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Description of Ewiss cheese, a new ewe's milk cheese processed by Swiss cheese manufacturing techniques: microbiological, physicochemical and sensory aspects

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ABSTRACT

Typically, Swiss-type cheese is made from cow's milk. However, in the present work an attempt to expand the sheep supply chain and product offering in this field was made by developing a new type of cheese using Swiss-type cheese technology. The cheese was manufactured under industrial conditions, and fermentations were carried out using freeze-dried commercial starters that are traditionally used in the production of Swiss cheese. Two experimental "Ewiss cheese" (EC) products were produced using raw milk (RM-EC) and pasteurized milk (PM-EC), respectively. Fourteen microbial groups were investigated by plate counts from curd until ripened cheeses. According to microbiological analyses, no statistically significant differences were found between the 2 productions with respect to the group of lactic acid bacteria (LAB). The curds were mainly characterized by mesophilic LAB cocci (7.45 $\log_{10}$ cfu/g in RM-EC and 7.33 $\log_{10}$ cfu /g in PM-EC). However, at the end of the ripening period (9 mo), the cheeses exhibited a higher presence of mesophilic LAB rods. Undesired microbiological groups were found only in the curd of raw milk cheese in the range of 10^4–10^5 cfu/g, but reaching undetectable levels by plate count in the cheese at the end of ripening. RM-EC and PM-EC were characterized by 76% and 68% of dry matter, respectively. These cheeses contained 29.30% and 34.36% of protein, and 51.31% and 50.38% of fat, respectively. Textural analysis showed differences in terms of hardness, chewiness, and gumminess between the experimental cheeses and Swiss cheese sold on the market. These differences could be attributed to the higher protein content of ewe's milk. The main fatty acids in the cheeses were palmitic acid, myristic acid, oleic acid, and capric acid. Among the organic acids, RM-EC had higher concentrations of lactic acid, while PM-EC was higher in propionic acid. The ewe's cheeses emitted 46 volatile compounds, including acids, aldehydes, ketones, esters, alcohols, and other compounds. PM-EC was characterized by the main compounds of Swiss-type cheese: acetic acid, butyric acid, ethyl butyrate, ethyl caproate, propanoic acid, and tetramethylpyrazine. Sensory evaluation showed that the new dairy products were generally appreciated, and PM-EC was the most preferred by the judges. This research has enabled the development of new ewe's milk products, which could stimulate the valorization of a sector that has been long neglected and still has a large margin of improvement.
Key words: ewe's cheese, novel dairy products, Swiss-type cheese, microbiological safety, sensory evaluation

INTRODUCTION

The Italian agrifood industry is a key sector of the country's economy. Several local economies rely on the strategic production chains of foods and wines that have earned a recognition of quality status. Among these foods, dairy products, particularly cheeses, are of importance for milk producers, transformation industries, food traders, and distributors (Schimmenti et al., 2021). Over the past 20 years, cheese consumption has been on a steady rise (Devi et al., 2023).

Among cheeses, Swiss-type cheese is long-ripened, hard cheese known for its characteristic round, regular holes (eyes) and slightly sweet flavor. This cheese is consumed almost worldwide and is manufactured in almost all industrialised countries (Bisig et al., 2010), usually from raw cow's milk. Cows' milk stands as the predominant milk type globally for cheese making, primarily due to its ability to yield high-quality final products. This superiority arises from the specialized breeding practices within the cattle industry and the efficient milk processing techniques employed. These factors contribute to optimal yields and consistent quality. While there has

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been recent growth in the production of non-bovine milk varieties (Nuñez et al., 2016), cow's milk remains the dominant choice. However, in specific peripheral and mountainous regions, such as the Mediterranean areas, ewe breeding takes center stage in the local agricultural economy (Cusimano and Salamone, 2020). These regions prioritize ewe's milk for cheese production, emphasizing its importance in sustaining livelihoods and culinary traditions.

Currently, non-bovine milk (including goat and ewe milk) amounts to 133 million tons, representing more than 17% of total milk output worldwide (Nayik et al., 2021). Ewe's milk is not extensively utilized in comparison to cow or goat milk. While goat milk has gained prominence and offers various health benefits, ewe's milk remains relatively less utilized globally, resulting in a narrower range of derived products. However, its unique properties make it an interesting option for specific applications. The most famous ewe's cheeses produced in Europe are Roquefort in France, Pecorino in Italy, Feta in Greece, Manchego in Spain, and Bryndza in central and eastern Europe. In Italy, ovine milk is predominantly employed in the production of Pecorino cheese which comes in several varieties, made in various regions, particularly in the central and southern areas. However, the production of only typical cheeses does not allow for innovation in the ovine milk sector. Given the nutritional advantages of sheep milk over goat and cow milk (Li et al., 2022), research efforts are essential to unlock the potential value of sheep milk by creating innovative products through ewe's milk processing. In recent years, there has been growing interest in revitalizing sheep breeding and promoting ovine milk production in rural areas (Koluman and Paksoy, 2024). Thus, these areas, often characterized by challenging environmental conditions and dwindling agricultural activity, require the following targeted initiatives to sustain local economies and preserve cultural heritage: sheep breeding revival; ovine milk production; sustainable land use. The cheese industry's expansion is strongly influenced by consumers' demand for unusual (unique) cheeses (Devi et al., 2023). Thus, the production of new cheeses, particularly those made from sheep's milk, could be instrumental in enhancing the value of marginal rural areas.

The objective of this study was to create a new type of ewe's cheese using Swiss cheese technology. To validate the new protocol, ewe's Swiss-type cheese was produced using both raw and pasteurized milk. The cheeses were evaluated for their microbiological traits and compared based on their physicochemical parameters, fatty acid profile, organic acid, volatile compounds and sensory acceptability.

MATERIALS AND METHODS

Raw materials and starter culture preparation

The ewe's whole milk used in this study was obtained from "Valle del Belìce" sheep reared in several farms within Agrigento province (Sicily, Italy). The collected milk was transported in a temperature-controlled environment to "Cooperativa Agricola Tumarrano" located in Cammarata (Agrigento, Italy) where it was transformed. Raw milk before pasteurization was characterized as follows: pH 6.63, lactose 4.50%, fat 6.22%, protein 5.72%, casein 4.35%, and urea 32.55 mg/dL. Pasteurization treatment occurred at 72°C for 15 s in a P75 50/2 pasteurizer (Tecnolat S.p.a., Nocera Inferiore, Italy). Lactic acid fermentation took place using freeze-dried commercial starters consisting of *Streptococcus thermophilus* (LYOBAC-D GDA). To obtain the typical eyes expected in a Swiss-type cheese, propionic acid bacteria (PAB) were inoculated, and propionic fermentation occurred by means of freeze-dried commercial starters consisting of *Propionibacterium freudenreichii* (LYOBAC-D PROPRIONIBACTERIUM). Both freeze-dried cultures were purchased from the enterprise Alce International s.r.l. (Quistello, Italy) and were inoculated directly into the tank milk, after reactivation was performed following manufacturer's instruction. Briefly, lyophilized cells (5 units) were revitalized in 2 L of ewe's milk and kept under gentle agitation for 10 min before addition to the final milk volume.

