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Evaluating autochthonous lactic acid bacteria for the production of Moroccan *Jben* cheese

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Abstract

This study aimed to develop different starter cultures from lactic acid bacteria strains indigenous to Moroccan *Jben* cheese and assess their effects on the physicochemical, microbiological, and sensory properties of *Jben* cheese produced at a pilot scale. A total of nine lactic acid bacteria strains, previously isolated and selected based on their acidifying, proteolytic, and aromatic properties, were used. Compatibility testing and principal component analysis identified four strains, which were further evaluated for their kinetic parameters. These strains were then combined into different lactic starter cultures to produce four experimental batches of *Jben* cheese, with a fifth batch produced using traditional whey as a control. Antagonism testing revealed an incompatibility between *Lactiplantibacillus* and *Lactococcus lactis* strains 5.12 and 17.6. Kinetic analysis showed that strain *Lc. lactis* 12.1 had the highest growth rate (0.60±0.04) and the shortest lag phase. Microbiological analysis revealed moderate yeast and mold contamination in the experimental cheeses, with levels lower than commercial *Jben* cheeses. Physicochemical analysis showed significant differences in ash, dry matter, and acidity levels across batches depending on the different cultures used, while protein and lipid contents remained consistent. Sensory evaluations indicated no significant overall differences among the cheeses, however the control batch was rated higher for texture, while cheeses with autochthonous strains scored better in aroma, flavor, and overall preference.

Keywords: indigenous ferments; compatibility testing; bacterial growth kinetics; artisanal cheese standardization; sensory evaluation

Introduction

Milk production and dairy processing are integral components of Morocco's economic and culinary culture, with *Jben* cheese occupying a prominent position among dairy products (Benkirane et al., 2022). *Jben* can be produced from various milk sources, including bovine, caprine, or their combinations. Typically, it is coagulated using calf rennet or vegetable-derived coagulant (an aqueous extract from *Cynara cardunculus* flowers), without the addition of commercial starters (El Galiou et al., 2015). Some artisanal producers incorporate whey from the previous batches. This non-standardized cheese is manufactured using traditional techniques that can vary among producers, resulting in significant heterogeneity in the physicochemical and microbiological properties of *Jben* across different batches and manufacturers (Azzouz et al., 2024).

The use of autochthonous lactic acid bacteria offers significant advantages in cheese production. These specific cultures facilitate process standardization while maintaining the authentic organoleptic properties of the cheese. They also enhance hygienic quality and product consistency, addressing the need for standardization while preserving traditional methods (Benkirane et al., 2022).

This research is motivated by the significant variability observed in the physicochemical and microbiological qualities of commercially available *Jben* cheeses, which can be attributed to a lack of standardization. Azzouz et al. (2024) highlighted this variability by comparing ten *Jben* cheese samples from the same region in northern Morocco. Additionally, several studies have reported similar inconsistencies in quality across different regions of Morocco (El Marnissi et al., 2013; Noutfia et al., 2011; Rhiat et al., 2011). Furthermore, significant levels of contamination with undesirable microbial flora have been noted (Azzouz et al., 2024; El Galiou et al., 2015), emphasizing the need for improved standardization in *Jben* cheese production.

In this study, goat milk was selected for *Jben* production, in preference to bovine or camel milk, for several reasons. Primarily, in Morocco, goat milk is predominantly allocated to cheese production, with minimal consumption in its unprocessed form (Zine-eddine et al., 2021). Furthermore, goat farming is predominant in the northern region of Morocco, where this study was conducted (Majid, 2021). In addition to its local availability, goat's milk offers notable gustatory and nutritional properties, making it a suitable choice for cheese production (Yadav et al., 2016).

The goal of this study was to assess the impact of autochthonous lactic acid bacteria, used as starter cultures, on the physicochemical, microbiological, and sensory properties of *Jben* cheese during production and storage.

Materials and methods

Development of lactic starters for Jben cheese production

Strains

A collection of nine autochthonous isolates, previously obtained from artisanal *Jben* cheeses in our laboratory (Faculty of Sciences and Techniques, Tangier, Morocco) and exhibiting desirable technological properties, was used in this study - *Lactiplantibacillus plantarum* 18.2, 14.4, and 18.5 *Lactococcus lactis* 5.12, 15.4, 12.1, 16.3, and 17.6, and *Enterococcus faecium* 16.1.

Strain compatibility assessment

A compatibility assay was conducted to identify potential antagonistic interactions among the preselected candidate strains (n=9) intended for lactic starter development. The experimental protocol was adapted from Sukhikh et al. (2017) with modifications. The procedure involved spread-plating 200 µL of the test strain on MRS agar medium (Biokar, Beauvais, France). After surface drying, wells were aseptically created in the agar using a sterile Pasteur pipette (Labbox, Rungis, France). Each well was then inoculated with 50 µL of a different lactic strain suspension. The inoculated plates were incubated at 37 °C for 24 hours under aerobic conditions. Antagonistic activity was quantified by measuring the diameter of inhibition zones surrounding the wells.

Strain selection, kinetics study and starter culture development

Strain selection. The selection criteria for formulating diverse starter cultures were based on a combination of strain compatibility assessments (antagonism testing) and principal component analysis (PCA) (Statgraphics, The Plains, US). Initially, antagonism testing was performed to ensure that strains combined within the same starter culture did not exhibit inhibitory effects against each other. Following this, PCA was utilized to group bacterial strains based on previously determined technological properties, such as diacetyl production (DA), aroma, proteolysis, and pH reduction. To enhance functional diversity, strains from different PCA clusters were chosen to formulate three starter cultures: one with lactococcal strains only, one combining a lactococcal strain with a *Lactiplantibacillus* strain, and one with a lactococcal and an enterococcal strain. A fourth culture incorporated all the strains from the first three formulations.

