing whey loss in the final product [49]. LAB strains that promote superior curd formation are particularly effective in minimizing syneresis, thereby reducing whey separation and further enhancing protein retention in the curd [50, 51]. Additionally, the production of exopolysaccharides (EPS) by specific LAB strains contributes to improved texture and further reduction of syneresis, thereby enhancing protein retention in the curd [52, 53].

These findings are comparable to previously reported protein contents, which ranged from 14.53 to 16.78% in traditionally processed *Ayib* samples from various regions of Ethiopia [22]. The application of defined lactic acid bacteria starter cultures has been shown to enhance the amino acid profiles of fermented foods. A study on African yam bean seed condiments by [54] demonstrated that samples inoculated with specific lactic acid bacteria (LAB) strains exhibited a higher total amino acid content than uninoculated samples. This indicates that starter cultures can significantly increase the nutritional value of fermented products by improving the amino acid availability. Additionally, LAB starter cultures influence fermentation dynamics, which can lead to variations in protein content. A previous study by [55] showed that in the production of ugba (fermented African oil bean), LAB-fermented samples contained higher protein levels than those produced via spontaneous fermentation. Furthermore, research has indicated that co-fermentation of different LAB strains can enhance the overall fermentation efficiency and improve nutrient profiles, including protein content [56, 57].

The fat content across all the samples showed no significant differences ($p > 0.05$), with values ranging from 1.90% (AY3 and AY4) to 2.02% (AY1 and AY8). This suggested that the starter culture had minimal impact on lipid metabolism or fat content stabilization during the fermentation process. The fat content of *Ayib* in this study was relatively lower than the 1.35% fat content in *Ayib* that has been reported by Regu et al. [43] and 1.40 to 1.44% reported by Eshetu and Asresie [22].

A statistically significant difference ($P < 0.05$) was observed in the total solid content among the samples, which may be attributed to differences in microbial activity, fermentation conditions, and substrate utilization by the starter cultures. The total solids (TS) content ranged from 20.67% in product AY1 to 22.89% in product AY6 (Table 2). Product AY6 exhibited the highest total solids, which were significantly different ($p < 0.05$) from all the products, except product AY2. The control sample, derived from spontaneously fermented milk without any added starter culture, displayed a total solid content of 20.09%. This variation is attributed to differences in the composition and fermentation efficiency of the LAB starter cultures used. The study found a strong correlation between total solids and protein content, as AY6 also had the highest protein level, indicating effective protein retention during coagulation. Conversely, AYB's lower total solids and protein content highlight the importance of selecting LAB strains that optimize protein retention and minimize whey loss. Additionally, certain LAB strains contributed to reduced syneresis, promoting firmer curd formation, which is crucial for enhancing texture [2, 3]. A related study conducted by Bekele et al. (2019) also showed that the use of five different commercial starter cultures significantly affected the proximate composition of the total solid and protein content of cottage cheese. These findings are consistent with previously reported total solid contents of 20.5%, 21%, and 21.67–21.29% [22, 58, 59].

The ash content ranged from 1.33% in product AY3 to 1.13% in product AY1, with no significant differences between samples and control samples without starter culture inoculation. This indicates that the mineral content was largely unaffected by the starter cultures or fermentation process. The result of this study aligned with values of 1.15–1.17% in *Ayib* samples collected from Eastern Gojem, Ethiopia by [22], and 1.24% reported by Regu et al. [43]. Studies examining the physicochemical properties of traditional fermented foods revealed that the impact of lactic acid bacteria (LAB) on ash content is generally minimal [55]. Research on ugba, a Nigerian fermented food with LAB starter culture, found that the ash content was not statistically significant when compared to spontaneously fermented samples [55]. Similarly, a study by Botthoulath et al. [60] on fermented bamboo shoots using the probiotic *Lactiplantibacillus plantarum* showed improvements in various nutritional parameters, but no significant changes in ash content resulting from fermentation. This observation is consistent with the broader consensus that while LAB enhances flavor, texture, and nutritional value, particularly in terms

Table 5 Growth inhibition of indicator microbes by LAB starter culture candidates