Ewiss cheese production and sample collection

The experimental plan involved 2 distinct productions (Figure 1). The first production was carried out using raw milk (RM), while the second production employed pasteurized milk (PM). The novel ewe's cheese manufactured using Swiss-type cheese technology was named "Ewiss cheese" (EC). The production of this novel cheese followed the protocol outlined in Figure 2. Specifically, a volume of 500 L of ewe's milk was heated to 40°C using a Pol-P-Polivalente (Inox Art Production S.r.l., Cammarata, Italy). After heat treatment, starter inoculation was performed through direct inoculum into the tank. Curdling of the milk took place by adding 245 g of extra lamb rennet paste (Caglificio Clerici, Cadorago, Italy), title 1: 10,000. The curd was mechanically broken down to the size of grains and cooked at 55°C for 5 min under slow agitation as reported by Gaglio et al. (2019). The clot was then transferred into circular molds that were pressed to facilitate the draining of the whey and stewed at 45°C, turning upside down in intervals of 10 min until pH values reached 5.15–5.20. Brining was carried out by immersing the cheeses in saturated brine

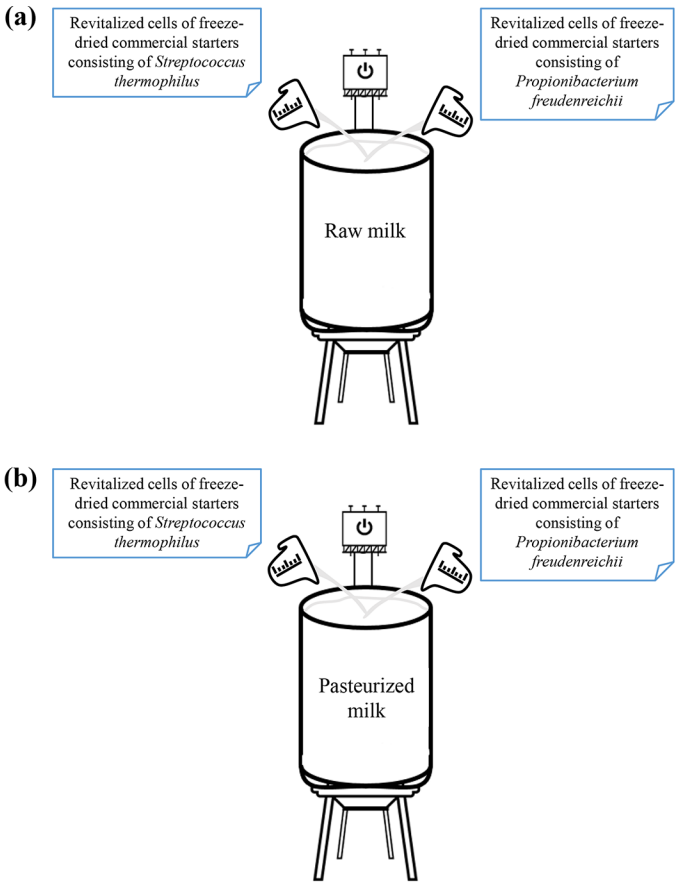


Figure 1. Experimental design of cheese productions. (a) production performed with raw milk and commercial starter cultures; (b) production performed with pasteurized milk and commercial starter cultures.

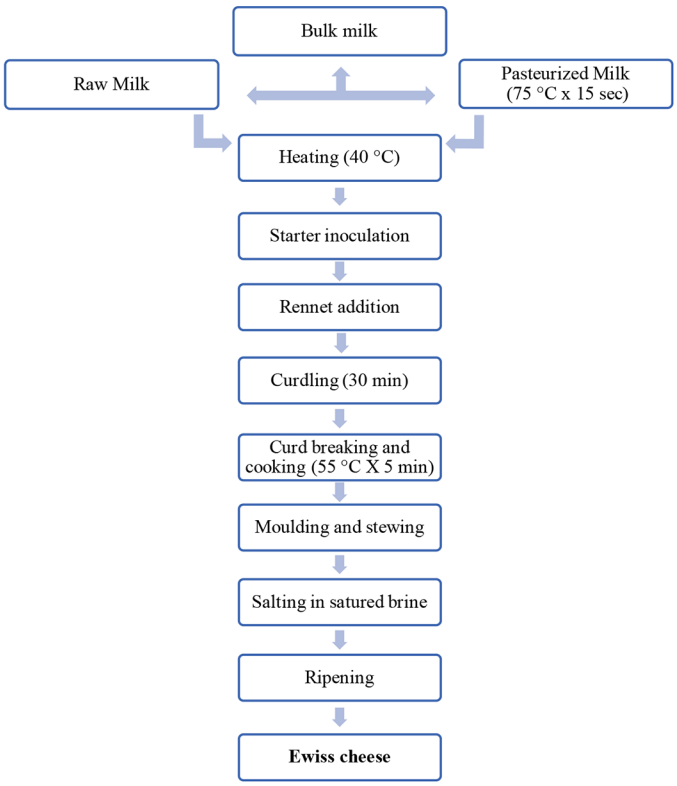


Figure 2. Flowsheet of Ewiss cheese production.

at 8–12°C for 7 d. After this, cheese ripening took place at low temperatures (4–7°C) for 2 wk for acidification, then at higher temperatures (approx. 20–22°C) for 8 wk for the development of PAB, and again at lower temperatures until 9 mo of ripening for the settlement of the eyes. Cheeses productions were performed in triplicate over 3 consecutive months (3 independent experimental replicates). Samples of commercial starter cultures (CSC), RM, PM, inoculated milk (IM), curd (C) and cheese (Ch) from RM-EC and PM-EC productions after 3, 6 and 9 mo were collected for analysis. All analyses performed on cheese at 9 mo of ripening were compared with those of a commercial cow's Swiss cheese at 9 mo of ripening (C-SC) (Lactalis Suisse, Küssnacht, Swiss).

Monitoring of physical parameters

All samples collected throughout the cheese-making process were kept under refrigerated conditions during transport to the laboratory of Agricultural Microbiology (University of Palermo) where they were analyzed. The changes in pH were monitored using a portable Hanna HI98165 pH meter (Hanna Instruments, Woonsocket, RI, USA). The temperature of the cheeses was monitored during the entire process, from the molding until the 9th mo on ripening, using Thermo Button 22 T 8 K data loggers (VWR International S.r.l., Milan, Italy) which were put inside the cheese core at the molding step.

Microbiological analyses

The number of viable cells of all samples collected along the production chain of RM-EC and PM-EC productions were estimated using the serial decimal dilution method. To perform this analysis, 1 mL of milk samples were directly serially diluted in Ringer's solution (Oxoid, Basingostoke, UK) while 10 g of freeze-dried commercial starters, curd and cheese samples were weighed aseptically and added with 90 mL of 2% sodium citrate solution. The samples were then homogenized in a stomacher BagMixer® 400 (Interscience, Saint Nom, France) at maximum speed for 2 min. Further serial dilutions were carried out in Ringer's solution (Hooi et al., 2004).

The following groups of microorganisms were enumerated: total mesophilic and psychrophilic microorganisms on Plate Count Agar (PCA) with 1 g/L skim milk (SkM) incubated at 30°C for 72 h and 7 d, respectively;

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mesophilic and thermophilic cocci on M17 agar with cycloheximide and anaerobically incubated at 30 and 44°C, respectively, for 48 h; mesophilic lactobacilli on de Man-Rogosa-Sharp medium (MRS) agar acidified at pH 5.4 with lactic acid (5 mol/L) anaerobically incubated at 30°C for 48 h; thermophilic lactobacilli on Whey-Based Agar Medium (WBAM) prepared as described by Settanni et al. (2012) anaerobically incubated at 44°C for 48 h; PAB on Pal Propiobac Plus media (PPP) incubated at 30°C for 7 d, in anaerobic jars; enterococci on Kanamycin Esculin Azide (KAA) agar incubated at 37°C for 24 h; total coliforms on Violet Red Bile Agar (VRBA) incubated at 37°C for 24 h; members of Enterobacteriaceae family on Violet Red Bile Glucose Agar (VRBGA) incubated at 37°C for 24 h; *Escherichia coli* and *Salmonella* spp. was plated on Hektoen Enteric agar (HEA) and incubated at 37°C for 24 h; coagulase-positive staphylococci (CPS) on Baird Parker (BP) agar with rabbit plasma fibrinogen, incubated at 37°C for 48 h; *Listeria monocytogenes* was investigated by plating on *Listeria* selective agar base (LSAB) with SR0140E supplement; pseudomonads on *Pseudomonas* Agar Base (PSAB) with selective supplement incubated at 25°C for 48 h; yeasts on Yeast Peptone Dextrose (YPD) agar with chloramphenicol (0.1 mg/mL) and incubated aerobically at 25°C for 48 h; molds on Potato Dextrose Agar (PDA) media incubated aerobically at 25°C for 7 d; clostridia were estimated using the most probable number (MPN) technique inoculated into reinforced clostridial medium (RCM) supplemented with 1.4% (vol/vol) Na-lactate and incubated at 37°C for 7 d.