Kinetics study. Selected strains (n=4) were then subjected to growth kinetics analysis. They were reactivated and standardized using the McFarland turbidity method. Subsequently, each strain was inoculated at a 10 % (v/v) concentration into appropriate growth media: Tryptic Soy Broth (TSB) (Biokar, Beauvais, France) for *Lactococcus* and *Enterococcus* species, and de Man, Rogosa and Sharpe (MRS) (Biokar, Beauvais, France) broth for *Lactiplantibacillus* species. Bacterial growth was monitored in real-time using an RTS-1C bioreactor (BioSan, Riga, Latvia), with optical density measurements recorded at 5-minute intervals. Incubation

parameters were maintained at 37 °C with continuous agitation at 2000 rpm for a duration of 24 hours, as per the protocol described by Bakrim et al. (2021).

Starter cultures development. The production of starter cultures involved a three-stage reactivation process for each individual strain. Initially, pure lactic acid bacteria strains were inoculated at a concentration of 1 % (v/v) into 10 mL of MRS broth (Biokar, Beauvais, France) and incubated at 37 °C for 24 hours. Subsequently, 10 % (v/v) inocula from the first stage were transferred into 20 mL of ultra-high temperature (UHT) treated milk (Jaouda, Taroudant, Morocco) and incubated at 37 °C for 24 hours. For the final reactivation, 10 % (v/v) inocula from the second stage were transferred into 100 mL of UHT milk (Jaouda, Taroudant, Morocco). These cultures were incubated at 37 °C for 24 hours and subsequently enumerated prior to use as starters. For enumeration, 10 mL of each culture was mixed with 90 mL of 0.85 % saline solution (Oxoid, Basingstoke, UK), and serial decimal dilutions were prepared up to a 10^{-5} dilution. The last two dilutions were used to inoculate MRS agar plates (Biokar, Beauvais, France), which were incubated at 37 °C for 24 hours before colony counting.

Following enumeration, the individual cultures were combined into specific formulations based on antagonism test results and PCA analyses and then employed in *Jben* cheese-making.

Jben cheese production

Microbiological assessment of milk

The microbiological assessment of the milk destined for *Jben* production involved enumerating the total mesophilic aerobic flora (TAMF) and testing for pathogenic bacteria, specifically *L. monocytogenes* and *Salmonella* spp. as previously described by Di Cerbo et al. (2020).

Jben production

*C*heese production was conducted at a farm situated in *Douar Dayedaate* (35.65605, -5.93345), within the commune of *Hjar Nhal*, *Tangier*. This farm maintains two distinct goat herds: *Saanen* and *Alpine* breeds, yielding a combined daily milk production of 55 liters. A total of five cheese batches were produced using goat milk. Four batches incorporated distinct combinations of selected strains, while a control batch was prepared using whey, following the traditional method. *Jben* production was carried out by inoculating raw goat milk (non-pasteurized) with starter culture mixtures, where each individual strain was added in equal proportions at a concentration of 0.5 % (v/v). The mixture was then gently stirred at 32 °C for 30 minutes. Salt (Rostom SARL, Salé, Morocco) was incorporated at a rate of 0.4 % (w/v), followed by the addition of rennet (Caille lait, Casablanca, Morocco) at 0.5 mL/L. The milk was allowed to coagulate for 18 hours. After coagulation, the curd was ladled into molds and left to drain overnight at 4 °C. The cheese was subsequently turned and stored at 4 °C.

Enumeration of yeasts and molds

Samples of 10 g were taken from the produced cheeses and blended with 90 mL of physiological saline solution (0.9 %) (Oxoid, Basingstoke, UK) in a stomacher Lab-Blender 400 (Gemini BV, Overijssel, Netherlands) for 3 minutes. After allowing the larger particles to settle, decimal dilutions were prepared. Enumeration of yeasts and molds was performed by surface plating on OGA medium (Biokar, Beauvais, France) for 4 days at 25 °C (ISO 6611-IDF 94, 2004).

Physicochemical composition

The dry matter content (IDF, 1982), ash content (IDF, 1964), and fat content (IDF, 1997) were determined following International Dairy Federation standards. The acidity and protein assays were conducted according to the standards established by the American Organization of Analytical Chemists (AOAC, 2000) and (AOAC, 1970), respectively. pH measurement was conducted in accordance with AFNOR standard procedures (1993).

Sensorial analyses

The sensory evaluation of the five cheeses was conducted using a panel of eight individuals experienced in cheese tasting. These analyses were performed on the first day following sample production. Various sensory criteria were considered, including visual analysis of external and internal appearance, evaluation of texture, aroma, flavor, and aftertaste. Each attribute was rated on a 7-point hedonic scale, ranging from 1 (low intensity/undesirable) to 7 (high intensity/desirable). Tasters were also asked to provide an overall acceptability score for each cheese sample. To minimize bias, samples were presented simultaneously in a randomized order.

Statistical analyses

All analyses were performed in triplicate, except for the protein quantification and bacterial kinetics study, which were conducted in duplicate. Statistical analyses were conducted using Statgraphics 19 software (The Plains, USA). The ANOVA test was employed to determine significant differences in microbiological and physicochemical parameters. Sensory data were analyzed using chi-square tests. Additionally, PCA was applied to cluster candidate strains based on their distinct technological traits. This approach enabled the selection of strains with complementary properties, ensuring the formulation of effective and diverse starter cultures.

Results and discussion

Strain compatibility

Compatibility tests assess how different strains interact in co-culture, guiding the selection of strains for specific applications like artisanal *Jben* cheese production. These tests play an important role in developing a starter culture,

optimizing cheese quality, aroma, and texture, while also preventing potential issues that could disrupt fermentation and desired microbial composition. To assess potential antagonistic interactions between the preselected strains (n=9), they were all co-cultured together. Following incubation, distinct zones of inhibition were observed surrounding wells inoculated with *L. plantarum* strains 18.2, 18.5, and 14.4 on culture plates containing *Lc. lactis* strains 5.12 and 17.6. This inhibitory effect may result from metabolites production like organic acids, bacteriocins, or other antimicrobial compounds, as well as competitive interactions. Incompatibility between *Lactobacillus* and other lactic acid bacteria species such as *Lc. lactis*, *S. thermophilus*, and *L. delbrueckii* subsp. bulgaricus has been previously documented in several studies, including those conducted by Sukhikh et al. (2017) and Horáčková et al. (2022). Conversely, no antagonism was identified between strains *Lc. lactis* M78 and *L. plantarum* H25, consistent with the observations of Samelis et al. (2023). The lack of inhibitory activity in *Lactococcus* selected strains and *E. faecium* 16.1 is advantageous as it allows preservation of desired bacterial diversity during cheese fermentation.

