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Use of an autochthonous defined whey starter culture for the industrial production of Gran Ovino, a Sicilian ewe's milk cheese

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ABSTRACT

This study reports the industrial scale-up of Gran Ovino (GO), an extra-hard Sicilian ewe's milk cheese originally developed at an artisanal level, with the aim of preserving its territorial identity while ensuring food safety and enhancing quality. Cheese production was carried out under industrial conditions using pasteurized milk and two different starter systems: a defined whey starter culture (DWSC) composed of selected *Streptococcus thermophilus* strains (EXP-GO) and a commercial starter culture (CTR-GO) as control. Microbiological analyses confirmed the hygienic and sanitary quality of both trials, with high levels of lactic acid bacteria (LAB) and absence of spoilage or pathogenic microorganisms. Metataxonomic profiling revealed the dominance of *Streptococcus* and *Lactobacillus* genera in ripened cheeses. After 12 months of ripening, EXP-GO cheese exhibited a more favourable fatty acid profile than CTR-GO, characterized by higher oleic acid levels and lower concentrations of trans fatty acid isomers. It also showed greater hardness and a more complex volatile organic compound (VOC) composition dominated by ketones, esters, and volatile acids. Sensory evaluation highlighted some differences in the appreciation of individual attributes between EXP-GO and CTR-GO. Overall, the use of DWSC enables the industrial production of Gran Ovino cheese with distinct compositional and sensory profiles, while maintaining its link to local tradition and biodiversity. These findings demonstrate that the use of DWSC enables the industrial production of Gran Ovino cheese with enhanced nutritional and sensory characteristics, while maintaining its link to local tradition and biodiversity.

1. Introduction

In today's global agribusiness landscape, continuous research is essential to ensure food quality, safety, and competitiveness (Gadanakis, 2024). Agrifood systems, including those in Italy, must respond to increasingly complex market dynamics and evolving consumer expectations. Innovation, particularly the ability to meet emerging needs, plays a pivotal role in this process (Ragazzi et al., 2008). At the same time, modern consumers are increasingly informed and selective, thanks to the widespread dissemination of scientific knowledge. This awareness has made them particularly sensitive to issues of food safety, authenticity, and sustainability (Grunert, 2015).

Sicily stands out as one of Italy's most active regions in agribusiness, thanks to its mild climate, rich biodiversity, and diverse geological features. In ovine dairy production, Sicily ranks second nationally after Sardinia (Sgroi & Modica, 2022), with 790,780 sheep recorded in 2023 (Rapporto Ismea Qualivita, 2024). Ovine farming remains a vital resource for rural areas affected by agricultural abandonment, and cheeses made from ewe's milk embody a unique link between people, territory and biodiversity. In this context, territorial identity emerges as a strategic driver for both business resilience and social sustainability, as it helps maintain rural livelihoods and prevent depopulation (Di Gregorio et al., 2023; Gargano et al., 2021).

Despite this potential, many Sicilian ewe's milk cheeses remain confined to niche-scale production, limiting their market impact and reducing profitability for producers. Industrializing selected products, while preserving their sensory identity and cultural value, represents a promising strategy to strengthen the regional supply chain (Tregear

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et al., 2007). In recent years, a growing body of research has investigated the development of new regional ewe's milk cheeses, inspired by dairy practices from other geographic areas, such as Quadrello di Ovino, Ovino Belmontese, and Ewiss (Busetta et al., 2025; Gaglio et al., 2024; Garofalo et al., 2021, 2024). In this context, Gran Ovino cheese was recently introduced at the artisanal level (Gaglio et al., 2019) as an innovative extra-hard sheep milk cheese inspired by the "Grana" cheesemaking technology characteristic of northern Italy.

Although not a traditional or long-established product, Gran Ovino is rooted in regional raw materials and microbial biodiversity, particularly the autochthonous lactic acid bacteria (LAB) naturally associated with Sicilian ewe's milk cheeses. Its industrial scale-up thus offers an opportunity to valorise local resources while improving process reproducibility, safety, and technological efficiency. However, compliance with food safety regulations requires the use of pasteurized milk in industrial production. In this context, maintaining the product's territorial identity depends largely on the use of autochthonous LAB. Accordingly, acidification was carried out using a defined whey starter culture (DWSC) composed of LAB strains previously isolated from traditional Sicilian ovine cheeses and selected for their favourable technological properties, thereby reinforcing the link between the cheese and its territory of origin. The performance of this DWSC was then assessed in comparison with a commercial starter culture under the same industrial conditions. The aim of the present study was to assess the industrial scale-up of Gran Ovino cheese using pasteurized milk and to evaluate the impact of an autochthonous DWSC on microbiological, physicochemical, nutritional, volatile, and sensory characteristics, compared with a commercial starter. This integrated approach provides insights into the potential of autochthonous LAB to support the industrial production of innovative ovine cheeses while maintaining their connection to the regional dairy ecosystem.

2. Materials and methods

2.1. Milk handling and inoculum preparation

Cheese productions were made using ewe's milk sourced from autochthonous Valle del Belìce sheep and transported to the Cooperativa Agricola Tumarrano sited in Cammarata (Agrigento, Italy) via tanker trucks at 4 °C. Aiming to industrialize Gran Ovino by preserving distinctive milk qualities, especially in terms of nutrition and sensory properties, two different productions were carried out. A control production of Gran Ovino (CTR-GO) was inoculated with freeze-dried commercial starter culture (LYOBAC-DT, Alce International s.r.l.), composed of defined strains of *Streptococcus thermophilus* (MO097), while an experimental production of Gran Ovino (EXP-GO) was inoculated with a DWSC composed of four selected strains of *Streptococcus thermophilus* (RC-UNIPASAAFM00189, RC-UNIPASAAFM00233, RC-UNIPASAAFM00239, and RC-UNIPASAAFM00249) previously isolated in regional ovine cheese products (Gaglio et al., 2014) and belonging to the University of Palermo culture collection. The four regional cultured strains were grown in M17 broth medium (Oxoid, Basingstoke, UK) and incubated overnight at 44 °C for 24 h. After growth, the cells were washed in peptone water (0.9 % v/v) and inoculated into whey-based medium (Settanni et al., 2012) at ~10^6 CFU/mL, then incubated at 44 °C for 24 h. The resulting multi-strain culture was used for experimental cheese making.

