Research article

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Revista Facultad Nacional de Agronomía

Impact of ripening environment and curd processing on the quality of artisanal Edam-type cheese

Impacto del entorno de maduración y el procesamiento de la cuajada sobre la calidad del queso artesanal tipo Edam

https://doi.org/10.15446/rfnam.v79.121856

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Keywords:
Microbiology
Moisture content
Proteolysis
Semi-hard cheese
Volatile compounds

CITATION: Costain Ramírez JE, Sepúlveda Valencia JU, Arango Bedoya O and Restrepo Molina DA (2026) Impact of ripening environment and curd processing on the quality of artisanal Edam-type cheese, Revista Facultad Nacional de Agronomía Medellín 79: e121856. doi: https://doi.org/10.15446/rfnam.v79.121856

ABSTRACT

Artisanal cheese production in Colombia is a critical economic and cultural pillar in various regions. Nevertheless, this sector is susceptible to persistent challenges, primarily due to the dominance of small-scale informal enterprises that hinder product diversification. Within this context, this study evaluated the effect of ripening conditions (controlled chamber versus natural environment) and curd processing methods (washed curd and cooked curd) on the physicochemical, microbiological, textural and aromatic characteristics of artisanal Edam-type cheese during 30 days, exploring alternatives to revitalize the regional dairy industry. A split-plot model with a factorial arrangement was used. Moisture, fat, protein, ash, sodium, lactose, proteolysis indices, microbiological quality, texture profile, and volatile organic compounds (VOCs) were observed. The cheeses ripened in a natural environment exhibited higher moisture content (44.4 vs. 43.5%), increased formation of soluble protein (6.97 vs. 6.52%), and a higher ripening index (RI) (26.73 vs. 24.61%). These treatments also showed a lower lactose content (36.29 vs. 40.50 mg kg^{-1}), more adhesive texture (maximum 20.13 vs. 11.44 N s), and safe microbiological counts at the end of the ripening period, compared to cheeses ripened in a controlled chamber. The results showed that optimizing local environmental conditions for the ripening of high-quality cheeses is a strategy with high potential for sustainability and profitability, as well as providing the capacity for direct technological and operational transfer to small and medium-sized artisanal industries.

Palabras clave:
Microbiología
Contenido de humedad
Proteólisis
Queso semiduro
Compuestos volátiles

RESUMEN

La producción de queso artesanal en Colombia es un pilar económico y cultural fundamental en diversas regiones. Sin embargo, este sector enfrenta desafíos significativos, principalmente por el predominio de pequeñas industrias informales que limitan la diversificación de sus productos. En este contexto, este estudio tuvo como objetivo evaluar el efecto de la maduración (en cámara controlada y ambiente natural) y del procesamiento de la cuajada (lavada y cocida) sobre las características fisicoquímicas, microbiológicas, texturales y de calidad aromática del queso artesanal tipo Edam durante 30 días, buscando alternativas para dinamizar la industria láctea regional. Se utilizó un modelo de parcela dividida con arreglo factorial. Se observaron la humedad, la grasa, las proteínas, las cenizas, el sodio, la lactosa, los índices de proteólisis, la calidad microbiológica, el perfil de textura y los compuestos orgánicos volátiles (COV). Los quesos madurados en condiciones ambientales naturales exhibieron una humedad ligeramente superior (44,4 frente a 43,5%), un aumento de las proteínas solubles (6,79 frente a 6,52%) y un índice de maduración más alto (IM) (26,73%) en comparación con los quesos madurados en cámara (24,61%). Estos tratamientos también mostraron un menor contenido de lactosa residual (32,29 frente a 40.50 mg kg^{-1}) y una textura más adhesiva (20,13 frente 11,44 N s) y recuentos microbiológicos seguros al final de la maduración, en comparación con los madurados en cámara controlada. Los resultados revelan un alto potencial para aprovechar las condiciones ambientales locales para el desarrollo de quesos madurados de alta calidad, convirtiéndose una estrategia sostenible y rentable que ofrece alternativas directamente transferibles a las pequeñas y medianas industrias artesanales.

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Received: September 18, 2025; Accepted: February 14, 2026
Rev. Fac. Nac. Agron. Medellín 79: e121856. 2026
ISSN 0304-2847 / e-ISSN 2248-7026

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The dairy chain is a crucial part of the global economy. It has a well-defined, high-value market, showing a growth of at least 10% between 2015 and 2021 (Nogueira et al. 2021). In Colombia, cheese production and consumption have increased by 50% in the 5 years, reaching 1.52 kg per capita, a figure surpassed only by milk. This production is carried out by small and medium-sized enterprises that use artisanal processes (50% of the industry is informal). Traditional techniques give the products special quality characteristics, giving them export potential (UPRA 2021; Asoleche 2023).

Edam-type cheese comes from the Netherlands and it is manufactured from partially skimmed raw cow's milk, resulting in a semi-hard cheese, brine-salted, and considered versatile in artisanal production (Amiri et al. 2022). This variety is characterized by a smooth texture, a deep yellow curd and distinctly mild, nutty flavor profile. Pasteurization processes are used to reduce the pathogens load and enzymatic activity that affect the safety and shelf life of the cheese. However, mesophilic starter cultures are added to control the ripening processes (Ahmed et al. 2023). This process must be gradual to prevent significant protein denaturation and excessive activation of indigenous enzymes (plasmin and cathepsin), as these can adversely impact cheese quality during storage (Rabbani et al. 2025). In semi-hard cheeses, the treatment of washed curd is performed to control lactose levels, regulate lactic acid formation, modify texture and color; furthermore, it influences proteolysis, rheology, and sensory characteristics (Natrella et al. 2023). Moreover, the ripening process is conducted in a specific environment where synergistic temperature and humidity levels. Alongside, the indigenous microbial consortia promote physicochemical, microbiological and biochemical pathways responsible for the formation of primary and secondary metabolites. These compounds established a unique and distinct sensory profile, defining the fundamental attributes of the cheese (De Santis and Fidaleo 2022).