PCA analysis and strain selection for starter culture development

The first PCA biplot (Figure 1a) aims to select between lactococcal strains for formulating the first ferment composed exclusively of lactococci. It illustrates their distribution across four quadrants. Strain 12.1, found in the bottom-left quadrant, is associated with pH reduction, highlighting its role in enhancing acidity. In contrast, strain 5.12, located in the top-left quadrant, correlates with diacetyl production, suggesting its potential to enhance the cheese's aromatic profile. While strain 12.1's strong acidification capacity might suggest pairing it with a less acidifying strain like 15.4, the absence of diacetyl production in strain 15.4 makes this combination less interesting. Therefore, combining strain 12.1 with strain 5.12, known for its diacetyl production, appears to be a more suitable strategy to achieve both desired acidity and aroma profiles in the final product.

The second biplot (Figure 1b) was generated to guide the selection of one *Lactococcus* and one *Lactiplantibacillus* strain for the development of the second starter culture. Given the observed incompatibility of *Lactococcus* strain 5.12 with all tested *Lactiplantibacillus* strains, strain 12.1 was chosen as the preferred *Lactococcus* candidate. Among the *Lactiplantibacillus* strains, strain 14.4 was excluded due to its relatively neutral aroma profile. Strains 18.2 and 18.5, both exhibiting more pronounced aroma characteristics, were considered more suitable. Ultimately, strain 18.2 was selected due to its higher diacetyl production capacity, which could effectively compensate for the lack of diacetyl production by strain 12.1.

A third PCA biplot was generated to compare *E. faecium* strain 16.1 with *Lactococcus* strains 5.12 and 12.1, guiding the selection process for the third starter culture (Figure 1c). Strain 5.12, situated in the bottom-left quadrant, exhibits moderate acid production compared to the higher acidifying

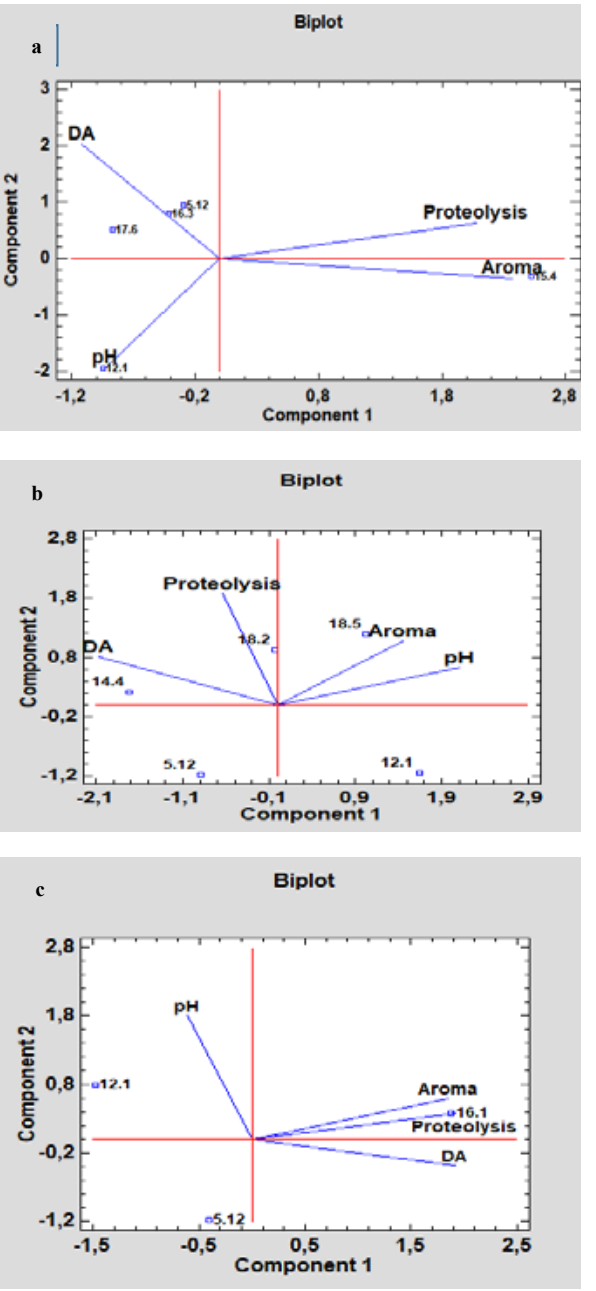


Figure 1a. *PCA biplot for selecting lactococcal strains to be used in the first combination of lactic starter culture. DA= Diacetyl acetoin production b. PCA biplot for the characterization of lactococcal and Lactiplantibacillus strains for the development of a second autochthonous starter culture for Jben cheese. DA = Diacetyl-acetoin production. c. Principal Component Analysis for comparing lactococcal strains and E. faecium 16.1 for the development of the third lactic starter culture. DA = diacetyl-acetoin production*

strains 12.1 and 16.1. Its leftward position reflects a less pronounced proteolytic activity than strain 16.1, although it shares similarities with strain 12.1 in this regard. Furthermore, it produces less diacetyl than strain 16.1 and exhibits

a comparable aroma profile to strain 12.1. Strain 12.1, located in the upper-left quadrant near strain 16.1, is distinguished by its high acid production. While strain 12.1 could be selected for its inability to produce diacetyl, strain 5.12 was ultimately chosen due to its lower acidifying capacity, in order to balance the acidity and avoid producing overly acidic cheese.