LAB isolate	Zone of inhibition (Diameter in mm)			
	E. coli	S. Typhimurium	S. aureus	L. monocytogenes
GB-15	18.2 ± 0.02	18.2 ± 0.02	15.5 ± 0.01	16.8 ± 0.03
SB-7	18.0 ± 0.01	18.0 ± 0.01	16.5 ± 0.03	18.7 ± 0.01
G-23	17.5 ± 0.03	16.8 ± 0.02	15.8 ± 0.02	17.2 ± 0.02
BB-60	17.3 ± 0.01	16.8 ± 0.01	12.5 ± 0.03	17.2 ± 0.03
NZ-44	16.2 ± 0.03	16.7 ± 0.03	16.8 ± 0.01	18.2 ± 0.01
BB-33	16.2 ± 0.02	16.7 ± 0.02	15.5 ± 0.03	17.0 ± 0.03
BZ-26	16.2 ± 0.03	16.2 ± 0.01	15.3 ± 0.02	16.0 ± 0.02

Where; *GB-15=L. rhamnosus, SB-7=L. paracasei, G-23=L. reuteri, BB-60= L. sake*, NZ-44=*L. curvatus, NN-33=L. plantarum, BZ-26=L. casei.* Diameter is presented as the mean± SD of triplicate analyses

Table 6 Antioxidant potential of each LAB isolate

LAB isolate	DPPH
GB-15	39.41 ± 0.66[c]
SB-7	40.38 ± 0.56[c]
G-23	32.65 ± 0.48[e]
BB-60	36.21 ± 0.36[d]
NZ-44	57.77 ± 0.56[a]
BB-33	23.82 ± 0.34[f]
BZ-26	49.03 ± 0.65[b]

[a-f] Products that share the same superscript letter do not exhibit a significant difference

of protein and total solids they do not significantly affect the mineral composition [57]. Zeng et al. [57] and Zeng et al. [57] research on Wanergao, a traditional Chinese fermented food, also demonstrated that although microbial activity influenced other physicochemical properties, there were no significant changes in ash content.

The pH levels of the products were significantly different $P < 0.05$. The pH values ranged from 4.49 in product AY5 to 3.89 in product AY6. Specifically, lower pH values of 3.89, 3.95, and 4.03 were observed for products AY6, AY2, and AY4, respectively. These findings are consistent with the results reported by Eshetu and Asresie [22], which noted a pH of 4.49 in *Ayib* samples collected from Eastern Gojem, Ethiopia, and a pH of 4.29 reported by Regu et al. [43]. Notably, the pH values of products AY6 and AY2 were lower than those reported by Eshetu and Asresie [22]. The observed variations in pH may be attributed to the acidification capabilities of lactic acid bacteria (LAB) present in the starter cultures during lactose fermentation.

A significantly higher titratable acidity of 0.93% ($P < 0.05$) was recorded for product AY6, whereas the lowest titratable acidity of 0.58% was observed for product AY8. The total titratable acidity of *Ayib* in this study was relatively higher than the 0.68% reported by Regu et al. [43] and 0.43–0.44% values reported by Eshetu and Asresie [22]. This variation in titratable acidity may be due to the fermentation time and the differing acidification capabilities of the starter cultures employed in the production and treatment of *Ayib*.

These findings highlight the influence of mixed starter culture on the nutritional and physicochemical characteristics of *Ayib*. Samples such as AY6 demonstrated superior nutritional profiles with higher total solids, protein content, and acidity, making it a promising formulation for enhancing *Ayib* quality. The uniformity in fat and ash contents suggests that these properties are less affected by fermentation dynamics.

Antimicrobial activity of starter culture candidate LAB isolates

To assess the antagonistic properties of the LAB isolates, all candidate starter culture isolates were evaluated for their antimicrobial activity against common food-borne pathogens as shown in Table 5. The antimicrobial effectiveness of these starter culture LAB isolates was tested against both Gram-positive organisms, specifically *Staphylococcus aureus* and *Listeria monocytogenes*, as well as Gram-negative organisms, *Escherichia coli* and *Salmonella* Typhimurium. The results of these antimicrobial assessments are summarized in Table 5.