All media and supplements used in this study were purchased from Oxoid, except PPP provided by Laboratoires Standa (Caen, France). All plate counts were performed in duplicate.

Monitoring of commercial starter culture and identification of autochthonous milk LAB

All presumptive LAB and PAB developed on MRS, M17 and PPP, respectively, inoculated with the cell suspensions of RM, IM and cheese samples at 9 mo of ripening were collected, purified, and tested for Gram reaction and catalase activity (Barbaccia et al., 2021). Differentiation of the isolates collected and dominance of *S. thermophilus* and *P. freudenreichii* (added as fermenting agents) over indigenous raw milk LAB were carried out by random amplification of polymorphic DNA (RAPD)-PCR analysis as described by Garofalo et al. (2023). Briefly, RAPD profiles obtained from LAB isolated from RM before commercial starter culture addition were compared with pure cultures of *S. thermophilus* and *P. freudenreichii* strains originating from freeze-dried commercial starters. Genotypic identification of the LAB resistant to the pasteurization process was performed at the AGRIVET Centre (Palermo, Italy) following the approach reported by Gaglio et al. (2016).

Physicochemical composition of cheeses

The physicochemical properties of the cheeses at the different stages of ripening were investigated. The pH was measured by immersing a portable Hanna HI98161 pH meter (Hanna Instruments, Woonsocket, RI, USA) into homogenized cheese sample. Measurements were taken in 3 different parts, and the results were averaged. Dry matter, fat, ash content, and total acidity were detected according to the AOAC International method (AOAC International 2012a; AOAC International 2012b; AOAC International 2012c; AOAC International 2012d). The salt content was determined following the method described by Hooi et al. (2004). Determination of nitrogen fractions was performed according to the IDF standard (IDF, 1993). All compositional analyses were performed in duplicate.

The color of cheeses was detected by using a tristimulus chromometer Minolta CR-400 (Minolta, Osaka, Japan). L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) were measured. The Chroma value was calculated as: $Chroma = \sqrt{a^2 + b^2}$ according to the standard CIE (Commission internationale de l'éclairage).

Texture analysis, free fatty acid profile and organic acids composition

Texture analysis was performed on all cheese samples, while the fatty and organic acid profile was investigated in cheese samples after 9 mo of ripening. Cheese texture was measured by means of the TA.XTplusC Texture Analyzer (Stable Micro Systems, Godalming, UK). The analysis included the measurement of primary parameters such as hardness, adhesiveness, springiness, cohesiveness and resilience, as well as secondary parameters such as chewiness and gumminess. Cheese samples (3 cm × 3 cm × 3 cm in size) were taken from the center of the cheese using a sharp knife. Samples were tested after they reached room temperature. The employed compression ratio was 50% from the initial height of the samples using 2 compression cycles at a constant crosshead speed of 2 mm/s. The texture analyses were performed twice for 2 independent samples from each batch of cheese. A total of 4 readings were taken for each treatment. Measured parameters were obtained by the Exponent software (Stable Micro Systems) version (6.0.6.0) from TPA curves.

The fatty acid profile of cheeses was determined by Gas Chromatography (GC-MS/MGas Chromatography-Mass Spectrometry (GC-MS/MS) on 10 g of grated

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cheese samples with the esterification of fatty acids to their methyl esters based on the methodology of De Jong and Badings (1990) with modification. Briefly, the sample injected (1 µL) with a split ratio of 1:40, through a GC-MS/MS (Agilent, 7890B GC −7010B MS Agilent Technologies Inc., Santa Clara, CA, USA) with a flame ionization detector with an autosampler (Gerstel, Germany). The separation of the fatty acids was achieved in a capillary Agilent J& W DB-WAX column (60 m x 0.25 µm x 0.25 µm) using helium as the carrier gas (1 mL/min). The oven temperature was held at 50°C for 1 min, raised to 200°C at a rate of 25°C/min held for 10 min and then to 230°C at a rate of 3°C/min held this temperature for 26 min. The inlet temperature and detector were set to 250°C and 300°C, respectively. Fatty acid identification was verified by comparing the sample peak retention times with the reference standards (Supelco 37 Component FAME Mix, Sigma-Aldrich, St. Louis, MO, USA).

Organic acids and lactose were determined on a homogeneous puree of cheese samples through an HPLC Shimadzu Nexera 2 (Kyoto, Japan), as described by Manolaki et al. (2006) with some modifications. In brief, the chromatograph included a ICSep ICE-Coregel 87H column (30 cm x 7.8 mm x 9µm, Transgenomic, USA) and a Refractive Index Detector (RID). The mobile phase was 4 mM H2SO4 (0.4 mL/min). The data were recorded and analyzed by LabSolutions software, and the concentrations were tentatively calculated using standard curves.

Detection of volatile compounds

The volatile composition of Ewiss cheese was determined by the headspace solid-phase microextraction method (HS-SPME) and gas chromatography/mass spectrometry (Agilent 7890B GC, 7010B MS triple quadrupole, MS Agilent Technologies Inc.) after 9 mo of ripening. The samples were kept at 60°C for 15 min and the volatile compounds were adsorbed with a coated fiber (Carboxen TM/PDMS StableFlexTM) for 45 min. The samples were then injected into a capillary column (60 m x 0.25 mm i.dx 0.25µm, J&W Scientific, Folsom, CA, USA) with a desorption time of 5 min. The column temperature was raised to 90°C with an increase of 3°C per min after waiting 4 min at 40°C and then maintained at 130°C for 4 min with an increase of 4°C per min. Finally, the temperature was raised to 240°C increasing by 5°C and held at this temperature for 8 min. The carrier gas was He (1 mLvb/min). The energy of the electrons was 70 eV and the mass range was 30–600 m/z. The partition ratio was 1:10. The volatile compounds were identified using the NIST library and expressed as percentages of the peak area of the total ion chromatogram in a heat map.

Sensory evaluation

A sensory evaluation was conducted in a sensory analysis room allocated in the Department of Agricultural, Food and Forest Science (SAAF) of the University of Palermo under artificial light. Panel members (n = 14, 8 females, 6 males, ages ranging from 25 to 54) were recruited from students and staff of the same department. To recognize specific attributes of the Swiss cheese typology, assessors were instructed to use procedures consistent with international standards for the training of descriptive panels (ISO 8589:2007, ISO, 2007). All samples were cut 24 h before evaluation, placed into the evaluation containers, and refrigerated overnight. Samples were removed from refrigeration 1 h before evaluation to allow equilibration to room temperature. Cheeses (15 g) were served in plastic dishes coded with 3 random digits. Panelists used unsalted crackers and water to clean their palates between samples and were unaware of the tested sample. Samples were analyzed for their olfactory traits (odor intensity, butter odor, milk odor, unpleasant odor), appearance attributes (rind, color, eye size eyes, eye distribution, elasticity, and firmness), and gustatory features (saltiness, sweetness, bitterness, typical Swiss cheese flavor, persistence of taste, unpleasant flavors, and overall satisfaction). All attributes were rated on a 9 cm line scale, in which direction was from left to right with increasing intensities from “low” at 0 to “high” at 9. The results obtained by the panelists were averaged.

Statistical Analyses

Microbiological counts, and physicochemical parameters were analyzed by ANOVA (ANOVA); the model included the effects of milk treatment (raw and pasteurized). Tukey’s post hoc test was used to estimate significant differences. Statistical significance was set at $P < 0.05$. The concentrations of Volatile Organic Compounds (VOCs) emitted from ovine cheeses were visually depicted using a heat map generated through ascending hierarchical clustering. The color gradient represented the VOC concentrations, transitioning from yellow (indicating lower concentrations) to red (indicating higher concentrations). All statistical processing was performed with XLStat software version 2020.3.1 for Excel (Addinsoft, New York, NY, USA).

RESULTS

Acidification process, ripening temperature, and microbiological characteristics

During cheese making, the acidification process was followed in both RM-EC and PM-EC trials, and the pH kinetics were quite similar. Raw milk had an initial pH of 6.7 and reached a pH of 6.4 at molding. Temperature was monitored both during production and during ripening. Data loggers present in the cheese paste allowed to monitor core temperature throughout the ripening period. The results showed an average temperature of 44.7°C immediately after molding. After 24 h, the temperature reached 16°C and decreased slowly until the third mo of ripening with an average temperature of 14°C. Data loggers recorded a slow rise in temperature from the 3rd mo of ripening and the temperature registered at the 9th mo was 18°C.