Cheese ripening has garnered significant scientific and technical interest due to its protracted kinetics and high operational costs (Helal and Tagliazucchi 2023), representing up to 60% of production costs (Teixeira et al. 2020). Consequently, the acceleration of the ripening period has been extensively investigated through the incorporation of exogenous enzymes, modified starter cultures, adjunct cultures, and the modulation of thermal and high-pressure treatments. These methodologies diverge from traditional ripening techniques typically conducted in controlled environments with temperatures ranging from 8 to 14 °C and relative humidity (RH) levels between 74 and 90% (Cardinali et al. 2022; Helal and Tagliazucchi 2023). This creates alternatives for ripening under environmental conditions in the high Andean zone of the municipality of El Contadero, reducing energy consumption associated with the cheese-making process (Corrieu et al. 2018). In this way, the aim of this study was to evaluate the effect of the curd type and the ripening environment about the physicochemical, microbiological, and textural characteristics of Edam-type cheese to reduce production costs.

In this context, it is expected that ripening under a high-Andean bioclimatic environment (low temperatures and high relative humidity), coupled with the technological conditioning of the curd (washed-curd and cooked-curd) can modulate the residual lactose concentration and the initial moisture content of the cheese. These conditions promote optimal enzymatic activity and facilitate controlled, progressive proteolysis and lipolysis. Consequently, there is an increase in the availability of precursors for volatile compounds responsible for flavor and aroma development. At the same time, a compact protein matrix would be preserved, exhibiting a uniform and cohesive texture, as well as a sensory profile comparable to that obtained in ripening systems operated under controlled conditions.

MATERIALS AND METHODS

Raw material

Fresh cow's milk was obtained from the Asociación de Lácteos Contadero (Asolacc) cooling plant, municipality of El Contadero (0°54'40.4 N 77°33'02.2" W), located at an altitude of 3,100 meters above sea level (masl), with a temperature of 6 to 18 °C and of 88 to 92% relative humidity (RH), characteristic of a specialized dairy production region. The temperature and RH were monitored with a digital thermohydrometer (UNI-T UT330TH) every 2 hours. The milk used was batch-characterized prior to process. The milk exhibited a pH of

6.72, total solids (TS) of 12.16%, total protein of 3.36%, fat of 3.28%, lactose of 4.53%, aerobic mesophiles of 7.15×10^4 CFU (Colony Forming Units) mL^{-1}, and somatic cells of 3.35×10^5 cs mL^{-1}.

Cheese making

Three batches of 100 L were produced at the processing plant of the Centro Internacional de Producción Limpia Lope, SENA (CIPLL - 1°12'24.6"N 77°15'06.5" W), yielding 60 cheeses weighing approximately 500 g each. The manufacturing process applied was adapted as described by Helal and Tagliazucchi (2023). The three production batches (n=3 x 100 L) were manufactured using milk from the same source. The milk was standardized to 2.8% fat content. Subsequently, uniform LTLT (low-temperature, long-time) pasteurization protocol was applied at 65±2 °C for 30 min. This thermal treatment was performed identically across all batches prior to curd differentiation (washed curd and cooked curd). The milk was cooled to 35 °C and inoculated with commercial starter lactic culture composed of *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *Lactis* which was added to act for 30 min. The starter culture presented a microbial activity of $\geq 5.0\times10^{10}$ CFU g^{-1} according to the manufacturer. Then, $CaCl_2$ (0.02%), KNO_3 (0.015%) and annatto (0.005%) were added before coagulation stage Chymosin rennet (CHY-MAX Powder Extra-NB, Chr Hansen, 2235 IMCU g^{-1}) was applied for 40 min. Washed curd was heated indirectly to 39 °C (0.7 °C min^{-1}). This process involved the removal of 30% of the whey, replaced by the addition of water at 65 °C until the target temperature was reached (39 °C). Furthermore, cooked curd was heated directly in the presence of all the whey at a rate of 0.7 °C min^{-1} until a final temperature of 39 °C was reached. The curd was pressed hand-crafted in stainless steel molds using 12.75 kPa. The obtained cheeses were placed in saturated brine (20% w/v; pH=5.5; 6 °C) for 7 hours. The cheeses were ripened for 30 days, in controlled chamber (10 °C and 90% RH) and natural environment (10.6 to 18 °C and 82.7 to 94.1% RH).

Experimental treatments

The experiment was structured as a split-plot design within a randomized complete block framework. Each independent milk batch (100 L) constituted one block. The trials were conducted on different days to control for variability inherent to the raw material, including the physicochemical composition and hygienic–sanitary quality. The 'curd type' factor (washed-curd and cooked-curd) was randomly assigned to the main-plots within each block. The 'ripening environment' factor (natural environment and controlled chamber) was arranged in subplots within each main plot. Consequently, the cheeses produced from half of the milk volume per batch were distributed between both ripening conditions. For this purpose, three milk batches were processed to function as block factors. The design comprised six main plots and 60 individual cheeses, which were treated as analytical subsamples to increase the precision of the response variable estimations. The cheeses from each batch were organized as: five of washed curd in a natural environment (WCNE), five of washed curd in a controlled chamber (WCCC), five of cooked curd in a natural environment (CCNE), and five of cooked curd in a controlled chamber (CCCC).

Sampling

Physicochemical and textural analyses were performed at 0, 15, and 30 days of ripening. For each sampling point, one-half of the cheese was taken as a representative sample for the analytical procedures. Microbiological loads s was assessed at days 0 and 30 of ripening using the remaining half of the cheese. The physicochemical and textural evaluations were conducted in triplicate, while the microbiological analyses were performed in duplicate. Specifically, the determination of volatile compounds (VOCs) was conducted in duplicate.

Physicochemical analyses in cheese

The following physicochemical characteristics were determined: moisture content; water activity (a_w) and ash; titratable acidity through alkaline titration with 0.1 N NaOH according to the methodology proposed by AOAC (1990); pH using an insertion electrode (HI99161, Hanna, Romania); fat content using the Van Gulik method; total protein using the Kjeldahl method multiplied by 6.38 (AOAC 1990); soluble protein at pH=4.6 as proposed by Moavro (2019) through extraction with sodium citrate, precipitation with glacial acetic acid, and measurement by micro-Kjeldahl, and NaCl indirectly through the determination of Na^+ ions using atomic absorption spectrometry according to NTC 5151 (ICONTEC 2003). The ripening index (RI) was calculated as the ratio of

soluble protein content to total protein content, as shown in Equation 1.

$$RI(\%) = \frac{P_{Soluble}}{P_{Total}} \times 100 \quad (1)$$

Where $P_{soluble}$ is the soluble protein (%), and P_{Total} is the total protein (%).