All seven LAB isolates demonstrated notable antagonistic effects against the tested gram-positive and gram-negative microorganisms. Among these isolates, GB-15 and SB-7 exhibited the most pronounced antagonistic effects, measuring an inhibition zone of 18.2 mm and 18.0 mm, respectively, against the Gram-negative bacteria *E. coli* and *S.* Typhimurium. Additionally, the isolates SB-7 and NZ-44 showed superior antagonistic activity against the Gram-positive bacteria *S. aureus* and *L. monocytogenes* (Table 5). These findings indicate the potential of these LAB isolates in enhancing the safety and storage stability of *Ayib* products through their antimicrobial properties.

The antimicrobial activity of lactic acid bacteria (LAB) can be attributed to their metabolic byproducts, which include organic acids, bacteriocins, hydrogen peroxide, ethanol, and diacetyl, among others [61]. Different studies have demonstrated that lactic acid bacteria-derived bacteriocins are effective in inhibiting the growth of pathogens such as *Listeria monocytogenes*, *Salmonella spp.*, *Escherichia coli*, and *Staphylococcus aureus* in diverse food matrices. This inhibition

contributes to improved food safety and overall food quality [62–64]. Studies have indicated that LAB strains isolated from curly kale juice inhibited *Staphylococcus aureus* by 13–22.5 mm, *Listeria monocytogenes* by 0–20.6 mm, and exhibited inhibition against *Escherichia coli* and *Salmonella enteritidis* by 0–15.6 mm [65]. These findings highlight the potential of LAB to enhance food safety through their antimicrobial properties.

Antioxidant potential of each isolate

Table 6 presents a comprehensive overview of the antioxidant potential of probiotic lactic acid bacteria (LAB) isolates, measured through their DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. The data revealed significant variability in the antioxidant capacities among the different LAB strains examined.

Among the isolates, strain NZ-44 emerged as the most effective, exhibiting a remarkable DPPH value of 57.77%, which underscores its superior ability to neutralize free radicals. Conversely, strain BB-33 displayed the lowest antioxidant potential with a DPPH value of 23.82%. The remaining isolates, GB-15, SB-7, G-23, BB-60, and BZ-26, demonstrated intermediate levels of antioxidant activity. These findings align with those of a previous study by Abduxukur et al. [66] on lactic acid bacteria derived from Xinjiang traditional fermented dairy products, which reported an antioxidant activity of 29.94%. Additionally, a separate study by Abubakr et al. [67] noted a 50.8% antioxidant activity of LAB in fermented skim milk, while *L. plantarum* strains isolated from Tibetan kefir showed an antioxidant capacity ranging from 14.7 to 58.1% [68]. In contrast, lower antioxidant potentials of 2.55–6.88% were reported by Kim et al. [69] for certain probiotic LAB cell-free supernatants, indicating a broad spectrum of antioxidant capabilities among the different strains.

The observed variability in antioxidant potential among the LAB isolates can be attributed to several factors. Strain-specific characteristics played a crucial role in determining the ability of each isolate to synthesize antioxidant compounds. Additionally, growth conditions, such as the composition of the culture medium, temperature, and pH significantly influence the production of these antioxidants. The diversity of antioxidant compounds generated by LAB, including organic acids, enzymes, and exopolysaccharides, further affects their overall antioxidant capacity.

Probiotic LAB strains that exhibit high antioxidant potential have a range of applications in various sectors. In the food industry, these isolates can function as natural preservatives, effectively inhibiting oxidative spoilage and enhancing the shelf life of food products. Their incorporation into functional food products boosts their nutritional value and offers health benefits. Furthermore, the antioxidant properties of these LAB strains may positively impact gut health and overall well-being when used as probiotics. Beyond the food industry, these LAB isolates hold promise in the pharmaceutical sector for the development of antioxidant supplements or therapeutic interventions targeting oxidative stress-related diseases.