Compatibility testing and PCA analysis enabled us to select four distinct strains for starter culture development from the initial nine preselected strains. These strains were combined into three different batches: *Lc. lactis* 12.1 and *Lc. lactis* 5.12 in the first batch, *Lc. lactis* 5.12 and *E. faecium* 16.1 in the second, and *Lc. lactis* 12.1 paired with *L. plantarum* 18.2 in the third.

The fourth batch combined four strains: *Lc. lactis* 5.12, *Lc. lactis* 12.1, *E. faecium* 16.1, and *L. plantarum* 18.2. This approach is crucial as it explores complex interactions between different bacterial strains within a cheese model, influencing sensory, physicochemical, and microbiological characteristics, as well as overall cheese quality. Additionally, it assesses the previously observed incompatibility between strains 5.12 and 18.2 under controlled environment.

Kinetic parameters of selected strains

The analysis of kinetic parameters provided greater insight into the potential and performance of the selected strains. Significant variations in maximum growth rate (μmax) ($p<0.05$) were observed among the four LAB strains (Table 1), with *Lc. lactis* 12.1 achieving the highest rate of 0.60 ± 0.04 h^{-1}, closely followed by *L. plantarum* 18.2 at 0.47 ± 0.01 h^{-1}. These results indicate that *Lc. lactis* 12.1 and *L. plantarum* 18.2 have the ability to reproduce more rapidly, potentially accelerating the milk acidification process during cheese production. *Lc. lactis* 12.1 exhibited a significantly higher growth rate compared to strains isolated from Suero costeño fermented milk (Valencia-García et al., 2018) yet lower than those reported for most *Lc. lactis* strains by Ruiz Rodríguez et al. (2019). Additionally, *L. plantarum* 18.2 showed growth rates comparable to those isolated from cereals but higher than those from fruits and vegetables (Coda et al., 2010; Fessard and Remize, 2019). These results attest to substantial diversity within these species in terms of growth rates, highlighting the strain-specific nature of this parameter.

The strain *Lc. lactis* 12.1 exhibited an exceptionally brief lag phase (Figure 2). This means that the strain quickly enters an active phase of multiplication after being introduced into the fermentation medium. It is worth noting that a short lag phase can be beneficial for cheese production, as it enables faster acidification of the milk, promoting milk coagulation and preventing the growth of undesirable microorganisms.

The generation times also varied significantly between strains ($p<0.05$). *Lactococcus. lactis* 12.1 had the shortest generation time at 1.17±0.07 hours, followed closely by *L. plantarum* 18.2 at 1.49±0.03 hours. In contrast, *Lc. lactis* 5.12 and *E. faecium* 16.1 exhibited longer generation times of 1.89±0.22 hours and 5.63±0.95 hours, respectively (Table 1).

Table 1. *Growth and acidification kinetics of selected strains*

	Maximum growth rate (h^{-1})	Generation time (h)
Lc. lactis 12.1	0.60±0.04[a]	1.17±0.07[a]
L. plantarum 18.2	0.47±0.01[b]	1.49±0.03[b]
Lc. lactis 5.12	0.37±0.04[c]	1.89±0.22[c]
E. faecium 16.1	0.13±0.02[d]	5.63±0.95[d]

Means within a column with different superscripts differ significantly ($p<0.05$)

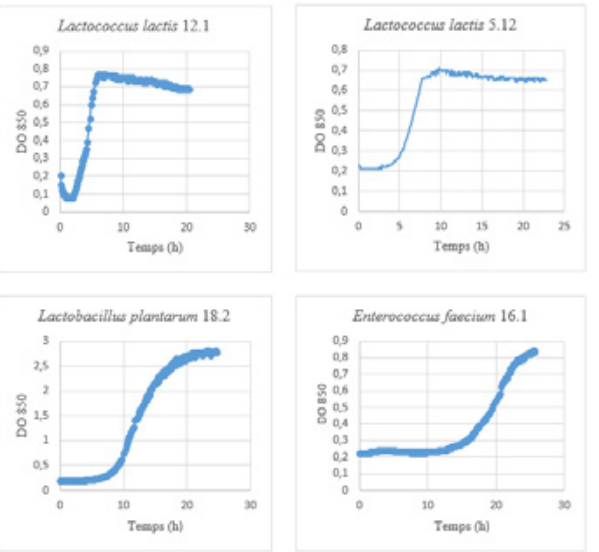


Figure 2. *Growth curves generated from the Biosan RTS-1C bioreactor for strains Lc. lactis 12.1, L. plantarum 18.2, Lc. lactis 5.12, and E. faecium 16.1*

Inocula quantification

The histogram below (Figure 3) presents the enumeration results of the selected bacterial strains and whey used for ferment development after an 18-hour incubation in MRS culture medium.

The enumeration results of inocula showed high counts with significant differences ($p<0.05$) (Figure 3). Notably, the whey exhibited an exceptionally high count of 9.04 log CFU/g, likely due to the presence of multiple autochthonous strains from the previous day's curd. In contrast, the pure selected strains - *Lc. lactis* 12.1, *L. plantarum* 18.2, *E. faecium* 16.1, and *Lc. lactis* 5.12 - showed slightly lower counts of 8.77, 8.59, 8.35, and 8.02 log CFU/g, respectively.

Microbiological quality of raw milk

The microbiological criteria for total aerobic mesophilic flora in raw milk vary by country, with thresholds set at

4.7 log cfu/mL in Belgium and France, and 5 log cfu/mL in Algeria. Our study showed a higher value of 5.7 log cfu/mL, exceeding these standards, likely due to the milk being from the previous day's milking and refrigerated. Currently, in Morocco, *Salmonella* spp. is the sole microorganism routinely monitored in raw milk. In this study, neither *Listeria monocytogenes* nor *Salmonella* spp. were detected in the milk samples, which aligns with the established safety standards.