Lactose was determined through high-performance liquid chromatography (HPLC) coupled with a refractive index detector (RID). For sample preparation, to 5 g of cheese, 15 mL of deionized water at 70 °C were added. The mixture was sonicated for 5 minutes and homogenized with an Ultraturrax for 2 minutes. The sample was diluted with deionized water up to 25 mL, centrifuged at 5,500 rpm for 10 minutes at ambient temperature and then, using the same parameters, a second centrifugation was performed at 4 °C. From the supernatant, 500 µL were taken and mixed with 50 µL of Carrez I, 50 µL of Carrez II, and 900 µL of deionized water to prepare 1.5 mL of solution to control the dilution factor (Monti et al. 2017). When a dense precipitate was present, the sample was centrifuged for 10 minutes and filtered through a nylon membrane (0.22 µm). The filtered solution was injected into an HPLC system equipped with an Aminex HPX-87H column (300 mm × 7.8 mm × 9 µm), with H_2SO_4 as the mobile phase, an injection volume of 15 µL, flow rate of 0.6 µL min^{-1}, and oven temperature of 65 °C for 35 min. Lactose content was determined from the calibration curve based on the dilution of the lactose standard. The concentration range was established between 50 and 3,000 mg kg^{-1}, with an adequate linear relationship (r^2>0.99). The limit of detection (LOD) and the limit of quantification (LOQ) were estimated at 0.98 and 3.27 mg L^{-1}, respectively. No co-eluting interferences were observed at the lactose retention time. The results were expressed in mg kg^{-1} of cheese on a wet basis as indicated in Equation 2.

$$L = C \times 0.025 \times \frac{1{,}000}{m} \quad (2)$$

Where L is lactose (mg kg^{-1}), C is lactose concentration in the sample (mg L^{-1}), 0.025 is the volume capacity, and m is the mass of the cheese sample in grams.

Volatile organic compounds (VOCs) determination

VOCs were studied through gas chromatography coupled to a mass spectrometry (detector GCMS - QP2010S Shimadzu, Kyoto, Japan) with a spitless injector operating at 250 °C. For absorption, 5 g of cheese was left in the headspace for 50 min at 45 °C, inserting an SPME (Solid-Phase Microextraction) head equipped with a DVB/CAR/PDMS, Stableflex (Gray-Notched) fiber. Desorption was performed directly in the GC injector, equipped with a DB-WAX capillary column (30 m × 0.25 mm × 0.25 µm), using helium UHP (Ultra High Purity) as the carrier gas and a column flow rate of 1 mL min^{-1}. A temperature gradient was performed for 50 min, starting at 40 °C for 3 min, with an increase of 5 °C min^{-1} up to 150 °C, then at 10 °C min^{-1} up to 250 °C with 15 min as retention period. To ensure the absence of significant carry-over, blank analyses were performed every five samples. Analytical conditions were maintained constant for all injections, and results were expressed as relative abundances due to the lack of an internal standard. The exploratory identification of VOCs was performed by comparing the retention indices and the experimental mass spectra (match factor >80%) with those reported in the NIST-11 and Wiley 9 mass spectral libraries.

Texture profile analysis (TPA)

TPA was performed using a texture analyzer (Lloyd LS1SH Ametek) equipped with a 30 mm diameter compression plate. Cubes of 15 mm were cut 2 mm from the cheese rind and left at ambient temperature for 30 min to apply two consecutive compressions. The compressions were set at 70%, with a speed of 1 mm s^{-1} and a load cell of 250 N. Five replicates were recorded for chewiness, gumminess, hardness, adhesiveness, cohesiveness, and springiness.

Microbiological analysis

Plate counts were performed for *Escherichia coli* according to NTC 4458 (ICONTEC 2018), *Staphylococcus aureus* according to ISO 6888-1 (ISO 2021), detection of *Salmonella* spp. using ISO 6579-1 and detection of *Listeria monocytogenes* through ISO 11290-1 (ISO 2017). Microbiological analyses were conducted using a three-class sampling plan (n=3, c=1, m, M) in accordance with Colombian regulations.

Statistical analysis

Using a 2×2 factorial design, the curd type was evaluated employing the block-by-curd interaction as the main plot

error term, and the effects of ripening, time, and their interactions were analyzed using the sub-plot residual error (Model 1 - Equation 3). For all cheeses, including those without ripening (day 0), the effect of curd type was evaluated using the block-by-curd interaction as the error term; conversely, the effects of ripening and curd-by-ripening interaction were assessed using the corresponding sub-plot residual error (Model 2 – Equation 4). For each response variable (physicochemical and textural), data were analyzed through analysis of variance (ANOVA) according to the experimental design.

Principal component analysis (PCA) was performed using the statistical package RStudio 4.4.1 for the volatile compounds. When differences were detected, Tukey's post hoc test was applied with a $P<0.05$ to determine distinctions between treatments. Prior to the analysis, the assumptions of normality were validated using the Shapiro-Wilk test, and homogeneity of variances between blocks was verified with Levene's test. A split-plot model was implemented through a completely randomized block design, assigning "curd type" to the main plots. Finally, microbiological analysis data were analyzed considering national regulations.

Model 1:

$$Y_{ijkl} = \mu + b_i + p_j + (bp)_{ij} + m_k + t_l + (pm)_{jk} + (pt)_{jl} + (mt)_{kl} + (pmt)_{jkl} + \varepsilon_{ijk} \quad (3)$$

Model 2:

$$Y_{ijkl} = \mu + b_i + p_j + (bp)_{ij} + m_k + (pm)_{jk} + \varepsilon_{ijk} \quad (4)$$

Where Y_{ijkl} is the response variable; μ is the overall mean; b_{ij} is the random effect of the i-th block (batch); p_j is the fixed effect of curd type; $(bp)_{ij}$ is the main-plot error; m_k is the fixed effect of the ripening type; t_l is the fixed effect of time; $(pm)_{jk}$, $(pt)_{jl}$, $(mt)_{kl}$ is the two-way interactions; $(pmt)_{jkl}$ is the three-way interaction; and ε_{ijk} is the subplot error.