Table 7 Antioxidant potential of the formulate

Formulates	DPPH %
F1	80.47 ± 0.79^{c}
F2	61.43 ± 0.95^{d}
F3	58.70 ± 0.65^{d}
F4	94.21 ± 1.15^{b}
F5	83.52 ± 1.06^{c}
F6	99.27 ± 1.39^{a}
F7	93.95 ± 1.46^{b}
F8	51.55 ± 1.35^{e}
F9	82.4 ± 1.16^{c}
Cont.	15.99 ± 0.50^{f}

Where: F1, F2, F3, F4, F5, F6, F7, F8, and F9 are probiotic lactic acid bacteria starter culture formulates. Cont. = Control (MRS broth without inoculum)

[a-f] Products that share the same superscript letter do not exhibit a significant difference

Antioxidant potential of the formulates

The data provided in Table 7 shows the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, which is a widely used measure of antioxidant potential, for the different LAB (lactic acid bacteria) formulations (F1-F9) and the control sample.

As illustrated in Table 7, the mixed/formulated lactic acid bacteria (LAB) displayed a markedly improved antioxidant potential in comparison to the control sample. Notably, formulation F6 exhibited the highest level of antioxidant activity, achieving a DPPH scavenging rate of 99.27%. These findings indicate that the F6 formulation possesses remarkable free radical scavenging abilities, reflecting a strong antioxidant profile.

The antioxidant potential of the formulated mixtures (F1-F9) was generally higher than that of the individual isolates, suggesting a synergistic effect among the LAB strains. Notably, F6 and F7 exhibited the highest antioxidant activities, surpassing even the most potent individual isolate, NZ-44. This suggests that the combination of different LAB strains in these formulations can significantly enhance their antioxidant properties.

Other LAB formulations also exhibited commendable antioxidant potential, with formulation F4 recording a DPPH scavenging activity of 94.21%, F7 at 93.95%, and F5 at 83.52%. In stark contrast, the control sample displayed a considerably lower antioxidant activity of only 15.99%.

Table 8 Correlation of titratable acidity, protein, storage stability, and antioxidant potential

Sample 1	Sample 2	*N*	Correlation	95% CI for	*P*-Value
TA	DPPH%	10	0.986	(0.941, 0.997)	0.000
Protein	DPPH%	10	0.725	(0.176, 0.930)	0.018
Storage stability	DPPH%	10	0.926	(0.710, 0.983)	0.000
Protein	TA	10	0.740	(0.207, 0.934)	0.014
Storage stability	TA	10	0.942	(0.767, 0.986)	0.000
Storage stability	Protein	10	0.741	(0.209, 0.935)	0.014

Where: *TA* Titratable acidity, *DPPH* 2,2-diphenyl-1-picrylhydrazyl

A related finding was reported by Zhou et al. [70], which indicated a DPPH scavenging activity of 95.98%. Furthermore, previous research on probiotic yogurt identified DPPH scavenging activities ranging from 62.5 to 94.85% Adelekan et al. [71], which are markedly similar to the values observed for the F6, F4, and F7 LAB formulations in this study. Additionally, another investigation into lactic acid bacteria isolated from traditional dairy products reported DPPH scavenging activities between 23.1% and 62.9%, again falling short of the remarkable antioxidant potential exhibited by formulations such as F6, F4, and F7. Further related research findings on lactic acid bacteria from traditional fermented food products revealed DPPH scavenging activities ranging from 28.7 to 87.8% [72]. Notably, *L. acidophilus* isolated from fermented food demonstrated a DPPH scavenging activity of 50.8% [67].

These comparisons highlight the exceptional antioxidant properties of the LAB formulations developed in this study, which could significantly enhance the quality and health-promoting attributes of the traditional Ethiopian dairy product *Ayib*. The capacity of these LAB formulations to effectively scavenge free radicals and exhibit potent antioxidant activity represents a promising finding that merits further investigation and potential application in the development of functional dairy products.

The notable antioxidant potential exhibited by the LAB formulations, particularly F6, F4, and F7, could contribute to the overall quality and stability of the *Ayib* product while providing potential health benefits to consumers. These findings underscore the importance of developing and evaluating novel LAB formulations to enhance the antioxidant properties of traditional dairy products, ultimately leading to the creation of more nutritious and health-promoting food items. In this study, the antioxidant content of the products was not quantified due to the production of antioxidant compounds occurring primarily during the fermentation process and to a lesser extent during the storage of the fermented products. To accurately assess the antioxidant potential of the formulations in the final product, it is essential to optimize the processing conditions of *Ayib.*

Pearson correlation coefficient of antioxidant potential with protein, TA and storage stability of the products

The correlation analysis presented in Table 8 examines the relationships between TA, protein content, storage stability, and antioxidant potential, as measured by the DPPH scavenging percentage. The analysis reveals several significant correlations that underscore the interplay between these variables.