Microbiological and physicochemical results of Jben cheese production

Enumeration of yeasts and molds

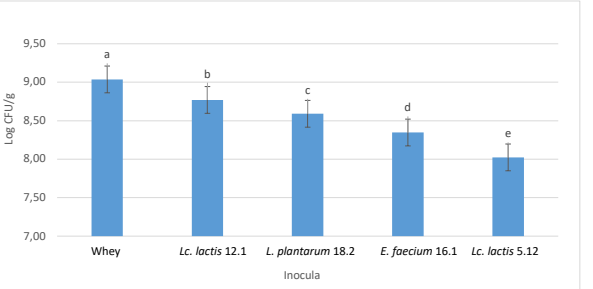
The cheeses were moderately contaminated with yeasts and molds (less than 5 log CFU/g), with significant differences observed between batches on the 1st and 5th days of storage ($p<0.05$) (Figure 4). The use of unpasteurized and refrigerated milk likely contributed to the initial contamination. Interestingly, the fifth batch, which included whey, exhibited different yeast and mold morphology (results not shown) compared to the batches with autochthonous strains, suggesting potential contamination from the whey or the equipment. The average count in this batch decreased significantly ($p<0.05$) from day 1 to day 5, reaching the lowest average of 3.67 log CFU/g, indicating possible antagonistic effects of the autochthonous microbiota in whey on yeasts and molds. In contrast, the batches made with autochthonous strains all showed an increase in yeast and mold counts, although they did not reach a significant difference threshold. Nevertheless, the values reported for the experimental batches remain much lower than those reported for commercial *Jben* cheeses (7.15 log CFU/g vs. 3.48 log CFU/g) (Azzouz et al., 2024), emphasizing the stricter hygiene practices implemented during controlled production.

Physicochemical results

The data in Table 2 present physicochemical parameters of produced cheeses, analyzed at two different time points: the first day (T1) and the fifth day (T5) of storage.

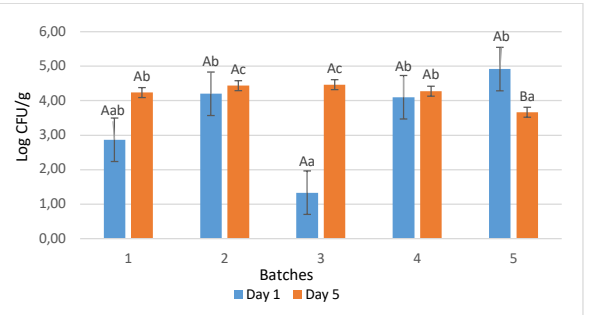
The pH measurements revealed significant variations among the different batches on both the first and fifth days, as well as within batches 3 and 5 between day 1 and day 5. Indeed, Batch 3 was the only one to experience a pH decrease, from 4.07 to 4.01. In contrast, batch 5 showed a significant pH increase, from 4.07 to 4.22. Batches 1, 2, and 4 maintained relatively stable pH values during the storage period, with a slight, non-significant increase.

Acidity assessment revealed significant differences ($p<0.05$) exclusively between batch 5 and batches 1, 3, and 4 on the fifth day of storage. Over time, a significant difference ($p<0.05$) was observed in batches 2, 3, and 5. Initially, average acidity levels were around 2.12, decreasing to stabilize at an average of 1.93 by the fifth day. The acidity levels achieved in our cheeses were comparable to those of commercial *Jben* cheeses analyzed in a previous study (Azzouz et al., 2024), highlighting the adequacy of the manufacturing process. Lactic acid concentration decreased over time due to lactose consumption by lactic bacteria and its release into whey.



Different letters above the histogram bars indicate statistically significant differences in inoculum counts ($p < 0.05$).

Figure 3. *Quantification of individual LAB strains for inclusion in lactic starter cultures and whey LAB*



Different uppercase letters above the bars represent significant temporal (between day 1 and 5) differences in yeast and mold counts ($p<0.05$), while lowercase letters indicate significant differences between batches in yeast and mold counts on the same day of analysis (Day 1 or Day 5) ($p<0.05$). The numbers on the horizontal axis represent the following cheese batches: 1 = Cheese made with Lc. lactis 12.1 and Lc. lactis 5.12; 2 = Cheese made with Lc. lactis 5.12 and E. faecium 16.1; 3 = Cheese made with Lc. lactis 12.1 and L. plantarum 18.2; 4 = Cheese made with Lc. lactis 5.12, Lc. lactis 12.1, E. faecium 16.1, and L. plantarum 18.2; 5 = Whey (control batch)

Figure 4. *Evolution of the yeast and mold averages in the five cheese batches between the first and fifth day of storage*

This evolution may also be influenced by the presence of yeasts and molds in the cheese, as these microorganisms contribute to the degradation of lactic acid, leading to its decline over time (López-Díaz et al., 2023). Moreover, the high acidity observed in batch 5 from the first day of storage can be attributed to the significantly higher concentration of lactic bacteria found in the whey (9.04 log CFU/g) compared to other cultures (8.02-8.77 log CFU/g).

The dry matter results indicate significant differences both between batches on days 1 and 5 and in their evolution over time ($p<0.05$). On day 1, dry matter levels were low, ranging from 31.79 % to 36.01 %. This was influenced by factors such as moderate salting, extended coagulation, the absence of mechanical drainage techniques, and ladle molding. However, these levels were comparable to those of commercial *Jben* cheeses (Azzouz et al., 2024). Batches produced from autochthonous strains showed higher dry matter percentages on day 1, ranging from 35.53 % to 36.01 %, which differed significantly from that of the fifth batch, which

had the lowest dry matter content at 31.79 %. This difference can be attributed to the higher water content of whey (used as inoculum in batch 5) compared to the autochthonous starter cultures grown in milk, as well as to the varying lactic acid concentrations between batches, which affect whey expulsion. Indeed, by day five, the cheese with the highest lactic acid content (2.02 g/100 g) also had the highest dry matter content (41.3 %). Additionally, dry matter levels increased significantly ($p<0.05$) between day 1 and day 5 across all batches, due to factors such as salt, humidity, temperature, and enzymatic changes during storage, which influence interactions among casein molecules (Johnson, 2003; Leclercq-Perlat, 2015; Hay, 2017).