2.2. Cheesemaking and sample collection

Two separate production trials were conducted in accordance with the manufacturing protocols detailed in Fig. 1. In detail, 250 L milk from the evening milking was skimmed by natural skimming at a temperature of 4 °C for approximately 12 h so that the fat could naturally rise to the surface (natural creaming). After fat separation, partially skimmed milk was mixed with 250 L whole milk from the morning milking kept under

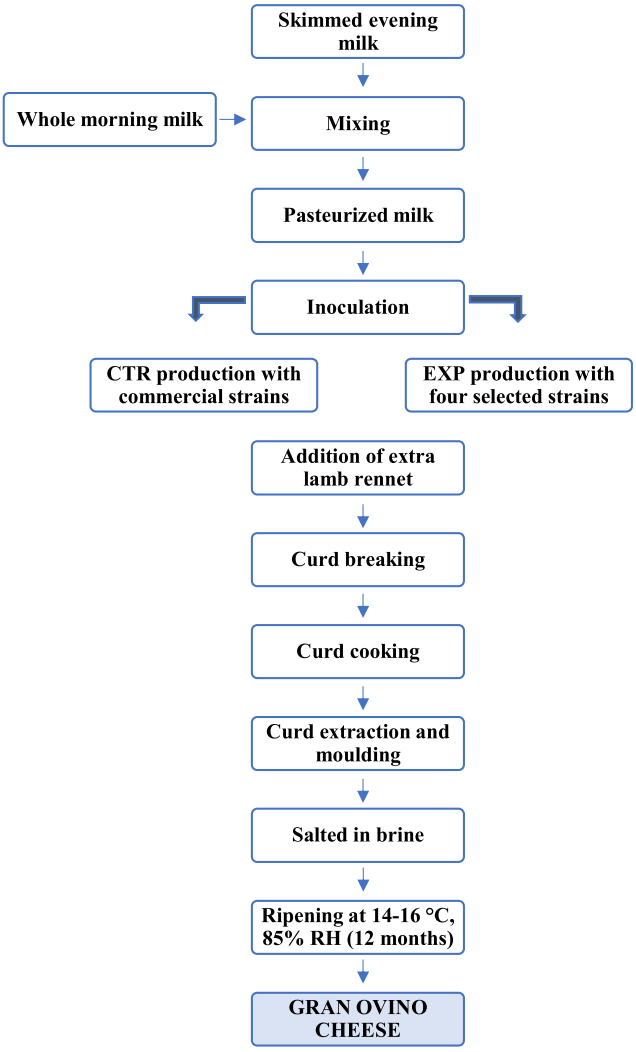

Fig. 1. Flowsheet of Gran Ovino cheese production.

low agitation to avoid both the aggregation of fat globules and their consequent surfacing. The milk, once blended, was pasteurized at 75 °C for 15 s in a P75 50/2 pasteurizer (Tecnolat S.p.a., Nocera Inferiore, Italy) and then transferred into a multi-purpose tank and cooled to 38 °C. Following heat treatment, starter cultures were added: for the CTR-GO trial, a direct inoculation into the tank was performed according to the manufacturer's instructions (typically yielding an inoculation level of ca. 10^7–10^8 CFU/mL) while for the EXP-GO trial, 8 L of DWSC (1 %, v/v) were added, the microbial load of the DWSC was previously determined by plate counts and averaged approximately 10^7 CFU/mL. Both cheesemaking trials CTR-GO and EXP-GO were carried out on the same day using the same batch of bulk milk to ensure a reliable comparison of the effects of the different starter cultures. Coagulation was achieved by adding 125 g of lamb rennet paste (Caglificio Clerici, Cadorago, Italy), title 1:10,000 previously dissolved in 1 L of tap water. After coagulation, the curd was cut using two mechanical lyres, one equipped with horizontal blades and the other with vertical blades, until granules of approximately rice-grain size were obtained (Fig. 2a). Subsequently, it was cooked for 5 min at a maximum temperature of 53 °C, with the temperature increasing by 1 °C per minute. The clot was then transferred into circular molds (approximately 13 kg) and left under whey until reaching a pH value close to 5.4 (Fig. 2b,c). Once the desired pH was reached, the forms were left at room temperature for 18 h, then placed in brine for 24 h. The ripening occurred at 14–16 °C and 85 % relative humidity for 12 months, during which the cheeses reached a final

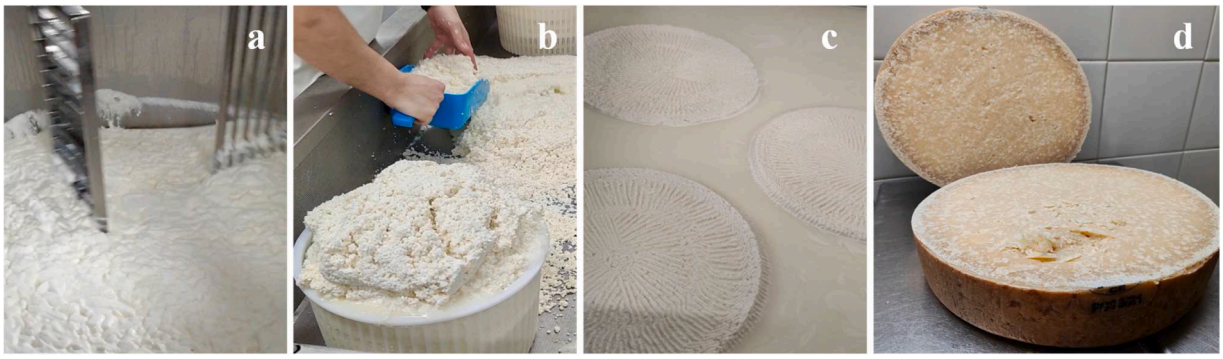

Fig. 2. Phase of Gran Ovino cheese production. A) curd breaking; B) moulding; C) salting; D) Gran Ovino cheese after 12 months of ripening.

weight of 11 kg (Fig. 2d).