Titratable acidity and DPPH% is exceptionally strong, correlation coefficient of 0.986 ($p < 0.001$). This indicates a highly significant positive relationship, suggesting that as the TA increases, the antioxidant potential, as measured by DPPH scavenging activity, also increases. This could be attributed to the presence of organic acids, which are known to enhance the antioxidant capacity of food products by neutralizing free radicals [68]. The correlation between protein content and DPPH% is moderate, with a coefficient of 0.725 ($p = 0.018$). This indicates a statistically significant positive correlation, suggesting that higher protein levels may contribute to increased antioxidant activity. Proteins can function as antioxidants through multiple mechanisms. The hydrolysis of proteins in fermented foods yields various peptides with antioxidant properties. Additionally, proteins have the capacity to donate electrons to free radicals, thereby stabilizing these reactive species [68]. The correlation between storage stability and DPPH% is also strong, with a coefficient of 0.926 ($p < 0.001$). This suggests that products with better storage stability tend to exhibit higher antioxidant potential. The stability of a product can influence its ability to retain antioxidant compounds over time, which is crucial for maintaining quality during storage. Antioxidants prevent spoilage by microorganisms and enhance the storage stability of the products [68].

Conclusions

In conclusion, this study effectively demonstrates the potential of seven lactic acid bacteria (LAB) strains (NZ-44, SB-7, BB-60, BB-33, GB-15, G-23, and NZ-26) in the development of compatible mixed cultures for *Ayib* production, free from antagonistic interactions. The implementation of these strains has led to significant improvements in the nutritional profile, shelf life, antioxidant potential, and sensory qualities of *Ayib*. The result revealed that both total solid and protein content enhancement in products AY6 and AY2. Furthermore, the LAB strains successfully reduced pH and increased titratable acidity, essential factors for the safety and quality of fermented dairy products. Sensory evaluations indicated that LAB-treated products enjoyed high acceptability and extended shelf lives of up to 18 days,

attributable to the antimicrobial properties of the strains, which effectively inhibited spoilage microorganisms. The strong antimicrobial activity against both Gram-positive and Gram-negative bacteria, particularly from strains GB-15 and SB-7, further enhances the safety and longevity of *Ayib*. Additionally, certain LAB formulations exhibited impressive antioxidant activity, suggesting potential health benefits that support the creation of functional dairy products. Overall, the findings from this study will contribute to the future applications and utilization of bio-protective starter cultures within the dairy industry.

Abbreviations

LAB	Lactic acid bacteria
TTA	Total titratable acidity
DPPH	2,2-diphenyl-1-picrylhydrazy
E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus*
NABRC	National Agricultural Biotechnology Research Center

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Authors' contributions
ZA Conceived the study, developed the experimental design, conducted data analysis, and drafted the manuscript. AT Supervised the microbial and biochemical assays, contributed to data analysis, and reviewed the manuscript. AD Supervised the microbial and biochemical assays, contributed to data analysis, and reviewed the manuscript. TD Edditted the munscript, fieldwork, particularly in cheese production, and lead the sensory evaluation, and contributed to the statistical analysis of sensory data. TH Edditted the munscript, assisted in fieldwork, particularly in cheese production, and lead the sensory evaluation, and contributed to the statistical analysis of sensory data.

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Data availability
The datasets generated and analyzed during this study are available from the corresponding author, Zerihun Asefa, upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the ethical standards set by the Ethics Review Committee of Addis Ababa University, and all procedures involving human participants, such as sensory evaluations, were approved. Written informed consent was obtained from all participants prior to their involvement in sensory evaluation panels. Participants were fully informed about the objectives, methods, and potential risks of the study, and their right to withdraw at any time was emphasized.

Consent for publication
All information presented in this manuscript is original and has been generated in accordance with ethical research practices.

Competing interests
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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