Table 1 shows the levels of microbiological counts. Raw milk hosted levels of total mesophilic microorganisms (TMM), mesophilic coccus and rod LAB at 10^6 cfu/mL and thermophilic coccus and rod LAB at 10^4 cfu/mL. Following pasteurization, the levels of these bacteria decreased by approximately 3 log cycles. Regarding undesired bacterial groups, especially total coliforms, members of the Enterobacteriaceae family, pseudomonads, and *E. coli* they were found at 10^3 cfu/mL in RM and completely disappeared in PM. The dried starter cultures LYOBAC-D GDA and LYOBAC-D PROPRIONIBACTERIUM showed a dominance of thermophilic LAB cocci and PAB, respectively, with levels above 10 $\log_{10}$ cfu/g. After addition, the resulting levels of thermophilic LAB cocci and PAB were approximately 10^7 cfu/mL in both RM and PM. The curds of the 2 cheese trials showed significant differences in TMM. The curds obtained from RM-EC production achieved higher values and showed a rising trend up to the 6th mo of ripening in the cheese, after which it decreased slightly. The same behavior was found for PM-EC production, but in the 2 productions, the levels differed by about 1 log cycle. Total psychrophilic microorganisms showed a decreasing trend in 9-mo ripened cheeses. Among the heterogeneous group of LAB, mesophilic cocci dominated samples of both trials, whereas rod-shaped thermophiles presented the lowest values in cheeses at the end of maturation. Additionally, 6- and 9-mo ripened cheeses obtained by PM exhibited slightly higher values than the respective cheeses processed by RM.

In PM-EC production, PAB reached the highest levels in the Ch-3 samples and remained high (around 10^7 cfu/g) even in the Ch-9 samples. Enterococci differed significantly among cheese productions and were below the detection limits (<2 $\log_{10}$ cfu/g) in curd from PM-EC production.

Among the undesirable bacteria investigated, curd and cheese samples from PM-EC production did not show detectable levels. However, total coliforms and enterobacteria were detected in curd (5.15 and 5.39 $\log_{10}$ cfu/g, respectively) and Ch-3 (2.87 and 3.87 $\log_{10}$ cfu/g, respectively) from RM-EC production, but were no longer counted in Ch-6 and Ch-9 samples. No pathogenic microorganisms of dairy interest such as *L. monocytogenes*, *Salmonella* spp. and CPS were ever found in any sample.

Persistence of commercial starter cultures and identification of autochthonous milk LAB

After enumeration, 52 presumptive LAB (Gram-positive and catalase negative) and 61 presumptive PAB (Gram-positive and catalase positive) were isolated from commercial starter cultures, RM, RM-EC and PM-EC at 9 mo of ripening. All isolates were subjected to RAPD-PCR to follow the added commercial starter strains throughout the production of cheeses by polymorphic profile comparison. The dendrogram reported in Figure 3 shows only 12 of the 113 isolates analyzed. These 12 isolates represent bacteria collected from various samples at least once. The remaining 101 strains were excluded from Figure 3 because they exhibited identical RAPD profiles as other cultures from the same sample. Two major RAPD clusters which included the strains *S. thermophilus* and *P. freudenreichii*, both isolated from freeze-dried commercial starters, were identified. In particular, only *P. freudenreichii* were detected both in PM and RM cheeses at 9 mo of ripening.

Four different strains (ESC1, ESC2, ESC17 and ESC22), isolated from RM, were identified by 16S rRNA gene sequencing as *Enterococcus faecalis* (Ac. No. PP430126 and PP430128) and *Lactococcus lactis* (Ac. No. PP430125 and PP430127). As reported in Figure 3, these strains were not detected in any of the samples analyzed after milk inoculation with commercial starter cultures. These results confirmed the dominance of the added *P. freudenreichii* strain, originating from commercial starter culture, over autochthonous milk LAB.

Physicochemical characteristics

The results of the physicochemical analysis are shown in Table 2. During ripening, pH increased in both productions by about half a point. Moreover, no statistically significant differences with commercial cow's cheese were observed in the pH value of the ewe's cheese. A similar trend was observed for dry matter content. RM-EC production, however, had higher values compared with PM-EC production. Notwithstanding, both deviated

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Table 1. Microbial loads[a] of samples

	Microorganisms[a]													
Samples	TMM	TPM	Mesophilic cocci	Mesophilic rod	Thermophilic cocci	Thermophilic rod	Propionic acid bacteria	Enterococci	Total coliforms	Enterobacteriaceaee	*E. coli*	Pseudomodads	Yeasts	Molds
RM	5.95[a]	4.83[a]	5.86[a]	5.91[a]	4.36[a]	4.84[a]	<2[a]	2.41[a]	3.03[a]	3.25[a]	2.91[a]	3.39[a]	2.79[a]	2.15[a]
PM	3.17[b]	2.11[b]	3.07[b]	3.21[b]	1.94[b]	1.55[b]	<2[a]	<1[b]	<1[b]	<1[b]	<1[b]	<1[b]	<1[b]	<1[b]
SEM	0.382	0.375	0.382	0.370	0.334	0.355	—	0.331	0.415	0.445	0.426	0.506	0.383	0.296
P - value	<0.0001	0.001	<0.0001	<0.0001	0.001	<0.0001	—	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001
C RM-EC	7.54[a]	4.59[a]	7.45	5.14	6.84[a]	4.86[a]	7.64[a]	5.24[a]	5.15[a]	5.39[a]	4.04[a]	4.51[a]	3.57[a]	1.65[a]
C PM-EC	7.37[b]	4.24[b]	7.33	5.05	6.42[b]	4.15[b]	5.94[b]	<2[b]	<1[b]	<1[b]	<2[b]	<2[b]	<2[b]	<2[b]
SEM	0.028	0.056	0.021	0.030	0.063	0.103	0.234	0.718	0.706	0.738	0.553	0.618	0.489	0.226
P - value	0.04	0.03	0.061	0.423	0.012	0.005	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Ch-3 RM-EC	7.75	4.32[a]	7.40[b]	7.78[a]	7.63	6.15[b]	8.23[b]	6.85[a]	2.87[a]	3.87[a]	<2	<2	<2	<2
Ch-3 p.m.-EC	7.42	3.35[b]	8.12[a]	6.92[b]	7.59	6.87[a]	8.71[a]	3.72[b]	<1[b]	<1[b]	<2	<2	<2	<2
SEM	0.058	0.134	0.104	0.118	0..009	0..105	0.069	0.430	0.394	0.530	—	—	—	—
P- value	0..071	<0.0001	0.004	<0.0001	0.196	0.005	0.003	<0.0001	<0.0001	<0.0001	—	—	—	—
Ch-6 RM-EC	8.01[a]	4.34[a]	7.53[b]	7.76[b]	6.21[b]	6.89[b]	7.21[b]	4.44[a]	<1	<1	<2	<2	<2	<2
Ch-6 p.m.-EC	7.46[b]	3.54[b]	8.25[a]	8.24[a]	7.47[a]	7.57[a]	8.20[a]	3.67[b]	<1	<1	<2	<2	<2	<2
SEM	0.079	0.112	0.100	0.068	0.173	0.094	0.136	0.107	—	—	—	—	—	—
P - value	0.003	0.001	<0.0001	0.002	<0.0001	<0.0001	<0.0001	<0.0001	—	—	—	—	—	—
Ch-9 RM-EC	7.07	4.35[a]	6.44[b]	7.17	6.15[b]	6.10[b]	7.08[a]	4.04[a]	<1	<1	<2	<2	<2	<2
Ch-9 p.m.-EC	6.93	3.09[b]	7.09[a]	7.12	6.54[a]	6.87[a]	6.86[b]	3.21[b]	<1	<1	<2	<2	<2	<2
SEM	0.032	0.174	0.091	0.040	0.065	0.108	0.033	0.118	—	—	—	—	—	—
P - value	0.215	<0.0001	0.001	0.744	0.044	0.001	0.014	0.002	—	—	—	—	—	—

Results indicate mean values of four plate counts (carried out in duplicates for two independent productions).