RESULTS AND DISCUSSION

Physicochemical characteristics analyses

The mean values of the physicochemical characteristics are completed with 95% confidence intervals, exact *P-values,* and standardized effect sizes (Cohen´s *d*) as presented in Table 1 and Table 2. The curd type (P=0.029), the ripening environment (P=0.026), and the time (P=0.043) were determining factors in the evolution of the cheese moisture. The initial moisture content was between 46.1 and 47.1%, reducing significantly (P=0.013, d=4.63) to values of 41.6% in CCCC and 44% for CCNE. The lowest moisture content was observed in cooked curd cheeses matured in a chamber after 30 days of maturation. Moisture loss, which can occur even during short ripening periods, is favored by a combination of factors, including the development of acidity in the curd, the easy evaporation of water into the environment due to the cheeses not being packaged in protective containers, the salt diffusion inside the cheese and the higher surface-to-volume ratio of small pieces. In addition, greater moisture gradients and increased air exchange rates in controlled chambers, contribute significantly to increased moisture loss (Levak et al. 2023). The decrease in the rate of moisture loss during ripening is attributed to a lower moisture gradient, and less water available, due to the promoting rind formation, reduction in osmotic pressure, and the reuse of water caused by amino groups formed during secondary proteolysis (Mazzocca et al. 2024). A_w for all samples decreased after 15 days, possibly related to proteolysis and the release of free water that evaporates on the surface. The increase at the end of ripening could have been a consequence of high relative humidity in the environment (>90%), causing casein hydration by recycling water molecules through carboxylic acids and amino groups formed (Mazzocca et al. 2024)

Fat increased significantly from 24.5% [23.8, 25.3] (day 0) to 27.1% [26.5, 27.6] (day 30) (d=−3.09, P<0.001, large). Total protein increased from 23.6% [22.5, 24.8] to 26.0% [25.4, 26.6] (d=−2.31, P=0.001, large). Ash increased from 3.37% [3.28, 3.46] to 3.47% [3.42, 3.52] (d=−1.24, P=0.034, large). NaCl showed no significant change (d=−0.30, P=0.544, small); however, it does have an effect on texture, syneresis, moisture, and flavor characteristics (Ramírez-Navas et al. 2017). Increases in fat, protein, and ash were consistent with concentration effects from moisture loss (d=2.68, P<0.001, large) rather than true compositional change curd type had a negligible effect on moisture in the natural environment (WC: 44.4% [41.2, 47.6] vs. CC: 44.0% [42.3, 45.7]; d=0.41, P=0.653) but a large significant effect in the controlled chamber (WC:43.9% [43.0, 44.8] vs. CC: 41.6% [40.8, 42.3]; d=6.85, P=0.001), indicating that the curd type × environment interaction determines moisture retention.

Table 1. Physicochemical composition of artisanal Edam-type cheese. Ripening environment effect at day 30 (Natural Environment vs Chamber Controlled).

Variable	Treatments		Contrasts assessment			Treatments		Contrasts assessment		
	WCNE	WCCC	P-value	Cohen¨s d	Effect	CCNE	CCCC	P-value	Cohen¨s d	Effect
Moisture (%)	44.412 [41.201, 47.622]	43.876 [42.977, 44.774]	0.5522	0.56	medium	43.994 [42.323, 45.664]	41.573 [40.807, 42.339]	0.0130	4.63	large
Protein soluble (%)	6.537 [5.978, 7.096]	6.497 [5.27, 7.724]	0.9069	0.1	negligible	7.047 [5.886, 8.209]	6.543 [6.221, 6.866]	0.1965	1.47	large
Protein total (%)	25.498 [23.818, 27.179]	25.979 [24.733, 27.226]	0.3828	-0.81	large	25.365 [24.341, 26.39]	27.045 [23.762, 30.328]	0.1494	-1.72	large
RI (%)	25.663 [21.891, 29.434]	24.992 [21.314, 28.671]	0.6132	0.45	small	27.788 [23.153, 32.423]	24.228 [21.347, 27.109]	0.0595	2.29	large
Fat (%)	26.738 [24.085, 29.391]	26.732 [25.76, 27.703]	0.9926	0.01	negligible	26.975 [25.364, 28.585]	27.884 [25.195, 30.574]	0.2936	-1.02	large
Ash (%)	3.462 [3.431, 3.493]	3.493 [3.227, 3.758]	0.6682	-0.4	small	3.483 [3.117, 3.848]	3.447 [3.375, 3.519]	0.7179	0.34	small
pH	5.26 [5.086, 5.434]	5.237 [5.185, 5.288]	0.6282	0.45	small	4.97 [4.622, 5.318]	5.023 [4.738, 5.309]	0.6380	-0.42	small
Acidity (%)	0.588 [0.446, 0.731]	0.607 [0.406, 0.808]	0.7618	-0.27	small	0.674 [0.391, 0.958]	0.697 [0.64, 0.754]	0.7660	-0.28	small
a_w	0.974 [0.972, 0.976]	0.974 [0.968, 0.981]	0.9400	-0.07	negligible	0.972 [0.964, 0.98]	0.974 [0.968, 0.979]	0.6072	-0.46	small
NaCl (%)	1.137 [1.057, 1.217]	0.988 [0.474, 1.503]	0.3402	1	large	1.045 [0.569, 1.521]	1.135 [0.435, 1.835]	0.6739	-0.37	small

WCNE: washed curd in a natural environment. WCCC: washed curd in a controlled chamber. CCNE: cooked curd in a natural environment. CCCC: cooked curd in a controlled chamber. t: time. RI: ripening index. a_w: water activity.

Table 2. Physicochemical composition of artisanal Edam-type cheese. Curd type effect at day 30 (Washed curd vs. Cooked Curd).