The non-statistical differences in lipid and protein percentages are due to the identical manufacturing method and milk used for all batches. Protein percentages ranged from 7.65 to 8.80 g/100 g on day 1 and from 8.81 to 10.15 g/100 g on day 5. Lipid values fluctuated between 16.5 and 19.4 g/100 g on day 1 and between 19.3 and 20.7 g/100 g on day 5. The increase from day 1 to day 5 is due to the rise in dry matter over time. The protein levels in the four batches were lower than those in Provola Dei Nebrodi, Kars Gravyer, Galichki kashkaval and Kazakh cheeses (Santa and Srbinovska, 2014, Çetinkaya and Öz, 2019, Randazzo et al., 2021; Li et al., 2021). Lipid contents were also lower than those reported in other studies (Kalavrouzioti et al., 2005; Lesic et al., 2016; Brandielli et al., 2019). These differences are due to the types of milk, manufacturing methods, dry matter content, and other production parameters.

Regarding ash content, most batches showed a significant increase ($p<0.05$) from day 1 to day 5, except for the second batch. On average, ash percentages increased from 1.18 g/100g on day 1 to 1.45 g/100 g on day 5, primarily due to the increase in cheese dry weight during storage. Significant differences between samples ($p<0.05$) were also observed on both the first and last days of storage, likely because ash quantification is based on total matter rather than solely on dry weight. This effect is particularly noticeable in batch 5, which had a higher water content, resulting in relatively lower ash concentrations compared to other batches. Our cheeses had lower ash content than commercial *Jben* cheeses analyzed in a previous study (Azzouz et al., 2024) and other cheeses like Halitzia and Diyarbakir Örgü (Papademas et al., 2019; Hatipoglu and Çelik, 2020). These differences arise from inherent variations in manufacturing processes such as salting, curd pressing, and dry matter content.

Table 2. *Physicochemical analysis results of the five cheese batches*

Parameters	Days	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	P value
pH	T1	4.20 ± 0.00^{Ad}	4.14 ± 0.00^{Ac}	4.07 ± 0.00^{Aa}	4.08 ± 0.00^{Ab}	4.07 ± 0.00^{Aa}	p<0.05
	T5	4.22 ± 0.04^{Ac}	4.17 ± 0.01^{Abc}	4.01 ± 0.06^{Ba}	4.09 ± 0.06^{Ab}	4.22 ± 0.03^{Bc}	p<0.05
Acidity (g/100 g of DM)	T1	2.07 ± 0.08^{Aa}	2.12 ± 0.03^{Aa}	2.15 ± 0.02Aa	2.08 ± 0.06^{Aa}	2.16 ± 0.14^{Aa}	p>0.05
	T5	1.94 ± 0.09^{Ab}	1.92 ± 0.05^{Bab}	1.99 ± 0.07Bb	2.02 ± 0.07^{Ab}	1.82 ± 0.03^{Ba}	p<0.05
Dry matter (%)	T1	35.74 ± 0.84^{Aa}	35.53 ± 0.06^{Aa}	36.01 ± 0.33^{Aa}	35.72 ± 0.42^{Aa}	31.79 ± 1.78^{Ab}	p<0.05
	T5	39.20 ± 0.68^{Bab}	40.98 ± 0.84^{Bc}	40.42 ± 0.33^{Bbc}	41.28 ± 0.68^{Bc}	39.01 ± 0.89^{Ba}	p<0.05
Fat content (%)	T1	18.60 ± 0.85^{Aa}	19.10 ± 1.32^{Aa}	19.43 ± 0.84^{Aa}	18.80 ± 0.82^{Aa}	16.47 ± 1.68^{Ab}	p>0.05
	T5	19.57 ± 1.50^{Aa}	20.53 ± 1.27^{Aa}	20.43 ± 0.75^{Aa}	20.70 ± 1.48^{Aa}	19.33 ± 1.63^{Aa}	p>0.05
Protein content (%)	T1	7.65 ± 0.06^{Aa}	8.40 ± 0.00^{Aab}	9.30 ± 0.76^{Ab}	$8.80 \pm 1.07A^{ab}$	7.69 ± 0.00^{Aa}	p>0.05
	T5	9.12 ± 1.27^{Aa}	10.15 ± 2.21^{Aa}	9.74 ± 0.51^{Aa}	9.39 ± 1.01^{Aa}	8.81 ± 0.06^{Ba}	p>0.05
Ash (%)	T1	1.30 ± 0.06^{Ac}	1.20 ± 0.07^{Abc}	1.14 ± 0.09^{Aab}	1.24 ± 0.11^{Abc}	1.01 ± 0.02^{Aa}	p<0.05
	T5	1.56 ± 0.08^{Bc}	1.29 ± 0.06^{Ab}	1.62 ± 0.06^{Bc}	1.65 ± 0.09^{Bc}	1.12 ± 0.05^{Ba}	p<0.05

DM=Dry matter; The capital letters indicate differences between Day 1 and Day 5 for the same parameter and the same cheese batch, while lowercase letters represent differences between batches for the same parameter, in the same day. Batch 1 = cheese made with strains Lc. lactis 5.12 and 12.1; Batch 2 = cheese made with strains Lc. lactis 5.12 and E. faecium 16.1; Batch 3 = cheese made with strains Lc. lactis 12.1 and L. plantarum 18.2; Batch 4 = cheese made with a combination of all 4 strains; Batch 5 = cheese made with whey.