[a] $\log_{10}$ cfu/g for curd and ripened cheeses.

[b] Abbreviations: TMM, total mesophilic microorganisms; TPM total psychrotrophic microorganisms; *E.*, *Escherichia*. RM, raw milk; PM, pasteurized milk; C, curd; EC, Ewiss cheese; Ch-3, cheese ripened 3 mo; Ch-6, cheese ripened 6 mo; Ch-9, cheese ripened 9 mo; n.e., not evaluated. SEM, standard error of the mean. On the column: a, b = $P < 0.05$.

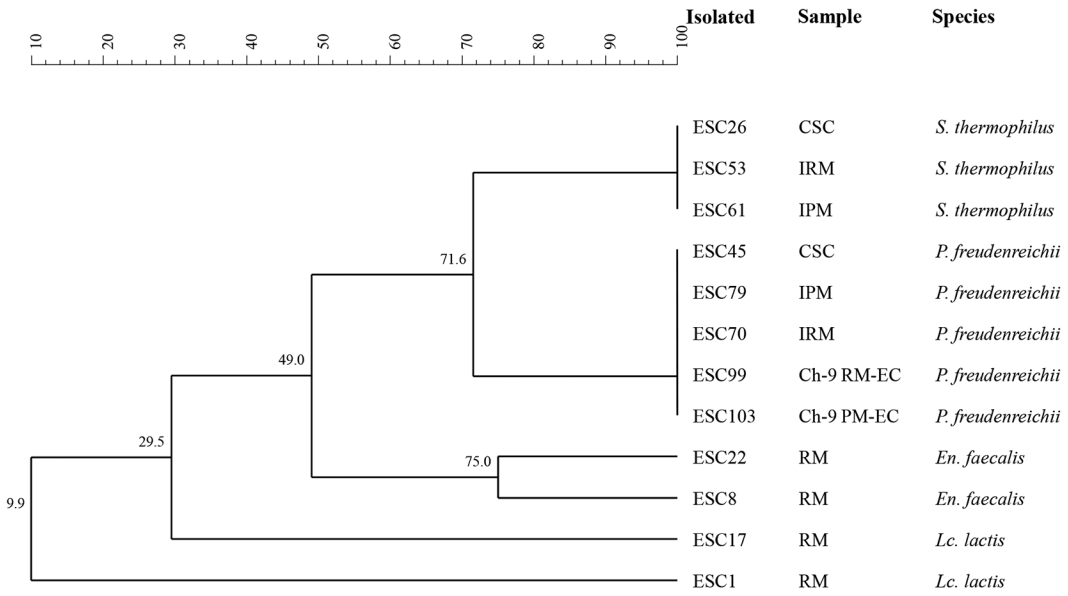


Figure 3. Dendrogram obtained with combined RAPD-PCR patterns generated with 3 primers for LAB and PAB strains isolated during cheese productions. Abbreviations: CSC, commercial starter culture; RM, raw milk; IRM, inoculated raw milk; IPM, inoculated pasteurized milk; Ch-9 RM-EC, Ewiss cheese at 9 mo of ripening produced with raw milk; Ch-9 p.m.-EC, Ewiss cheese at 9 mo of ripening produced with pasteurized milk; *En.*, *Enterococcus*; *Lc.*, *Lactococcus*.

significantly from the C-SC, which resulted in a much lower value. Fat content, instead, first increased in both productions by 2 percentage points then decreased in the 9-mo ripened cheese within a value superimposable to that of commercial cheese. Protein values were almost similar for all cheeses. Throughout the ripening process, the behavior of the 2 cheeses diverged in terms of salt content. For RM-EC, the salt content increased until the 6th mo and remained stable thereafter. However, in the case of PM-EC, salt content showed a slight decline at the 6th mo, followed by a subsequent rise of nearly one point by the 9th mo of ripening. In terms of total acidity, the maximum percentage (3.65%) was observed in the 9th mo ripened PM-EC sample.

Colorimetric parameters (Table 2) indicated that, considering cheeses at the end of ripening, lightness was comparable with the commercial cow's cheese; instead, the redness attribute was higher in the PM-EC cheese (78.62) while C-SC registered the highest value for yellowness parameters (22.39). Lastly, Chroma and Hue angle were significantly higher in cow's commercial cheese (22.54 and −0.12, respectively) than in ewes' cheeses. During the aging period these 2 parameters both tended to increase in novel cheeses.

Textures, fatty acids profile and organic acids composition

Texture profile analysis of the Swiss-type cheeses is presented in Table 3. The texture of Ewiss cheeses changed during ripening, specifically hardness, gumminess and chewiness increased from the 3rd mo of ripening until the end of maturation. Moreover, all 3 of these characteristics exhibited significant differences compared with the C-SC. No significant differences were found between the 9-mo ripened cheeses in terms of adhesiveness, springiness and cohesiveness. Lastly, resilience was lowered during ripening in the experimental ewe's cheeses, leaving the C-SC a significantly higher value with respect to others.

The fatty acid profile is shown in Table 4. Only cheese at the end of aging was assayed. Palmitic acid (C 16:0) was the most abundant fatty acid in all the cheeses found in the C-SC at 35.91%, in RM-EC at 34.81% and in PM-EC at 26.88%. It was followed by oleic acid *cis* (C 18:1) in C-SC and PM-EC cheeses. Meanwhile, RM-EC featured a higher myristic acid (C 14:00) content (14.83) than oleic acid (12.33). Other fatty acids detected were similar among cheeses, except for capric acid (C 10:0), occurring in low amounts in C-SC (3.31) and in considerably higher amounts in ewe's milk cheeses (11.17 and 9.68 in RM-EC and PM-EC, respectively).

As stated previously, the composition of organic acids is given only for cheese at the end of maturation (Table 5). When analyzed, the 3 cheeses showed statistically significant differences in all organic acids. Lactic acid concentration was more abundant in RM-EC production (3467.2 ppm). The butyric acid concentration in ewe's cheeses (326.1 and 312.3 ppm in RM-EC and PM-EC, respectively) was nearly double that found in C-SC

Table 2. Physicochemical parameters of Ewiss cheeses

Samples	Parameters											
	pH	Dry matter (%)	Fat (% in DM)	Protein (% in DM)	Salt (% in DM)	Ash (% in DM)	Acidity (%)	Lightness (L^*)	Redness (a^*)	Yellowness (b^*)	Chroma	Hue angle
Ch-3 RM-EC	4.75^a	67.86^a	53.42^a	29.87^b	3.46^a	7.30^a	1.89^b	76.61	−3.60^b	11.90^b	12.43^b	−0.30
Ch-3 p.m.-EC	4.67^b	64.19^b	48.55^b	43.50^a	2.52^b	6.30^b	2.03^a	75.75	−4.75^a	14.81^a	15.56^a	−0.32
SEM	0.50	6.62	5.19	4.05	0.31	0.69	0.23	7.73	0.44	1.37	1.44	0.196
P - value	0.01	<0.0001	0.023	<0.0001	<0.0001	<0.0001	0.40	0.284	0.009	0.01	0.01	0.03
Ch-6 RM-EC	5.11	69.97^b	55.02^a	31.77^b	3.54^a	6.86^a	1.72^b	81.80	−3.13^b	13.00^b	13.37^b	−0.24
Ch-6 p.m.-EC	5.00	66.42^b	51.19^b	39.42^a	2.28^b	6.28^b	2.62^a	82.61	−3.88^a	14.68^a	15.19^a	−0.26
SEM	0.46	6.25	4.88	3.54	0.31	0.60	0.23	7.54	0.36	1.29	1.34	0.03
P - value	0.218	<0.0001	0.031	<0.0001	<0.0001	<0.0001	0.004	0.616	0.005	0.001	0.001	0.196
C-SC	5.56^b	63.93^c	48.88	28.64^b	2.21^c	5.52^c	2.08^c	79.16	−2.67^c	22.39^a	22.54^a	−0.12^c
Ch-9 RM-EC	5.52^b	76.00^a	51.31	29.30^b	3.54^a	6.72^a	2.98^b	77.09	−3.39^b	14.95^b	15.33^b	−0.23^b
Ch-9 p.m.-EC	5.65^a	67.99^b	50.38	34.36^a	3.10^b	6.40^b	3.65^a	78.62	−4.15^a	16.26^b	16.79^b	−0.26^a
SEM	0.02	1.55	0.46	0.81	0.17	0.16	0.20	0.50	0.19	1.01	0.97	0.02
P - value	0.001	<0.0001	0.171	<0.0001	<0.0001	<0.0001	<0.0001	0.351	0.001	<0.0001	<0.0001	<0.0001

Results indicate mean values of three determinations carried out in duplicate for each of the two independent cheese-making.