Variable	Treatments		Contrasts assessment			Treatments		Contrasts assessment		
	WCNE	CCNE	P-value	Cohen¨s d	Effect	WCCC	CCCC	P-value	Cohen¨s d	Effect
Moisture (%)	44.412 [41.201, 47.622]	43.994 [42.323, 45.664]	0.6533	0.41	small	43.876 [42.977, 44.774]	41.573 [40.807, 42.339]	0.0012	6.85	large
Protein soluble (%)	6.537 [5.978, 7.096]	7.047 [5.886, 8.209]	0.1908	-1.39	large	6.497 [5.27, 7.724]	6.543 [6.221, 6.866]	0.8885	-0.13	negligible
Protein total (%)	25.498 [23.818, 27.179]	25.365 [24.341, 26.39]	0.7888	0.24	small	25.979 [24.733, 27.226]	27.045 [23.762, 30.328]	0.2964	-1.07	large
RI (%)	25.663 [21.891, 29.434]	27.788 [23.153, 32.423]	0.2036	-1.25	large	24.992 [21.314, 28.671]	24.228 [21.347, 27.109]	0.5225	0.57	medium
Fat (%)	26.738 [24.085, 29.391]	26.975 [25.364, 28.585]	0.7631	-0.27	small	26.732 [25.76, 27.703]	27.884 [25.195, 30.574]	0.1987	-1.42	large
Ash (%)	3.462 [3.431, 3.493]	3.483 [3.117, 3.848]	0.8309	-0.2	small	3.493 [3.227, 3.758]	3.447 [3.375, 3.519]	0.5398	0.58	medium
pH	5.26 [5.086, 5.434]	4.97 [4.622, 5.318]	0.0504	2.62	large	5.237 [5.185, 5.288]	5.023 [4.738, 5.309]	0.0804	2.58	large
Acidity (%)	0.588 [0.446, 0.731]	0.674 [0.391, 0.958]	0.3291	-0.95	large	0.607 [0.406, 0.808]	0.697 [0.64, 0.754]	0.1879	-1.51	large
a_w	0.974 [0.972, 0.976]	0.972 [0.964, 0.98]	0.3858	0.87	large	0.974 [0.968, 0.981]	0.974 [0.968, 0.979]	0.6764	0.37	small
NaCl (%)	1.137 [1.057, 1.217]	1.045 [0.569, 1.521]	0.4956	0.67	medium	0.988 [0.474, 1.503]	1.135 [0.435, 1.835]	0.5110	-0.59	medium

WCNE: washed curd in a natural environment. WCCC: washed curd in a controlled chamber. CCNE: cooked curd in a natural environment. CCCC: cooked curd in a controlled chamber. t: time. RI: ripening index. a_w: water activity.

The fat content was comparable to that observed in whole cheddar cheeses (29.1–31.3%) (Singh et al. 2023), indicating high transfer rates, related to the effect of pasteurization. LTLT pasteurization at 65 °C 30 min^{-1} promotes partial denaturation of β-lactoglobulin and its association with k-casein via disulfide bonding. This increases surface hydrophobicity of the casein matrix, enhancing fat globule retention during curd formation (Amiri et al. 2022).

An increase in soluble protein was observed during ripening, with values in cooked curd cheeses matured in the natural environment 7.047% [5.886; 8.209] compared to chamber controlled = 6.543% [6.221; 6.866]; *P*=0.197, Cohen's d=1.47 (large effect). The observed increases could be attributed to the microorganisms added, the action of rennet and natural milk enzymes (proteases) that act on casein. Promoting the formation of long peptides that are subsequently degraded into short peptides and amino acids through secondary proteolysis mediated by enzymes (proteases and peptidases). The latter originating from starter lactic acid bacteria, non-starter lactic acid bacteria, and adjunct cultures (Amiri et al. 2022; Ahmed et al. 2023).

RI increased substantially from day 0 (9.91% [8.87, 10.95]) to day 30 (25.67% [24.46, 26.88]) across all treatments (pooled: d=–9.43, *P*<0.001, large). At day 30, the environment effect was significant only for cooked-curd cheeses (CCNE: 27.79% [23.15, 32.42] vs. CCCC: 24.23% [21.35, 27.11]; d=2.29, *P*=0.060), confirming that natural-environment ripening accelerates proteolysis specifically in cooked-curd cheeses. RI as an indicator of protein degradation is higher in cheeses with higher moisture content, low salt content, and high temperature (>13 °C), maintaining an environment suitable for the development of microorganisms and enzymes (Mazzocca et al. 2024)

The pH value at the beginning of ripening (5.12 to 5.19) was similar to that observed in Edam cheese (5.10 to 5.15) (Garbowska et al. 2016). There are significant changes due to the combined effects of curd type (*P*<0.0804, effect large). In cheeses with cooked curd, a reduction to pH=4.97 was observed, which could be attributed to greater retention of moisture and residual lactose, which stimulates lactic acid formation through the action of microorganisms present in the cheese (Garbowska et al. 2016). Conversely, curd washing improves pH control, increasing up to 5.26 during the ripening time, possibly due to the lower lactose content, which limits lactic acid production (Levak et al. 2023).

During manufacturing and the initial stages of ripening (pressing, salting) performed at ambient temperature, high acidity was observed as result of the high acidification caused by the added lactic acid bacteria, lactose metabolism, and lactic acid formation, as reported by Meireles et al. (2024). and fat lipolysis (Amiri et al. 2022). However, after 15 days, a reduction in acidity was observed, possibly related to the inhibitory effect of microorganisms because of rapid moisture loss, lactose depletion, low pH (optimum 6 to 7.5), and amino acids formation and ammonia in secondary proteolysis, which have a buffering effect (Amiri et al. 2022). The increase in acidity at the end of ripening is possibly associated with the action of non-starter lactic acid bacteria and adjunct cultures, which promote the production of volatile fatty acids (Meireles et al. 2024) with a total of 11 acids observed, mainly acetic, butyric, pentatonic, and hexanoic.

Lactose

Figure 1 shows the temporal evolution of lactose content (mg kg^{-1}) during cheese ripening. In all treatments, the variation in lactose content was adequately described by a first-order kinetic model, characterized by an exponential decrease over time. The curd washing process resulted in a reduction in lactose content in the cheese, with 45.8 mg kg^{-1} in washed curd compared to 60.1 mg kg^{-1} in cooked curd (Natrella et al. 2023). The lactose content at the end of ripening was approximately 33 mg kg^{-1}, decreased by the action of starter and non-starter lactic acid bacteria are essential for preventing the development of undesirable secondary microbiota, through the formation and contribution of intermediate and final compounds (Piñeiro-Lago et al. 2020; Decadt et al. 2024).