Table 3. *Sensory evaluation of the five cheese batches*

Criteria/Batches	1	2	3	4	5	p value
External appearance	5.9	5.9	5.3	5.8	6.5	0.48
Internal appearance	6.1	5.4	5.9	6.0	6.8	0.61
Texture	5.8	5.8	5.9	5.6	5.9	0.51
Flavor	5.6	5.3	5.8	5.6	5.3	0.79
Aroma	5.4	5.6	5.8	5.6	5.1	0.58
Aftertaste	5.6	5.3	5.3	6.0	4.9	0.83
Overall appreciation	7.3	7.0	7.4	8.0	7.1	0.20

The numbers in the first row of the table correspond to the following cheese batches: 1 = Cheese made with Lc. lactis 12.1 and Lc. lactis 5.12; 2 = Cheese made with Lc. lactis 5.12 and E. faecium 16.1; 3 = Cheese made with Lc. lactis 12.1 and L. plantarum 18.2; 4 = Cheese made with Lc. lactis 5.12, Lc. lactis 12.1, E. faecium 16.1, and L. plantarum 18.2; 5 = Whey (control batch). The results represent the average scores from eight panelists, using a 7-point hedonic scale
(1 = undesirable, 7 = desirable), for each attribute of the five Jben cheese batches. The p-values were generated by the chi-square test

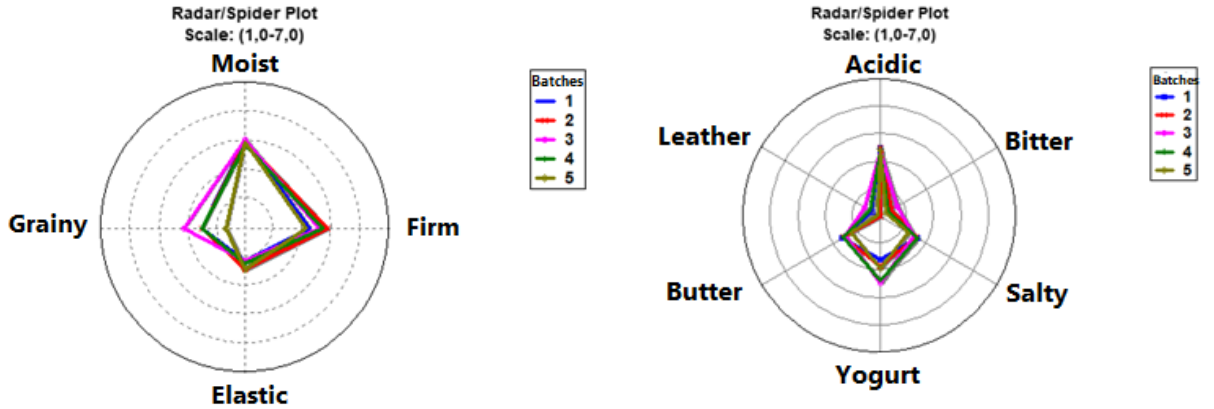


Figure 5 (a) *Spider plot illustrating texture characteristics of the produced cheeses. (b) Spider plot illustrating aroma and flavor characteristics of the produced cheeses*

Figure 5a illustrates the texture characteristics of the five cheese batches, with average scores given by eight panelists on a 1 to 7 scale (1 = low intensity, 7 = high intensity). Figure 5b depicts the aroma and flavor attributes, also based on average ratings from the eight panelists on a 1 to 7 scale. The points on the graphs represent the mean scores for each characteristic across the batches. The numbers on the right of the spider plots correspond to the different cheese batches 1 = Cheese made with Lc. lactis 12.1 and Lc. lactis 5.12; 2 = Cheese made with Lc. lactis 5.12 and E. faecium 16.1; 3 = Cheese made with Lc. lactis 12.1 and L. plantarum 18.2; 4 = Cheese made with Lc. lactis 5.12, Lc. lactis 12.1, E. faecium 16.1, and L. plantarum 18.2; 5 = Whey (control batch).

Sensorial results

Table 3 summarizes the average scores assigned by eight panelists for the five batches of *Jben* cheese produced.

Chi-square statistical analysis revealed that variations in the sensory criteria of the cheeses are not conclusively attributed to differences in inocula ($p<0.05$). Similar conclusions were found for Ibores and Pecorino Siciliano cheeses (Gonzalez et al., 2003; Guarcello et al., 2016). However, the general appreciation criterion had the lowest p-value, indicating greater variability in overall evaluations. The batch with four native strains had the highest average score. While overall differences were not statistically significant, detailed sensory analysis revealed subtle preferences among tasters. Batch 5 stood out in texture due to higher moisture content, resulting in a creamier texture similar to *Jben* cheese, while other batches were firmer and less moist, likely due to higher lactic acid production. Batches made with native strains were favored for flavor, aroma, and aftertaste, likely due to their aromatic and acidifying properties. All batches, except for batch 2, received higher overall appreciation scores than the whey batch (N°5). The lower score for batch 2, produced with enterococcal strain 16.1, is likely due to an ethanol smell noted by some tasters, which contributed to its reduced appreciation. Indeed, Giraffa (2006) has already shown that enterococci are known to produce these types of flavors. However, the highest score for batch 4 suggests a synergistic aromatic effect from the four strains. Batch 3's high score indicates strain 18.2's significant role in aroma and flavor, aligning with El Galiou et al. (2023), who found *Lactobacillus* spp. strains enhance aroma and flavor in the produced cheeses.

A focused analysis using spider plots provided deeper insights into the cheeses' sensory characteristics, emphasizing key textural and gustatory aspects.

The data represented in the first spider plot (Figure 5a) describe the textural characteristics of the five produced cheeses, rated from 1.9 to 5.0 (on a 1 to 7 scale). According to the results, batch 5 was found to be the least firm, with a notably lower rating compared to the other batches (3.8). Conversely, batch 2 demonstrated the highest firmness (4.8). Regarding granularity, batch 3 received the highest rating, with a score of 3.8. In contrast, batch 5 received the lowest rating (1.9). Regarding moisture and elasticity, these two characteristics showed relative stability across the different batches.

The second spider plot (Figure 5b) represents the attributes related to flavor and aroma. The points on the graph indicate the values assigned to each characteristic for each batch of cheese, with ratings ranging from 1.0 to 4.1 (on a scale of 1 to 7). Overall, acidity and salinity levels appear relatively balanced across all batches, except for batch 5, which received the lowest score for salinity. However, the bitterness criterion stood out significantly in the third batch, receiving a noticeably higher rating than the others. Butter aroma was detectable at similar intensities in most batches, except for batch 5, where it was notably subdued. Batches 3 and 4 stood out with a more pronounced "yoghurt" characteristic, unlike batch 1, where this trait was less marked. Regarding the "leather" attribute, it was more prominent in batch 3, while it was less noticeable in batches 2 and 5.