Abbreviations: RM, raw milk; PM, pasteurized milk; EC, Ewiss cheese; Ch-3, cheese ripened 3 mo; Ch-6, cheese ripened 6 mo; Ch-9, cheese ripened 9 mo; DM, dry matter; SEM, standard error of the mean. On the column: a, b = $P < 0.05$.

(151.4 ppm). Cheese made from PM showed a higher concentration of propionic acid (1978.9 ppm) as well as fumaric acid (502.4 ppm); this latter acid, however, was not found in C-SC.

Composition of volatile organic compounds

The volatile organic compounds (VOCs) of the 3 kinds of cheese were analyzed using SPME-GC/MS, and the results are shown in Figure 4. The study evidenced differences in quality between the C-SC, RM-EC, and PM-EC cheeses. A total of 46 compounds were identified among the chemical classes of carboxylic acids, aldehydes, ketones, esters, alcohols, and other compounds. Carboxylic acids were the main class of the VOC in all samples analyzed, followed by ketones. Esters were not found in the C-SC, but were found in the RM-EC (8.25) and PM-EC (3.04) samples. The PM sample had 33.10% propionic acid, which was the most abundant compound among the acids found in percentage terms. Acetoin was the most abundant ketone found in the C-SC, while the experimental cheeses had a higher percentage content of 2-heptanone. Benzaldehyde was the most notable constituent within the group of aldehydes found in C-SC. However, a negligible amount was found in sheep's milk cheeses. The study found that the presence of ethyl propionate, ethyl caproate, and ethyl caprylate was significantly higher in RM cheese than in PM sheep cheeses. Conversely, ethyl butyrate and propyl propionate were only detected in PM sheep cheeses. Alcohols were the most abundant group in the RM sample, with ethyl alcohol, (S)-(+)-2-heptanol, and 2-butanol as the most abundant components.

Sensory assessment

The spider plot in Figure 5 shows the sensory assessment of the new ewe's milk cheese and that of the commercial cheese. The sensory characteristics of the cheeses were evaluated at the end of ripening. Ewe's cheeses were compared with C-SC, and the results did not reveal statistically significant differences ($P > 0.05$) for most of the attributes considered. Overall, none of the cheeses exhibited unpleasant odors, and the sensory characteristics varied slightly among the cheeses evaluated. Cheeses made from RM showed a higher odor intensity, firmness, and saltiness. Sweetness and milk odor were predominant in C-SC, while cheese made from PM received the lowest scores for color and eye distribution. Statistically significant differences ($P < 0.01$) were detected in the size of the eyes, elasticity of the cheeses, and typical Swiss cheese flavor; as expected, C-SC received the highest scores, while RM-EC the lowest. Even though there were no statistical differences, the PM cheeses were the most appreciated by the judges.

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Table 3. Texture attributes in Ewiss cheeses

	Texture (N/mm)						
Samples	Hardness (N)	Adhesiveness (N.sec)	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Ch-3 RM-EC	150.77^{a}	−5.80^{a}	0.79	0.70	105.95^{a}	84.10^{a}	0.37
Ch-3 p.m.-EC	93.85^{b}	−5.22^{b}	0.83	0.75	70.86^{b}	58.80^{b}	0.40
SEM	13.35	0.62	0.07	0.06	8.87	7.14	0.03
P - value	<0.0001	<0.0001	0.196	0.074	0.001	0.008	0.140
Ch-6 RM-EC	158.11	−2.66	0.83	0.59^{b}	92.98	77.34	0.29
Ch-6 p.m.-EC	157.4	−8.55	0.84	0.67^{a}	106.10	89.03	0.32
SEM	13.89	0.98	0.07	0.05	8.76	7.37	0.03
P - value	0.972	0.070	0.482	0.028	0.363	0.318	0.060
C-SC	127.39^{b}	−53.77	0.86^{a}	0.75^{a}	96.10^{b}	83.04	0.41^{a}
Ch-9 RM-EC	284.29^{a}	nd	0.77^{c}	0.48^{b}	136.44^{ab}	105.61	0.21^{c}
Ch-9 p.m.-EC	222.15^{a}	−11.91	0.80^{b}	0.65^{a}	144.61^{a}	115.79	0.30^{b}
SEM	18.24	9.29	0.01	0.03	6.93	4.85	0.02
P - value	0.002	0.182	<0.0001	0.001	0.04	0.086	<0.0001

Results indicate mean values of four determinations carried out in duplicate for each of the two independent cheese-making.

Abbreviations: RM, raw milk; PM, pasteurized milk; EC, Ewiss cheese; Ch-3, cheese ripened 3 mo; Ch-6, cheese ripened 6 mo; Ch-9, cheese ripened 9 mo; SEM, standard error of the mean. On the column: a, b = $P < 0.05$.

DISCUSSION

Ewe's cheese products are an integral part of the cultural heritage of many Italian regions. Hard and semi-hard cheeses, with a medium to long ripening time, are the most representative. The use of raw milk ties these products to their specific production areas and ensures a high microbiological biodiversity. The production and marketing of these dairy products contribute significantly to the country's economy (Pirisi et al., 2011). However, managing the sanitary quality of ewe's milk poses many difficulties, such as low per animal, milking systems, and herd rearing conditions and to control the microbiological quality of the milk, pasteurization treatment is often applied to ewe's milk.

The aim of this work was 2-fold: i) to develop a new ewe's milk product using the technology of a well-known and appreciated cheese such as Swiss-type cheese; and ii) to compare the new cheeses by processing both RM and PM. Considering that cheeses are unique ecosystems that contain different types of microorganisms (Beresford and Williams, 2004), the microbiological parameters were assessed first by plate counts.

The plate count analysis performed on milk samples revealed that RM hosted consistent levels of TMM and LAB, while PM was characterized by cell densities of these bacteria at 3 orders of magnitude lower. Similar results were previously reported by Garofalo et al. (2023) and Barbaccia et al. (2022) in RM and PM used to produce ovine pressed and stretched cheeses. Both PM and RM cheeses resulted safe in terms of health and hygiene. Although RM-EC initially showed the presence of undesirable bacteria in RM and curd samples, these were

Table 4. Free fatty acid profile in Ewiss cheeses

	Samples				
Fatty acids	C-SC	RM-EC	PM-EC	SEM	*P* - value
C6	2.09^{c}	3.30^{a}	2.86^{b}	0.09	<0.0001
C8	1.38^{c}	3.39^{a}	3.11^{b}	0.16	<0.0001
C10:0	3.31^{c}	11.17^{a}	9.68^{b}	0.60	<0.0001
C12:0	4.01^{c}	6.46^{a}	5.53^{b}	0.18	<0.0001
C14:0	13.32^{b}	14.83^{a}	12.44^{c}	0.18	<0.00010
C15:0	1.23	1.21	1.18	0.01	0.105
C16:0	35.91^{a}	34.81^{a}	26.88^{b}	0.72	<0.0001
C16:1	1.76^{a}	1.17^{b}	1.01^{b}	0.06	<0.0001
C18:0	9.87^{a}	5.82^{b}	9.57^{a}	0.33	<0.0001
C18:1 *cis*	19.65^{a}	12.33^{c}	17.00^{b}	0.54	<0.0001
C18:1 *trans*	1.66^{b}	0.90^{b}	2.85^{a}	0.15	0.002
C18:2	1.95	1.60	3.12	0.17	0.145
C18:3 n3	0.45^{c}	0.60^{b}	1.11^{a}	0.05	<0.0001

Results indicate mean values of six determinations (carried out in triplicate for two independent productions). Data within a line followed by the same letter are not significantly different according to Tukey test.