Three independent cheese production trials were conducted on different days over three consecutive months (one per month), using distinct milk batches and processing runs. Each trial was considered a biological replicate, with samples collected and analysed separately without pooling. The resulting data were treated as independent observations (n = 3) and used as experimental units in all statistical analyses. All analytical determinations were performed in duplicate (two technical repeats) per each biological replicate.

2.3. Sample collection, temperature and pH monitoring

Samples of pasteurized milk (PM), inoculated milk (IM), cooked curd (CC), CTR-GO and EXP-GO cheeses at 8 and 12 months of ripening were collected. The pH was measured throughout the cheese-making process (from milk to curd) using a portable HI 9025 pH meter (Hanna Instrument, Ann Arbor, MI, USA). During ripening (up to the 12th month), the temperature of cheeses was monitored using Thermo Button 22 T 8 K data loggers (Plug & Track by Proges Plus, Willems, France) inserted in cheese during the phase of molding. Data were recorded every 24 h.

The microbial counts for liquid samples (PM, IM) and solid samples (CC and cheeses) at 8 and 12 months of ripening were determined. In particular, the pasteurized milk and inoculated milk samples (1 mL) were directly diluted (1:10) in Ringer's solution, while cooked curd and cheese samples (25 g) were first homogenized with 225 mL of 2 % (w/v) sodium citrate solution (Sigma Aldrich). The mixture was homogenized using a Stomacher machine (BagMixer 400, Interscience, Saint Nom, France) for 2 min at maximum speed. After homogenization, ten-fold serial dilutions were prepared, and the viable counts of specific microbial groups were assessed using the following media: i) De Man Rogosa and Sharpe (MRS) agar supplemented with cycloheximide (10 mg/mL) for enumeration of presumptive mesophilic lactobacilli, incubated for 48 h at 30 °C; ii) De Man Rogosa and Sharpe (MRS) agar supplemented with cycloheximide (10 mg/mL) for enumeration of presumptive thermophilic lactobacilli, incubated for 48 h at 44 °C; iii) M17 agar supplemented with cycloheximide (10 mg/mL) for enumeration of presumptive mesophilic lactococci, incubated for 48 h at 30 °C); iv) M17 agar supplemented with cycloheximide (10 mg/mL) for enumeration of presumptive thermophilic cocci, incubated for 48 h at 44 °C; v) Plate Count Agar (PCA) supplemented with 1 g/L skim milk for enumeration of total mesophilic microorganisms (TMM) and psychrotrophic microorganisms (TPM) incubated for 72 h at 30 °C and 7 d at 7 °C, respectively; vi) Kanamycin Aesculin Azide (KAA) agar for enumeration of enterococci, incubated for 48 h at 37 C; vii) *Pseudomonas* agar base (PAB) supplemented with cephaloridine sodium fusidate cetrimide (CFC) for enumeration of *Pseudomonas* spp. incubated for 48 h at 25 C; vii) Violet Red Bile Glucose Agar (VRBGA) for enumeration of members of Enterobacteriaceae, incubated for 24 h at 37 °C; ix) *Escherichia coli*-Coliforms Chromogenic Medium (ECC) agar for *E. coli,* incubated aerobically for 24 h at 37 °C; x) *Listeria* selective agar base (LSAB) with SR0140E supplement for *Listeria monocytogenes* incubated for 24 h at 37 °C; xi) Dicloran Rose Bengal Chloramphenicol (DRBC) agar for enumeration of yeast incubated for 5 d at 25 °C; xii) Potato Dextrose Agar (PDA) for enumeration molds incubated for 7 d at 25 °C; xiii) Hektoen Enteric Agar (HEA), for enumeration *Salmonella* spp. incubated for 24 h at 37 °C.

In addition, the detection of L. *monocytogenes* and *Salmonella* spp. was carried out using a two-step enrichment procedure, as described in ISO 112,90-1:2017 and ISO 65,79-1:2017, respectively. All media, except for CHROM (provided by Condalab, Madrid, Spain) were purchased from Oxoid. Analyses were performed in duplicate for all samples.

Microbial DNA from cheese samples was extracted using the QIAamp DNA Investigator Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. At each sampling point (8 and 12 months of ripening), DNA was extracted from two cheeses, each representing a different production month (from January to March). The DNA extracted from the three independent cheeses was pooled, resulting in a single composite DNA sample for each ripening stage (8 and 12 months). This approach was adopted to obtain an overall qualitative overview of the microbial community. However, it does not allow the assessment of intra-batch variability and precludes inferential statistical comparisons among biological replicates. DNA concentrations were determined using the Nanodrop ND 1000 spectrophotometer (NanoDrop Technologies Wilmintog, DE, USA). After DNA extraction, for each sample, a 464-nucleotide sequence of the V3-V4 region (Baker et al., 2003; Claesson et al., 2010), of the 16S rRNA gene (*E. coli* positions 341 to 805) was amplified and sequenced on the Illumina MiSeq system were carried out at Fondazione Edmund Mach (FEM, San Michele a/Adige, Italy) sequencing platform leading to 250 bp paired-end reads. After sequencing, raw reads were analyzed by using the QIIME2 (Bolyen et al., 2019) software.

Quality and chimera filtering step was performed by the qiime DADA2 denoise-paired script (Callahan et al., 2016). Representative bacterial sequences were aligned using MAFFT and used for phylogenetic reconstruction in FastTree, leveraging the alignment and phylogeny plugins (Katoh & Standley, 2013; Price et al., 2009). Taxonomic and compositional analyses for bacteria were conducted using the feature-classifier plugin (available at https://github.com/qiime2/q2-feature-classifier). A pre-trained Naive Bayes classifier, based on the Greengenes 13_8 99 % Operational Taxonomic Units (OTUs) database which had been previously trimmed to the V4 region of 16S rDNA, bound by the 341F/805R primer pair, was applied to paired-end sequence reads to the generate taxonomy tables. The MiSeq Illumina sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) and are accessible under Accession Number (PRJNA1379453).