The regression analysis demonstrated an optimal convergence through the application of a first-order kinetic model, exhibiting coefficients of determination (R^2) consistently exceeding the 0.50 threshold. Cheeses made with cooked curd showed faster lactose depletion rates than those made with washed curd, both under

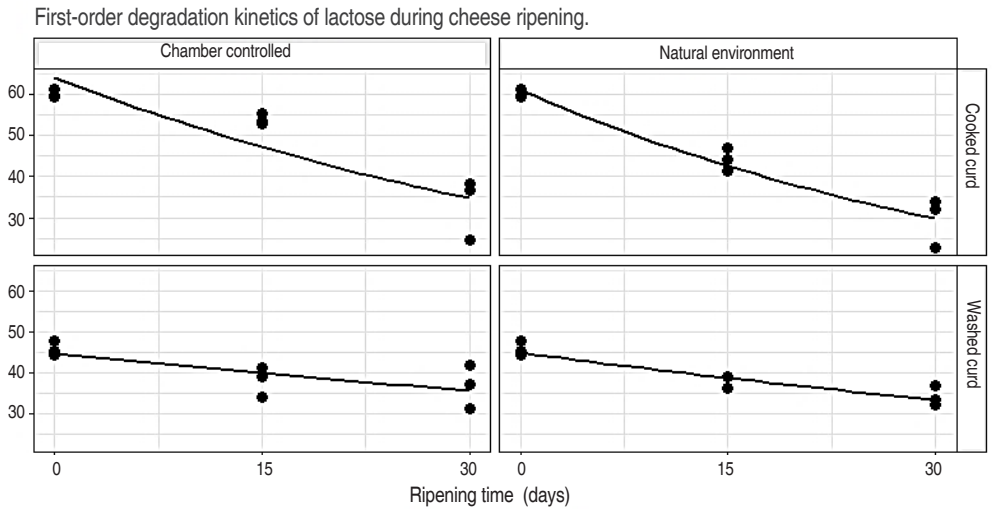


Figure 1. First-order degradation kinetics of lactose in WCCC, WCNE, CCCC, and CCNE cheeses. Point represents individual experimental values, and lines correspond to the fitted first-order model.

natural environmental conditions (k=0.0240) and in a controlled chamber (k=0.0205). The faster rate of lactose degradation observed in cooked curd cheeses could have been attributed to the cooked curd process, which increases the availability of residual lactose and enhances the metabolic activity of the cheese microbiota during ripening. In contrast, washed curd cheeses exhibited more limited lactose degradation, suggesting either lower substrate availability or reduced fermentative activity.

Volatile organic compounds (VOCs) determination

As shown in Figure 2, a total of 66 volatile compounds were identified throughout the ripening process, including 15 acids, 8 alcohols, 3 aldehydes, 8 ketones, 10 esters, 2 ethers, 7 hydrocarbons, 8 terpenes, and 5 unclassified compounds. Of these, 28 were identified based on mass spectra and retention indices. In unripened cheeses, terpenes such as dl-limonene, beta-myrcene, and p-cymene were detected, whereas after 15 days, a greater development of esters (methyl butanoate, methyl hexanoate, and ethyl hexanoate) and acids (butanoic, hexanoic, octanoic, and hexadecenoic acids) was observed. In the final stage of ripening, alcohols such as 3-methyl-1-butanol, 1,3-butanediol, and benzene ethanol were observed. The dl-limonene compound decreases but still accounts for about 40% of the total area at the end of ripening.

After 30 days of ripening, when comparing pairs of treatments significant differences were found (P<0.05, and effect large) in compounds such as cyclooctatetraene, -pinene, methyl octanoate, 2-methyl-1-propanol, hexylformate, toluene, and ethylbenzene. The presence of VOCs is related to glycolysis, lipolysis, and proteolysis developed by the microbiota present in the cheese (Tekin and Hayaloglu 2023). The formation of fatty acids during ripening depends on various reactions involving amino acid catabolism, lipolysis, and lactose fermentation (Amiri et al. 2022). In this way, it was related with the presence of short-chain and medium-chain fatty acids standing out, contributing to flavor and aroma components, mainly alcohols, secondary alcohols, ketones, and esters, which are easily detected due to low perception thresholds (Amiri et al. 2022; Singh et al. 2023).

Volatile free fatty acids represent 23.0% of total VOCs, particularly acetic acid, butanoic, hexanoic, and octanoic acids, which are related to the homo-fermentative and hetero-fermentative metabolism of native, starter, non-starter, and adjunct cultures. Important aromatic compounds were developed due to their easy hydrolysis, water solubility, and olfactory activity (Amiri et al. 2022; Tekin and Hayaloglu 2023). Some key aromas have been reported in cheese, such as 2-heptanone (spicy, fruity), p-cymen (sour), limonene (citrus), 3-methyl-1-butanol (floral), 3-methylbutanal (nutty), and 3-hydroxy-2-butanone (nutty).

Texture profile analysis (TPA)

The data distribution achieved an overall explanatory quality of 80.2%, with Dimension 1 accounting for 49.0%