The detailed analysis of sensory criteria using Table 4 provided additional insights. For texture attributes, the high scores for moisture can be attributed to the curds not being pressed, overly salted, or cut into small pieces during the production process. However, the low score for elasticity could be explained by the fact that the curds were not stretched, in accordance with the traditional methods of *Jben* production. Regarding granularity, the higher scores for the experimental batches are likely due to the increased lactic acid content in these batches compared to the control batch.

Table 4. Scores of organoleptic characteristics attributed to the produced cheeses by the panellists

Organoleptic characteristics	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	p value
Flavors						
Acidity	4.1±1.2	4.1±1.5	3.9±1.8	3.9±1.8	4.0±1.8	0.91
Saltiness	3.0±1.3	2.9±1.1	2.8±1.3	3.0±1.1	2.5±1.2	0.98
Bitterness	1.4±0.7	1.6±0.9	1.9±1.6	1.4±0.7	1.3±0.5	0.63
Aromas						
Yoghurt	3.0±1.4	3.3±1.7	4.0±1.9	3.9±1.8	3.4±1.7	0.83
Butter	3.0±2.1	2.9±2.0	2.8±1.8	2.9±1.9	2.5±2.0	0.99
Leather	1.4±0.7	1.0±0.0	1.8±1.5	1.5±1.1	1,1±0.4	0.64
Texture						
Moisture	5.0±1.1	4.9±1.1	5.0±1.1	4.8±1.0	4.9±1.6	0.97
Firmness	4.0±1.9	4.8±1.5	4.4±1.3	4.6±1.5	3.8±2.1	0.88
Granularity	3.0±1.6	3.0±1.7	3.8±2.1	3.0±1.7	1.9±1.5	0.42
Elasticity	2.5±1.5	2.9±1.6	2.5±1.4	2.6±1.6	2.9±1.6	0.97

The scores represent the averages of 8 values given by 8 testers. The results were subjected to a chi-square test, with the p - value indicated in Table 4.

For flavors and aromas, the high scores for yoghurt-like notes in batches 3 and 4 reflect the strong aromatic properties of the *L. plantarum* 18.2 strain. As for the butter note, it was high in all experimental batches made with autochthonous strains compared to the control, suggesting a notable influence of the indigenous strains on this characteristic flavor. Furthermore, the low score for saltiness in batch 5 can be attributed to its high moisture content, which diluted the relative salt concentration, resulting in a less salty perception. Lastly, the relatively high bitterness score in batch 3 is likely due to excessive proteolysis, as unbalanced proteolysis can cause the accumulation of bitter peptides, negatively impacting the overall flavor of the cheese (Smit et al., 2005).

Conclusion

This study highlights the impact of autochthonous starter cultures on the microbiological, physicochemical and sensorial characteristics of *Jben* cheese. The cheeses produced in this study exhibited lower levels of yeast and mold compared to commercially available ones, indicating good hygiene practices. Regarding physicochemical properties, moisture and acidity levels were significantly influenced by the different types of cultures used, highlighting the cheese-making abilities of these strains and suggesting opportunities for production improvements. Incorporating autochthonous strains did not lead to significant variations in sensory properties. However, batches made with these strains stood out with higher scores for flavors, aromas, and aftertaste. Additionally, the cheese produced from a combination of all selected strains received the highest overall appreciation score, indicating a positive impact on the sensory quality of *Jben* cheeses. Future research should focus on producing *Jben* cheese with pasteurized milk to ensure safety and assess the true impact of isolated autochthonous strains on the cheese-making process. Additionally, adjusting bacterial concentrations could help balance moisture and acidity, achieving a texture similar to traditional *Jben* while minimizing bitterness caused by excessive proteolysis. Finally, exploring optimal preservation methods for these strains will also support their large-scale application in the cheese industry.

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Evaluacija autohtonih bakterija mliječne kiseline za proizvodnju marokanskog *Jben* sira

Sažetak

Ovo istraživanje imalo je za cilj razviti različite starter kulture iz autohtonih sojeva bakterija mliječne kiseline marokanskog sira Jben i procijeniti njihove učinke na fizikalno-kemijska, mikrobiološka i senzorska svojstva sira proizvedenog u eksperimentalnim uvjetima. U istraživanju je korišteno ukupno devet sojeva bakterija mliječne kiseline, prethodno izoliranih i odabranih na temelju njihovih sposobnosti zakiseljavanja, proteolitičkih i aromatskih svojstava. Ispitivanjem kompatibilnosti i analizom glavnih komponenti identificirana su četiri soja, koja su dodatno procijenjena za svoje kinetičke parametre. Ti sojevi su zatim kombinirani u različite starter kulture kako bi se proizvela četiri eksperimentalna uzorka Jben sira, dok je peti uzorak proizveden korištenjem tradicionalnog načina kao kontrole. Ispitivanje antagonizma otkrilo je nekompatibilnost između sojeva *Lactiplantibacillus* i *Lactococcus lactis* 5.12 i 17.6. Kinetička analiza pokazala je da je soj *Lc. lactis* 12.1 imao najveću stopu rasta (0,60±0,04) i najkraću lag fazu. Mikrobiološka analiza otkrila je umjerenu kontaminaciju kvascima i plijesni u pokusnim sirevima, s razinama nižim nego kod komercijalnih Jben sireva. Fizikalno-kemijska analiza pokazala je značajne razlike u sadržaju pepela, suhe tvari i kiselosti između uzoraka, ovisno o različitim kulturama koje su se koristile, dok su sadržaji proteina i lipida ostali konzistentni. Senzorske procjene nisu ukazale na značajne razlike među sirevima, no kontrolni uzorak ocijenjen je višom ocjenom za teksturu, dok su sirevi s autohtonim sojevima imali bolje ocjene za aromu, okus i ukupnu preferenciju.

Ključne riječi: autohtoni fermenti; testiranje kompatibilnosti; kinetika rasta bakterija; standardizacija tradicionalnih sireva; senzorska evaluacija

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