Cheese samples were analysed for dry matter (DM), fat, total nitrogen (TN × 6.38) and ash content after 8 and 12 months of ripening according to the methodology of AOAC (Association of Official

Analytical Chemists, 2012 a, b, c, d). Salt content was determined following the method described by Hooi et al. (2004), by titrating chloride ions with 0.1 N $AgNO_3$ using K_2CrO_4 as an indicator. The endpoint, indicated by the appearance of a reddish-brown precipitate, corresponds to the formation of Ag_2CrO_4. Nitrogen fractions were determined according to the IDF standard (IDF, 1993). Cheese color was assessed by a tristimulus chromometer Minolta CR-400 (Minolta, Osaka, Japan) following the standard Commission Internationale de l'Eclairage (CIE, 1986). pH measurements were carried out by electrometric way with Hanna HI98161 pH meter (Hanna Instruments, Woonsocket, RI) into homogenized cheese samples.

Cheese hardness was measured with TA.XTplusC Texture Analyzer (Stable Micro Systems, Godalming, UK) evaluating the maximum compressive strength (compressive stress, N/mm^2) of samples 3 cm x 3 cm x 3 cm in size and kept at room temperature (25 °C) as in the work of Topçu and Saldamli (2006).

2.4. Analysis of cheeses free fatty acids content

Free fatty acid (FFA) profile of ripened cheeses (12 month) was determined by Gas Chromatography (GC) according to the procedure followed by Garofalo et al. (2024). Briefly, 10 g of grated cheese were processed following the method of De Jong and Badings (1990), with slight modifications. A 1 μL aliquot of the sample was injected at a split ratio of 1:40 into a GC–MS/MS system (Agilent 7890B GC - 7010BMS) equipped with a flame ionization detector and an autosampler (Gerstel, Germany). Fatty acid separation was performed on an Agilent J&W DB-WAX capillary column (60 m × 0.25 μm × 0.25 μm), using helium as the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially held at 50 °C for 1 min, then increased to 200 °C at 25 °C/min and maintained for 10 min, followed by a ramp to 230 °C at 3 °C/min, held for 26 min. The inlet and detector temperatures were set at 250 °C and 300 °C, respectively. The comparison of peak retention times with reference standards (Supelco 37 Component FAME Mix, Sigma-Aldrich, St. Louis, MO) provided the identification of fatty acids, and results were expressed as the relative percentage of total identified fatty acids.

2.5. Analysis of volatile organic compounds

VOCs were determined in the two trials and in a commercially available Grana Padano cheese (C-GP) purchased from a local supermarket (Decò - Gruppo Arena, Palermo, Italy), by using headspace solid-phase microextraction (HS-SPME) combined with gas chromatography coupled to mass spectrometry (GC–MS). The instrumentation included an Agilent 7890B gas chromatograph and a 7010B triple quadrupole mass spectrometer (Agilent Technologies Inc.). Samples were pre-incubated at 30 °C for 15 min to promote volatilization. A Carboxen/PDMS StableFlex fiber was then exposed to the headspace for 30 min to adsorb volatile compounds. Desorption was performed for 5 min using a splitless injector, followed by injection into a capillary column (60 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA, USA).

The oven temperature program began at 40 °C, ramped to 90 °C at 3 °C/min, held at 130 °C for 4 min, then increased to 240 °C at 5 °C/min and held for 8 min. Helium served as the carrier gas at a constant flow rate of 1 mL/min. Mass spectrometric data were collected in scan mode over a range of 30–600 *m/z*, using a split ratio of 1:10. Compounds were identified by comparing mass spectra with the NIST library, and results were reported as percentages of the total peak area of relevant signals (Gioacchini et al., 2010).

2.6. Sensory assessment of cheeses

The sensory evaluation compared the CTR-GO, EXP-GO, and C-GP cheeses. Samples (30 ± 5 *g*) were served in white polypropylene cups. The assessment was carried out by a semi-expert trained panel of 22 evaluators (12 women and 10 men, aged 23–54 years) from the Department of Agricultural, Food and Forest Science, University of Palermo. Panelists, including students, technical staff, and professors, received specific training for cheese sensory assessment in accordance with ISO 8589:2007.

All evaluations were performed in individual sensory booths under controlled conditions and standardized white lighting, using an iPad connected to the Smart Sensory Box software (Smart Sensory Solutions S.r.l., Sassari, Italy). Training sessions focused on ensuring consistent use of a 9-point hedonic scale to assess the pleasantness (liking) of selected sensory attributes rather than the descriptive intensity of those attributes. The evaluated attributes included: aspect (color and uniformity), smell (odor appreciation, butter odor appreciation, milk odor appreciation, and unpleasant odor perception), taste (saltiness appreciation, sweetness appreciation, acidity appreciation, bitterness appreciation, and spiciness appreciation), consistency (elasticity, solubility, and grittiness during mastication) and overall liking. This sensory approach was intended as a preliminary comparative assessment of attribute appreciation and not as a comprehensive descriptive analysis or consumer acceptance study.

2.7. Statistical analysis

Statistical analyses were performed considering the three independent cheesemaking productions as biological replicates. For each production, all analytical determinations were carried out in duplicate; these technical duplicates were used exclusively to ensure analytical accuracy and were averaged prior to statistical processing. Therefore, technical duplicates were not treated as independent observations. Data were analysed using XLStat software version 7.5.2 for Excel (Addinsoft, NY, USA) through ANOVA, and mean values were expressed together with the standard error of the mean (SEM), which was used to indicate the precision of the estimated mean for each treatment. Multiple comparisons were performed using Duncan's procedure and Tukey's test. Values of $p < 0.05$, marked by different letters, were assumed as indicators of statistical significance. Hardness parameters of cheese data were determined using Exponent software (Stable Micro Systems) version (6.0.6.0) from texture profile curves (TPA) curves.