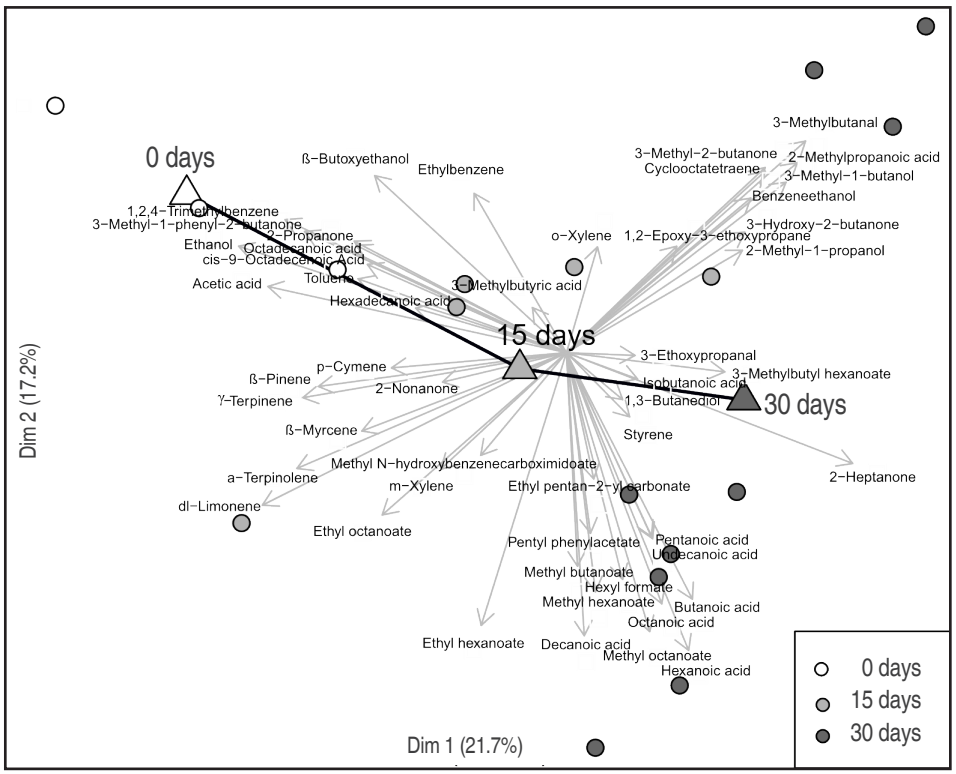


Figure 2. Distribution of areas in volatile compounds during ripening.

of the total variance (mainly associated with hardness and gumminess), while Dimension 2 explaining 31.2%, dominated by elasticity, chewiness, and adhesiveness. The low percentage of observations with significant T^2 values (1.7%), the absence of high residual Q values (3.8763), and the highly significant results of the permutation tests, demonstrate that the structure observed in the biplot reflects true multivariate differences in the textural profile (Figure 3). Overall, the results confirm that ripening time exerts the strongest multivariate effect on the TPA profile, followed by the ripening environment and the curd type.

During the ripening period, a decrease in springiness was observed, while chewiness, gumminess, hardness, adhesiveness, and cohesiveness increased. This tendency for gumminess and springiness was similar to that observed in cheddar cheese (Singh et al. 2023). The changes in the cheese during ripening were mainly related to moisture loss, the development of proteolysis and lipolysis with the formation of secondary compounds, changes in the moisture/protein ratio, the lubricating effect of fat, and the plasticizing effect developed due to moisture retained inside the cheese (Azevedo GD et al. 2024).

It is important to highlight that adhesiveness was higher in a natural environment (20.13 N s) compared to the chamber (11.44 N s), indicating that relative humidity is related to greater proteolysis, increased ionic strength which in presence of water and the formation of some water-soluble peptides, caused the cheese to be more viscous and stickier (Ahmed et al. 2023). The correlation between adhesiveness and the ripening index (RI, equivalent to SN/TN) was r=0.823 (Pearson, p_FDR<0.001, ***; Spearman ρ=0.886, p_FDR<0.001, ***), confirming the positive, strong and statistically robust relationship between proteolysis advancement and the increase in adhesiveness postulated in the manuscript. The correlation between adhesiveness and NaCl was r=0.159 (Pearson, p_FDR=0.5462, ns; Spearman ρ=0.247, p_FDR=0.4017, ns). No statistically significant association was found between NaCl and Adhesiveness. However, adhesiveness did show a significant negative correlation with moisture (r=-0.49, p_FDR=0.0213,*), indicating that water loss during ripening contributes to the increase in adhesiveness, consistent with the concentration of proteins and salts.