Abbreviations: C-SC, commercial Swiss cheese; RM-EC, raw milk Ewiss cheese; PM-EC, pasteurized milk Ewiss cheese; SEM, standard error of the mean. On the row: a, b, c = $P < 0.05$.

Table 5. Organic acids profile in ripened Ewiss cheeses

	Samples				
Organic acids	C-SC	RM-EC	PM-EC	SEM	*P* - value
Lactic acid	576.5^{c}	3467.2^{a}	741.4^{b}	234.32	<0.0001
Butyric acid	151.4^{c}	326.1^{a}	312.3^{b}	14.03	<0.0001
Fumaric acid	nd	367.3^{b}	502.4^{a}	37.53	<0.0001
Propionic acid	1525.7^{b}	715.7^{c}	1978.9^{a}	92.37	<0.0001
Glucose	157.4^{a}	20.5^{c}	42.6^{b}	10.61	<0.0001

Results are expressed in parts per million (ppm). Data within a line followed by the same letter are not significantly different according to Duncan test. Abbreviation: C-SC, commercial Swiss cheese; RM-EC, raw milk Ewiss cheese; PM-EC, pasteurized milk Ewiss cheese; SEM, standard error of the mean; nd, not detected. On the row: a, b, c = $P < 0.05$.

undetectable by the end of the ripening. Reassessing the safety of hard raw milk cheeses such as Cheddar, Swiss and Italian hard cheeses, when matured for a given period revealed that this process does not favor the survival of pathogens, as observed in previous studies (Brooks et al., 2012; Serrano et al., 2018; Metz et al., 2020). Regarding LAB, the 2 experimental cheeses exhibited differences in the microorganisms detected. In RM cheese, there was a higher occurrence of PAB (7.08 $\log_{10}$ cfu/g), which are typical in Swiss-type cheeses (Thierry et al., 2002). In contrast, the thermophilic cocci and rod-shaped lactic acid bacteria had a higher load in the PM cheese sample (6.54 and 6.87 log10 cfu/g, respectively). Moreover, RM-EC reported a higher cell density of enterococci, which are non-starter LAB. These enterococci play a significant role in the development of sensory characteristics during cheese ripening (Giraffa, 2003), consistent with the findings of Terzić-Vidojević et al. (2021) who recognized enterococci as an essential part of the natural microbial population of raw milk.

A total of 4 LAB strains isolated from RM were identified and allotted into *En. faecalis* and *Lc. lactis* species. These LAB species are typical of ovine milk microbiota (Busetta et al., 2022). However, none of these strains was found in RM-EC and PM-EC after 9 mo of ripening, confirming the ability of the added *P. freudenreichii* strain to persist and dominate during the ripening period (Thierry et al., 2011).

The physicochemical composition of the cheeses was comparable in terms of fat content – 51.31% in RM-EC and 50.38% in PM-EC (both measured in dry matter). Ewe's milk is known for its richness in fat, protein, minerals, and vitamins, setting it apart from milk of other species (Balthazar et al., 2017). However, protein content and acidity were higher in PM-EC. A similar finding was observed by Rezaei et al. (2020) when investigating the physicochemical properties of Motal cheese to assess the impact of pasteurization. Interestingly, cheese color did not exhibit significant differences between the 2 types; both displayed similar lightness, yellowness, and chroma index.

Understanding the rheological properties of cheese is crucial for assessing its consistency, structure, and overall quality. These properties are influenced by factors such as composition, processing techniques, and storage conditions. Notably, the composition of cheese, including its fat, protein, and moisture content, significantly impacts its texture and mouthfeel (Lucey et al., 2003).

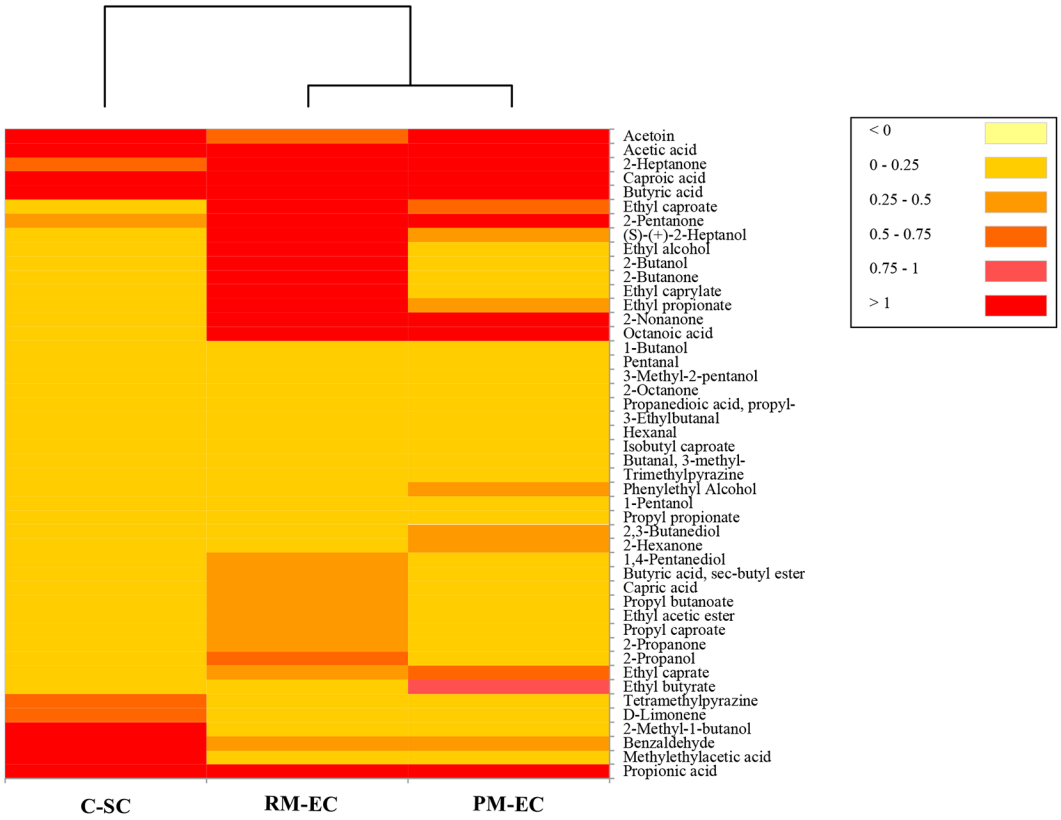


Figure 4. Volatile compounds of Ewiss cheeses using HS-SPME-GC-MS analysis. The heat map plot depicts the relative concentration of each VOCs. Abbreviations: C-SC, commercial Swiss cheese; RM-EC, raw milk Ewiss cheese, PM-EC, pasteurized milk Ewiss cheese.

In the context of this study, a texture analysis was conducted on 2 types of cheeses: RM-EC and PM-EC. Interestingly, they exhibited similar hardness and chewiness parameters. However, when considering other measured parameters—such as springiness, cohesiveness, and gumminess—differences emerged. PM-EC displayed slightly higher values in these aspects. This variation may be attributed to proteolysis, a process affecting the texture of the experimental cheese, as also observed by Awad et al. (2006).

Cheese not only provides essential compounds like calcium, proteins and vitamins, but it also contains numerous bioactive molecules. Among these, fatty acids play a crucial role (Summer et al., 2017). The fatty acid profiles of raw and pasteurised milk cheeses exhibit significant differences across nearly all examined fatty acids. In both cheese types, capric acid (C10:0), myristic acid (C14:0), palmitic acid (C16:0), and oleic acid (C18:1 *cis*) dominate. However, the RM-EC sample has a higher proportion of these compounds. This variation is attributed to metabolic activities by the natural microbiota in raw milk (Fuka et al., 2013). Unfortunately, pasteurization leads to the inactivation of these bacteria and enzymes, which are characteristic of raw milk (Psoni et al., 2006).