3. Results and discussion

3.1. Monitoring pH and temperature in both cheese productions

The physicochemical parameters of Gran Ovino cheese were monitored at different stages of production and ripening (after 8 and 12 months from production) for both control (CTR-GO) and experimental (EXP-GO) batches (Table 1). At the cooked curd stage, the pH values were comparable between treatments (5.86 for CTR-GO vs. 5.78 for EXP-GO cheeses), with the experimental curd showing slightly greater acidity. Soon after molding, the temperatures reached in cheese cores were similar, though marginally lower in CTR-GO cheese (51.5 °C). Approximately 24 h after production, following moulding, both groups exhibited a reduction in pH (5.26 vs. 5.18), with EXP-GO again presenting lower values. The recorded temperatures were identical (16.2 °C) indicating uniform handling conditions. During ripening at 8 month, pH values remained close (5.40 for CTR-GO vs. 5.34 for EXP-GO), while temperatures were stable across treatments (14.7 °C vs. 14.9 °C). After 12 months of ripening, the pH values converged further (5.45 vs. 5.40), and temperatures remained consistent (14.2 °C vs. 14.4 °C). The convergence of pH values after 12 months of ripening suggests that long-term biochemical processes, including proteolysis and buffering capacity of the cheese matrix, may mitigate initial differences (Watkinson et al., 2001).

Temperature profiles were nearly identical between treatments, confirming that ripening conditions were maintained uniformly. Throughout all stages, EXP-GO cheese showed slightly lower pH values compared to the control, indicating a tendency toward greater

Table 1
pH and temperatures detected during CTR-GO and EXP-GO cheese productions.

Samples	pH	T(°C)
Cooked curd		
CTR-GO	5.86	51.5
EXP-GO	5.78	52.6
SEM	0.06	0.30
p-value	n.s	n.s
Cheese soon after moulding		
CTR-GO	5.26	16.2
EXP-GO	5.18	16.2
SEM	0.10	0.05
p-value	n.s	n.s
Cheese ripened 8 months		
CTR-GO	5.40	14.7
EXP-GO	5.34	14.9
SEM	0.07	0.08
p-value	n.s	n.s
Cheese ripened 12 months		
CTR-GO	5.45	14.2
EXP-GO	5.40	14.4
SEM	0.06	0.08
p-value	n.s	n.s

Results indicate mean value of three independent measurements. Data within a column followed by different letters are significantly different according to Tukey's test ($p < 0.05$). Abbreviations: CTR-GO, control Gran Ovino production; EXP-GO, experimental Gran Ovino production; SEM, standard error of the mean. n.s, not significant.

acidification. These findings suggest that environmental and technological parameters were effectively controlled, and that the observed pH differences are more likely due to the influence of the starter strains rather than external variables. Moreover, the consistency of these parameters is comparable to that observed in industrial-scale cheese production, where strict process control is essential to ensure product uniformity (Bansal & Veena, 2024).

3.2. Microbial dynamics during cheeses production

The plate counts of the different microbial groups investigated from pasteurized milk to cheeses ripened 12 months in CTR-GO and EXP-GO productions, are reported in Table 2. Microbial counts across different growth media revealed consistent patterns between control and experimental Gran Ovino productions.

In pasteurized milk, presumptive LAB groups showed low counts (2–3 CFU/mL) with no detectable growth of spoilage or pathogenic microorganisms, including enterococci, pseudomonads, Enterobacteriaceae, *E. coli*, L. *monocytogenes*, yeasts, molds, and *Salmonella* spp.. In particular, *Salmonella* spp and L. *monocytogenes* were not detected in pasteurized milk sample, corresponding to undetectable levels (0 log CFU/ml, even after enrichment procedure was applied), while *E. coli* and all other investigated microbial groups were below 1 log CFU/ml. These results can be attributed to the thermal treatment and for this reason are not included in Table 2.

No statistically significant differences ($p > 0.05$) were observed between CTR-GO and EXP-GO for presumptive mesophilic and thermophilic lactobacilli (MRS 30 °C and 44 °C), presumptive mesophilic and thermophilic lactococci (M17 30 °C and 44 °C). Furthermore, also TMM (PCA 30 °C) showed superimposable loads with LAB, confirming that LAB were dominant in pasteurized milk. However, presumptive thermophilic lactobacilli showed counts approximately one log cycle lower. These findings are consistent with previous reports on ovine cheeses such as "Quadrello di Ovino" and Pecorino "Primosale" (Barbaccia et al., 2021; Garofalo et al., 2021).

Following inoculation, LAB counts increased markedly to 6.02–6.40 CFU/mL, reflecting starter culture activity. During curd cooking, counts further rose to 7.04–7.45 CFU/g, consistent with microbial concentration in the curd matrix. During ripening, a gradual decline in LAB

Table 2
Microbial count during different cheese productions.