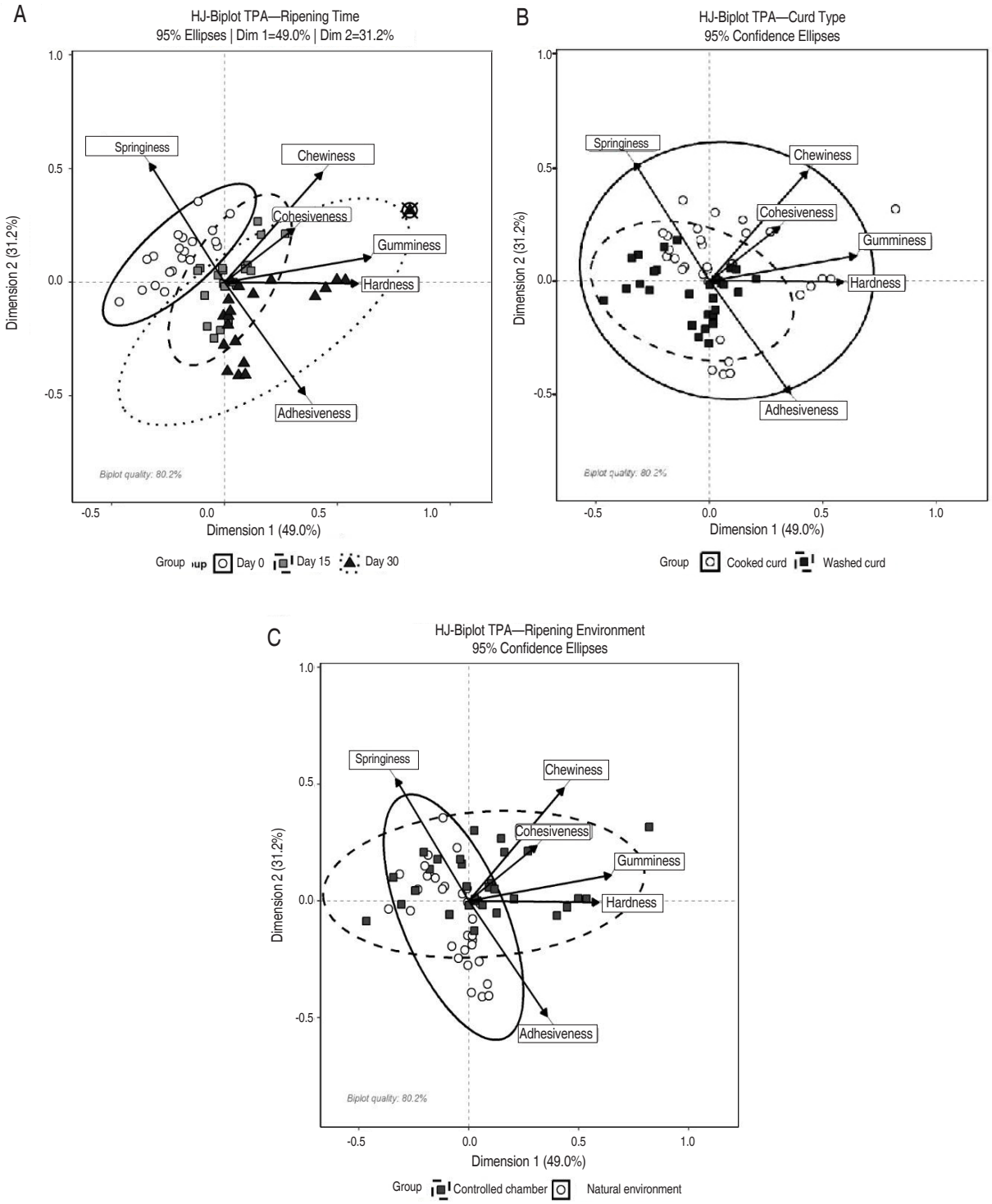


Figure 3. Biplot representation for ripening time in days (**A**), curd type (**B**), and ripening environment (**C**) on the change of textural attributes in cheese. Observations marked with ⊗are identified as atypical according to Hotelling´s T^2 (UCL, 95%).

On the contrary, springiness decreased to 0.17 in cooked curd cheeses and to 0.19 in washed curd cheeses, as a consequence of the continuous degradation of the protein matrix during ripening, which is associated with changes in moisture, protein, and fat content in the cheese (Amiri et al. 2022; Ahmed et al. 2023). Furthermore, the values obtained were lower than normal as a response to the intense acidification produced by the added cultures, which also influenced colloidal calcium (Ahmed et al. 2023; Lamichhane et al. 2018).

Microbiological analysis

At the end of the ripening period, *E. coli* was detected in one sample (Table 3), with counts slightly exceeding the regulatory limits (Ministerio de Salud y Protección social 2022). Thus, as the ripening days progressed, colony-forming units (CFU) decreased due to the activity of lactic acid bacteria (Singh et al. 2023) and changes in key parameters such as pH, water activity (a_w), salt concentration, and redox potential, as well as the formation of organic acids and potential bacteriocins, as previously described for ripened cheeses. This requires attention, as it is considered an indicator of sanitary quality, a potential pathogen, and a cause of textural defects, swelling, and unpleasant flavors in cheese (Mazzocca et al. 2024).

Table 3. Microbiological performance during cheese maturation.

Sample	Time (d)	*E. coli* (UFC g^{-1})	*L. monocytogenes* 25 g^{-1}	*Salmonella* spp 25 g^{-1}	*S. aureus* (UFC g^{-1})
WC	0	<10	Not present	Not present	<100
CC	0	160	Not present	Not present	<100
WCCC	30	<10	Not present	Not present	<100
WCNE	30	<10	Not present	Not present	<100
CCCC	30	<10	Not present	Not present	<100
CCNE	30	40	Not present	Not present	<100
Reference values		**<10**	**Absence**	**Absence**	**<100**

WC: Washed curd. CC: Cooked curd.

As for *S. aureus*, all samples were found to be below the detection limit reflecting the safety of the product; however, it is necessary to consider its high capacity for enterotoxin production even with low counts (Ministerio de Salud y Protección Social 2022). Moreover, the main pathogens commonly reported in cheese, such as *L. monocytogenes* and *Salmonella* spp, were absent during 30 days of maturation. However, exceptional care must be taken with these pathogens due to their ubiquitous nature and ability to survive in various conditions as these microorganisms can be found in any stage of processing (Mazzocca et al. 2024). The absence of the main microorganisms was limited mainly by pasteurization, salt content, and appropriate management in milk production and ripening processes.

Scope and generalization

The results obtained in this study are directly applicable to semi-hard cheeses produced under similar technological conditions, particularly those manufactured from pasteurized milk and subjected to washed-curd or cooked-curd processes with short to intermediate ripening times. Ripening under natural environmental conditions proved to be a viable alternative in temperate climates (12–18 °C) with moderate to high RH (70–85%), such as those found in Andean dairy regions, provided that good hygienic practices are maintained. At temperatures above 18 °C or RH below 65%, passive control measures (e.g., natural ventilation or shading) or a reduction in ripening time are recommended. Conversely, at temperatures below 12 °C or under very high RH (>90%), the use of protective barriers or controlled heating is recommended.

CONCLUSION

The ripening time and natural environmental conditions influenced moisture dynamics, which contributed to the

concentration of components, the progressive degradation of the casein matrix, an increase in the ripening index reaching values up to 27%, and the accumulation of soluble nitrogenous compounds associated with flavor development. These results suggest that moderate temperatures and relatively high humidity levels favor enzymatic activity without compromising product stability, supporting natural ripening as a viable and sustainable alternative for the artisanal production of semi-hard cheeses.

Curd processing significantly affected lactose metabolism and acidification. Washed-curd cheese exhibited lower lactose concentrations, contributing to a more stable pH during ripening, whereas cooked-curd cheeses showed faster degradation kinetics, indicating greater substrate availability for lactic fermentation. These biochemical changes were reflected in the textural profile, in which ripening time explained most of the multivariate variability: hardness, gumminess, and adhesiveness increased, whereas springiness decreased. Microbiological analyses confirmed acceptable microbiological quality throughout ripening.

ACKNOWLEDGMENTS

The authors expressed their gratitude to the Universidad Nacional de Colombia, the Universidad de Nariño, Centro Internacional de Producción Limpia LOPE, Asociación de Lácteos Contadero, and Announcement 15 for master's and doctoral degrees released by Minciencias SGR for their financial and technical support.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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