Organic acids play a pivotal role in determining cheese quality and serve as valuable indicators of starter activity during the ripening process. In ewe's milk cheese, water-soluble and short-chain organic acids arise through microbial metabolism. These acids include lactic, acetic, pyruvic, propionic, formic, and butyric acids, all of which contribute to the distinctive flavor and aroma of the final cheeses (Fox et al., 1993). An intriguing finding from our research is the elevated propionic acid content observed in cheese made from PM. This particular acid is responsible for the characteristic eyes and nutty flavor commonly associated with Swiss artisanal cheese (Thierry et al., 2002; Fröhlich-Wyder, 2004). Despite pasteurization inactivating approximately 95% of enzymes, their activity remains significant in long-ripened cheeses.

The production of cheese involves several factors that impact its aroma. Among these factors, the origin of the

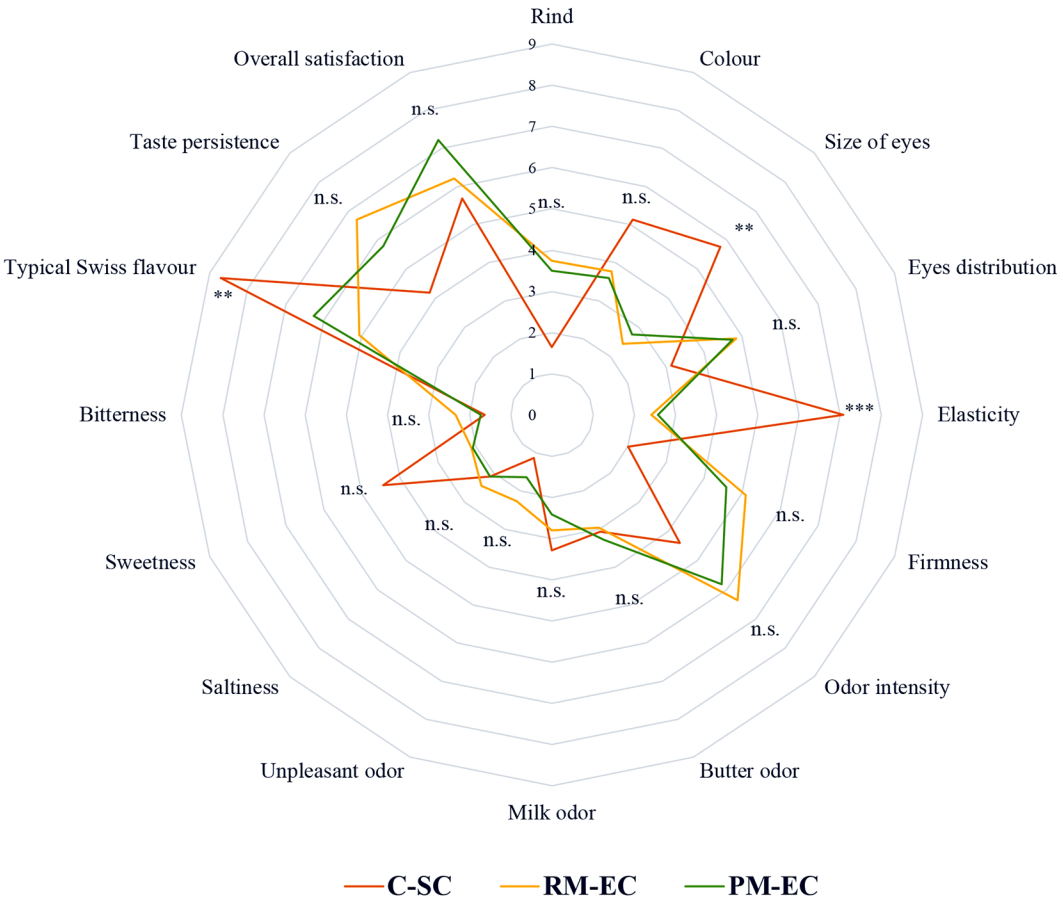


Figure 5. Sensorial assessment of Ewiss cheeses. Abbreviations: C-SC, commercial Swiss cheese; RM-EC, raw milk Ewiss cheese; PM-EC, pasteurized milk Ewiss cheese. *P* value: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; n.s., not significant.

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milk and its treatment—whether raw or pasteurized—are crucial parameters (Berard et al., 2007). According to existing literature, cheeses made from PM exhibit less development of volatile compounds during the ripening process compared with their counterparts made from raw milk. Interestingly, when comparing raw ewe's milk cheese to cheeses made from raw milk of other species, the former displays the highest volatile content (Ocak et al., 2015). Our research aligns with these findings. In our study, the RM-EC cheese demonstrated remarkable versatility in terms of volatile compounds analyzed. It exhibited elevated levels of caproic and capric acids, which significantly contribute to the taste and aroma of cheese (Tomar et al., 2020). Additionally, higher amounts of 2-butanone and 2-heptanone, important ketones, impart a buttery smell and herbaceous notes in cheese (Curioni and Bosset, 2002). However, it remains evident that the unique aroma of Swiss cheese is challenging to replicate (Castada et al., 2019).

Swiss cheese is characterized by several key volatile compounds, including acetic acid, butyric acid, ethyl butyrate, ethyl caproate, propanoic acid, and tetramethylpyrazine (Taylor et al., 2013). Interestingly, our study found that these compounds were present in both types of Ewiss cheeses (from RM and PM). However, contrary to our expectations, the PM-EC sample exhibited higher levels of some of these acids. In addition, it is worth noting that PM-EC also displayed elevated concentrations of ethyl butyrate and ethyl caproate, both of which significantly contribute to the flavor profiles of various cheeses, including Cheddar, Emmental, Grana Padano, and Pecorino. Even at low concentrations, these esters impart fruity notes to the cheese (Bontinis et al., 2012; Curioni and Bosset, 2002).

Flavor plays a pivotal role in the dairy industry, significantly impacting consumer acceptance and preference. The milk source (mainly cow, goat, or sheep) directly influences the taste and aroma of dairy products. Cheese aroma, in particular, results from a delicate balance of various aromatic compounds that, individually, cannot fully capture the complete sensory experience (Bintsis et al., 2004). Considering the exceptional quality of ewe's milk—known for its low allergenic activity and rich concentration of nutraceutical compounds—ewe's milk cheeses, whether raw or pasteurized, resonate with health-conscious consumers (Nudda et al., 2014). This underscores the importance of sensory analysis as an essential evaluation criterion. A product's global competitiveness hinges on its acceptance by discerning consumers. Our research reveals minimal differences between RM-EC and PM-EC cheeses. However, in terms of overall acceptance, the PM-EC sample received higher ratings from judges.

Variations in eye size and the distinctive Swiss flavor, likely influenced by milk type, suggest ample room for improvement in new cheese products. Swiss cheese production typically involves clarifying the milk before pouring it into the vat, a step that enhances eye development and distribution (Clark, 2009). This clarification process may also account for the observed differences. Despite these nuances, parameters such as color, absence of unpleasant odor, and taste persistence present a positive outlook for these novel cheeses. These attributes align with modern consumer expectations (Herz, 2006). Importantly, our findings encourage the production of ewe's milk cheeses, a category that remains relatively unknown and underappreciated. These cheeses, although associated with a robust animal aroma, hold promise even among young consumers unfamiliar with their unique qualities.

CONCLUSIONS

This research conducted an extensive analysis of the microbiological, physicochemical, VOC profile, and sensory characteristics of the novel Ewiss cheese. The cheese was manufactured from ewe's milk processed at an industrial level using freeze-dried commercial starters, following the Swiss cheese-making method. Notably, the microbiological analysis revealed no statistically significant differences between RM-EC and PM-EC productions in terms of LAB composition. LAB cocci predominantly drove the acidification process. Chemical and textural analyses highlighted distinctions between the experimental Ewiss cheese and the commercial Swiss cheese, primarily attributed to the higher protein content in ewe's milk. Impressively, sensory evaluation indicated that the new cheese received high praise from judges. In the realm of developing ewe's milk products for modern consumers, this study stands as the pioneering successful attempt to create a ewe's cheese by adapting the well-established and beloved Swiss cheese-making process.

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