Samples	Microorganisms				
	MRS 30 °C	MRS 44 °C	M17 30 °C	M17 44 °C	PCA 30 °C
Pasteurized milk					
CTR-GO	2.85	1.97	2.54	2.71	2.91
EXP-GO	2.65	1.86	2.14	2.53	2.85
SEM	0.09	0.09	0.13	0.11	0.06
p-value	n.s	n.s	n.s	n.s	n.s
Inoculated milk					
CTR-GO	6.20	6.10	6.10	6.40	6.74
EXP-GO	6.10	6.02	6.04	6.36	6.63
SEM	0.04	0.05	0.06	0.06	0.07
p-value	n.s	n.s	n.s	n.s	n.s
Cooked curd					
CTR-GO	7.25	7.12	7.04	7.10	7.35
EXP-GO	7.45	7.26	7.10	7.40	7.55
SEM	0.08	0.10	0.09	0.09	0.12
p-value	n.s	n.s	n.s	n.s	n.s
Cheese ripened 8 months					
CTR-GO	6.49	6.35	6.31	6.50	6.40
EXP-GO	6.35	6.20	6.10	6.30	6.12
SEM	0.09	0.08	0.09	0.10	0.10
p-value	n.s	n.s	n.s	n.s	n.s
Cheese ripened 12 months					
CTR-GO	5.37	5.00	5.50	5.30	5.20
EXP-GO	5.07	5.14	5.65	5.15	5.10
SEM	0.07	0.10	0.09	0.08	0.10
p-value	n.s	n.s	n.s	n.s	n.s

Loads are reported log CFU/mL for liquid samples, and log CFU/g for curd and cheeses. Results indicate as mean value (n = 6; two technical repeats from three independent productions). Data within a column followed by different letters are significantly different according to Tukey's test ($p < 0.05$).
Abbreviations: MRS 30 °C, de Man-Rogosa-Sharpe agar incubated at 30 °C for detection of mesophilic rod LAB; MRS 44 °C, de Man-Rogosa-Sharpe agar incubated at 44 °C for detection of thermophilic rod LAB; M17 30 °C, medium 17 agar incubated at 30 °C for detection of mesophilic coccus LAB; M17 44 °C, medium 17 agar incubated at 44 °C for detection of thermophilic coccus LAB; PCA 30 °C, plate count agar added with skimmed milk incubated at 30 °C for total mesophilic microorganisms; CTR-GO, control Gran Ovino production; EXP-GO, experimental Gran Ovino production; SEM, standard error of the mean; n.s, not significant.

populations was observed. At 8 months, counts decreased to 6.10–6.50 CFU/g. A similar trend was observed by Monfredini et al. (2012), supporting our findings. However, the slightly different rate of decline observed in our study may be explained by differences in starter culture composition and ripening conditions, particularly temperature and salt content. By 12 months the levels further declined to 5.00–5.65 CFU/g. An almost 1 log cycle reduction after 12 months of ripening is a common phenomenon in Grana type cheeses due to nutrient depletion and accumulation of inhibitory metabolites (Gatti et al., 2008; Santarelli et al., 2013).

3.3. Bacterial community of cheeses

The bacterial community of cheese samples after 8 and 12 months of ripening were analyzed using Illumina sequencing. This sequencing analysis allowed the classification of sequences into OTUs. The V3-V4 hyper variable region of the bacterial 16S ribosomal RNA gene was sequenced on four pooled samples. OTUs with a relative abundance (RA) above 0.1 %, a threshold indicative of abundant bacterial communities (Logares et al., 2014), are presented in Fig. 3. The results of the classification revealed nine taxonomic groups, mainly at the genus level, given the pooled sequencing approach, these results should be interpreted as qualitative indications of the microbial groups present rather than as quantitative comparisons between productions or ripening

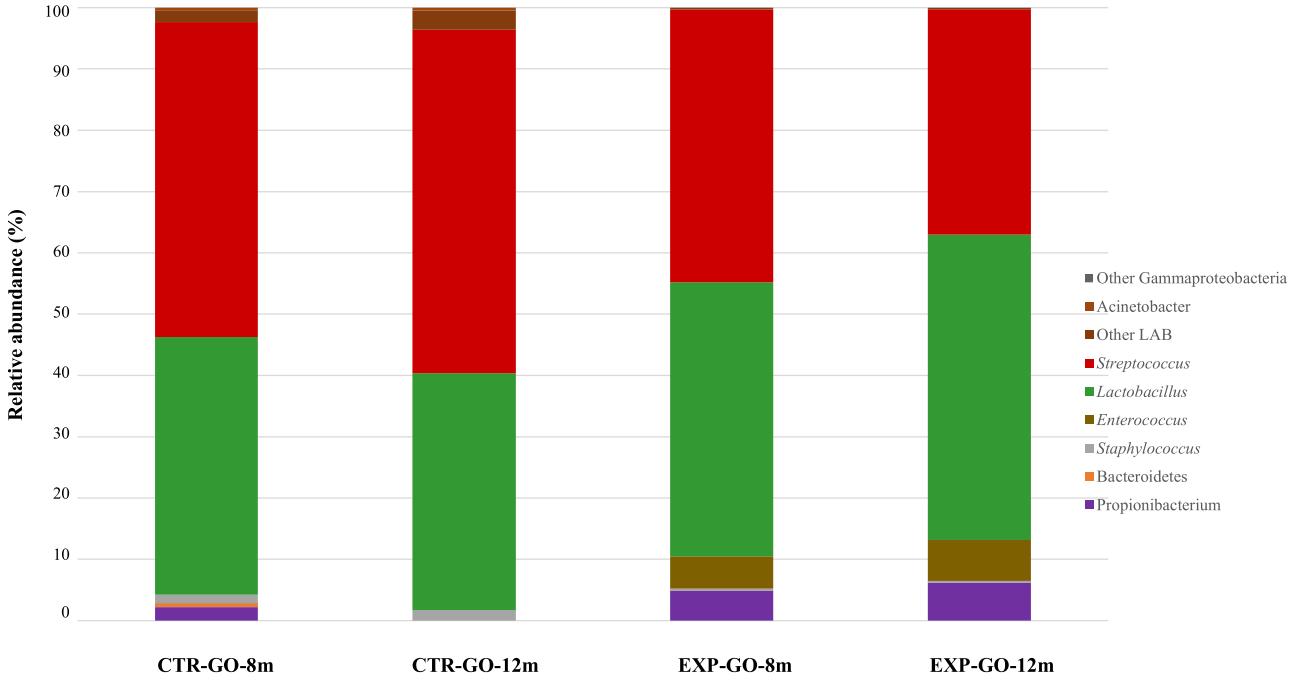

Fig. 3. Relative abundances (%) of bacteria identified by MiSeq Illumina. Abbreviations: CTR-GO-8 m, control Gran Ovino production after 8 months of ripening; EXP-GO-8 m, experimental Gran Ovino production after 8 months of ripening; CTR-GO-12 m, control Gran Ovino production after 12 months of ripening; EXP-GO-12 m, experimental Gran Ovino production after 12 months of ripening.

times. LAB were detected in all cheeses ripened 8 and 12 months in both CTR-GO and EXP-GO production. In particular, all cheeses were characterized by a high percentage of *Streptococcus* and *Lactobacillus.* Specifically, *Streptococcus* was more abundant in CTR-GO production at 8 and 12 months reaching value of 51.39 % and 55.97 % respectively. Although *Streptococcus* is a component of LAB starter group (Iyer et al., 2010), the high RA % observed in cheese samples may be attributed to DNA from dead cells, derived from pasteurized milk or bacterial strains used as fermenting agents. Similar findings were reported in Grana Padano and PDO Pecorino Siciliano (Bassi et al., 2015; Todaro et al., 2024), although those studies reported a lower relative abundance of *Streptococcus*, likely due to differences in milk treatment and starter culture management.

Lactobacillus was the second most abundant bacterial group, particularly in EXP-GO production (44.68–49.81 % of RA). Although not included in the starter culture, the detection of *Lactobacillus* is consistent with the persistence and proliferation of thermoduric strains naturally present in pasteurized milk. These microorganisms are capable of surviving heat treatment and continuing to grow during the cheese ripening process. Their resilience to thermal stress, along with their ability to thrive under the selective conditions typical of cheese maturation, such as low pH and high salt concentrations, has been well documented (Perin et al., 2017). Nevertheless, the presence of this group is not unexpected, as several studies (Alessandria et al., 2016; Levante et al., 2017) have reported high levels of *Lactobacillus* during the ripening of Grana Padano cheese. Similarly, *Propionibacterium* was detected in all samples analyzed, albeit at relatively low levels (< 7 % RA).

These bacteria are commonly found in raw milk, in small amounts, which makes their detection difficult using culture-dependent methods (Bücher et al., 2024). Like lactobacilli, propionibacteria include thermoduric strains capable of surviving moderate heat treatments such as pasteurization.

Once introduced into the cheese matrix, they can proliferate during ripening and contribute to the development of distinctive sensory attributes, including flavour and texture (Ritschard & Schuppler, 2024). *Acinetobacter,* a genus commonly associated with contaminated food matrices and dairy processing environments (De Paula et al., 2021), was detected only in CTR-GO samples. However, considering the pooled nature of the sequencing data and the lack of biological replication, this observation should be regarded as a preliminary qualitative indication and not as evidence of a treatment effect. Further studies based on independent replicated productions would be necessary to clarify whether the use of selected cultures may influence the occurrence of this genus in cheese.

3.4. Physicochemical composition of cheeses

Table 3 summarizes the physical parameters and chemical composition of CTR-GO and EXP-GO cheeses at two ripening stages: 8 and 12 months. After 8 months of ripening, EXP-GO cheese showed higher fat and salt contents (55.08 % and 3.37 %, respectively) than CTR-GO (52.02 % and 2.84 %), but a lower protein concentration (30.10 % vs. 33.75 %). These results are in line with those reported by Serrapica et al. (2020) for aged mountain Pecorino cheese.

Differences in gross composition were also reflected in physical attributes: EXP-GO appeared less firm and visually lighter, with a reduced red hue compared to CTR-GO. By the end of ripening, EXP-GO maintained its lighter appearance, while CTR-GO darkened, contrary to typical trends in the literature, which generally report a decline in L* values during maturation (Rinaldi et al., 2010).

At 12 months, EXP-GO continued to exhibit elevated fat and salt levels, along with a significantly higher pH and increased hardness compared to CTR-GO. The pH rose in both samples over time, attributed to lactic acid depletion and the alkalizing effect of protein breakdown products (Watkinson et al., 2001). Regarding textural composition, CTR-GO showed greater hardness (269.05 N) than EXP-GO (187.16 N), but by the end of ripening, EXP-GO became markedly firmer (335.86 N), indicating structural and compositional changes. This trend is consistent with the study of Madrau et al. (2006), which suggests that Pecorino cheeses harden progressively due to dehydration and protein matrix consolidation. However, the more pronounced increase observed in EXP-GO suggests that starter-driven biochemical activity may have

Table 3
Physicochemical composition of cheeses.

Samples	Parameters									
	Dry matter (%)	Fat in DM (%)	Protein in DM (%)	Ash (%)	Salt in DM (%)	Color			pH	Hardness (N)
						Lightness (L*)	Redness (a*)	Yellowness (b*)		
Cheese ripened 8 months										
CTR-GO	73.04^{a}	52.02^{b}	33.75^{a}	4.46^{a}	2.84^{a}	74.32^{a}	−3.25^{b}	16.44^{a}	4.67^{a}	269.05^{a}
EXP-GO	71.26^{b}	55.08^{a}	30.10^{b}	4.47^{a}	3.37^{b}	76.59^{a}	−4.39^{a}	14.58^{a}	4.76^{a}	187.16^{b}
SEM	0.20	0.34	0.42	0.01	0.06	0.39	0.13	0.26	0.01	9.33
p-value	0.002	0.001	0.004	0.846	0.003	0.269	0.002	0.121	0.196	0.04
Cheese ripened 12 months										
CTR-GO	75.02^{a}	47.65^{b}	28.87^{a}	4.56^{b}	2.98^{b}	67.20^{b}	−3.34^{a}	15.37^{a}	5.47^{a}	217.35^{b}
EXP-GO	73.09^{b}	51.99^{a}	29.52^{a}	4.99^{a}	3.41^{a}	78.83^{a}	−3.09^{a}	16.06^{a}	5.71^{b}	335.86^{a}
SEM	0.23	0.49	0.16	0.05	0.05	1.33	0.05	0.28	0.03	14.68
p-value	0.027	0.003	0.472	<0.001	0.008	0.006	0.360	0.691	<0.001	0.048

Results indicate as mean value (n = 6; two technical repeats from three independent productions). Abbreviations: CTR-GO, control Gran Ovino production; EXP-GO, experimental Gran Ovino production; SEM, standard error of the mean; DM, Dry matter. On the row: a, b = $p \leq 0.05$.

accelerated structural changes compared to traditional production systems. Overall, the data indicate that EXP-GO underwent accelerated ripening, likely influenced by the inoculated starter cultures, which enhanced salt and fat retention, promoted pH shifts, and resulted in a firmer and brighter final product.

Similar compositional and textural patterns have been reported by Beltrán Sanahuja et al. (2023), highlighting the importance of production practices and ripening conditions in shaping the final characteristics of ovine cheeses.

3.5. Free fatty acid profile

Free fatty acids contribute significantly to the development of cheese sensory properties and nutritional value (Li et al., 2024). The FFA profile of CTR-GO and EXP-GO is shown in Table 4, while C-GP is included as a descriptive reference. A total of thirteen fatty acids were identified, with saturated fatty acids (SFAs) representing the predominant class, followed by monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). Overall, the profiles observed in Gran Ovino cheeses suggest an accumulation of long-chain SFAs, potentially influenced by starter culture and processing conditions (Gebereyowhans, 2024).

Table 4
Fatty acid profile of cheeses.

Compounds	Samples			SEM	*p-value*
	CTR-GO	EXP-GO	C-GP		
Short- and medium-chain SFA					
Caproic acid (C6)	2.42^{a}	2.30^{a}	2.16^{a}	0.04	0.423
Caprylic acid (C8)	2.71^{a}	2.21^{b}	1.45^{c}	0.11	<0.0001
Capric acid (C10:0)	8.63^{a}	6.79^{b}	3.52^{c}	0.46	<0.0001
Lauric acid (C12:0)	4.94^{a}	3.99^{b}	4.19^{b}	0.09	<0.0001
Myristic acid (C14:0)	11.93^{b}	12.82^{a}	12.67^{a}	0.09	0.008
Long-chain SFA					
Pentadecanoic acid (C15:0)	1.08^{c}	1.55^{a}	1.34^{b}	0.04	<0.0001
Palmitic acid (C16:0)	28.07^{c}	30.34^{b}	34.77^{a}	0.60	<0.0001
Stearic acid (C18:0)	9.18^{c}	11.06^{a}	10.17^{b}	0.17	<0.0001
MUFA					
Palmitoleic acid (C16:1)	1.19^{b}	1.20^{b}	1.78^{a}	0.06	<0.0001
Oleic acid (C18:1 *cis*)	17.93^{c}	19.20^{b}	21.37^{a}	0.31	<0.0001
Oleic acid (C18:1 *trans*)	3.48^{a}	2.23^{b}	1.50^{c}	0.17	<0.0001
PUFA					
Linoleic acid (C18:2)	3.40^{a}	2.95^{b}	2.83^{b}	0.06	0.002
Linolenic acid (C18:3 n3)	1.07^{a}	1.07^{a}	0.41^{b}	0.07	<0.0001

Results indicate as mean value (n = 6; two technical repeats from three independent productions). Abbreviations: SFA, saturated fatty acids; FA, fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CTR-GO, control Gran Ovino production; EXP-GO, experimental Gran Ovino production; C-GP, Commercial Grana Padano; SEM, standard error of the mean. On the row: a, b = $p \leq 0.05$.

Short- and medium-chain FFAs differed between the two productions. Caprylic (C8:0) and capric (C10:0) acids were significantly lower in EXP-GO (2.21 % and 6.79 %, respectively) compared to CTR-GO (2.71 % and 8.63 %, respectively). These acids are typical of ewe's milk and contribute to the intense and characteristic flavours of the cheese (Güler, 2005). No significant differences were detected in caproic acid (C6) levels between samples. Myristic acid (C14:0) content was significantly higher in EXP-GO (12.82 %) than in CTR-GO (11.93 %) suggesting differences in lipid metabolism possibly associated with microbial activity or fermentation conditions (Bergamini et al., 2010). Similarly, EXP-GO showed significantly higher levels of long-chain SFAs, including pentadecanoic (C15:0), palmitic (C16:0), and stearic (C18:0) acids, which may contribute to the firmer texture observed instrumentally (Białek et al., 2020).

Among MUFAs, oleic acid (C18:1 *cis*) was significantly more abundant in EXP-GO (19.20 %) than in CTR-GO (17.93 %), a feature associated with improved nutritional properties and oxidative stability (Öz et al., 2022; Bemer, 2017). In contrast, trans isomers of C18:1 were lower in EXP-GO, which is considered nutritionally favorable (EFSA, 2010). Regarding PUFAs, linoleic acid (C18:2 n-6), an essential ω−6 fatty acid, was detected at higher levels in CTR-GO (3.40 %), whereas alpha-linolenic acid (C18:3 n-3), an ω−3 PUFA involved in brain development, neural function, and cardiovascular health (Pandohee, 2022), showed no significant variation between the two cheeses. Considering the nutritional relevance of the n-6/n-3 fatty acid ratio (Gutierres et al., 2025), these findings suggest a potential advantage of the experimental cheeses.

The C-GP sample exhibited a broadly comparable fatty acid composition. However, given the limited information available on its production and ripening conditions, it is considered only as a qualitative reference and is not included in the comparative interpretation.

3.6. Volatile organic compounds composition of cheeses

VOCs are key components in the cheese aroma, arising mainly as the result of microbial processes and enzyme action during ripening (Yavuz et al., 2021). In the present study, Gran Ovino cheeses (CTR-GO and EXP-GO) and a commercial Grana Padano cheese were analyzed and compared (Fig. 4). The differences in VOC profiles provide clear indications of the biochemical transformations that occurred during aging.

Whereas C-GP has a more standardized profile, with predominance of acids and ketones, but less variety of esters and alcohols, consistent

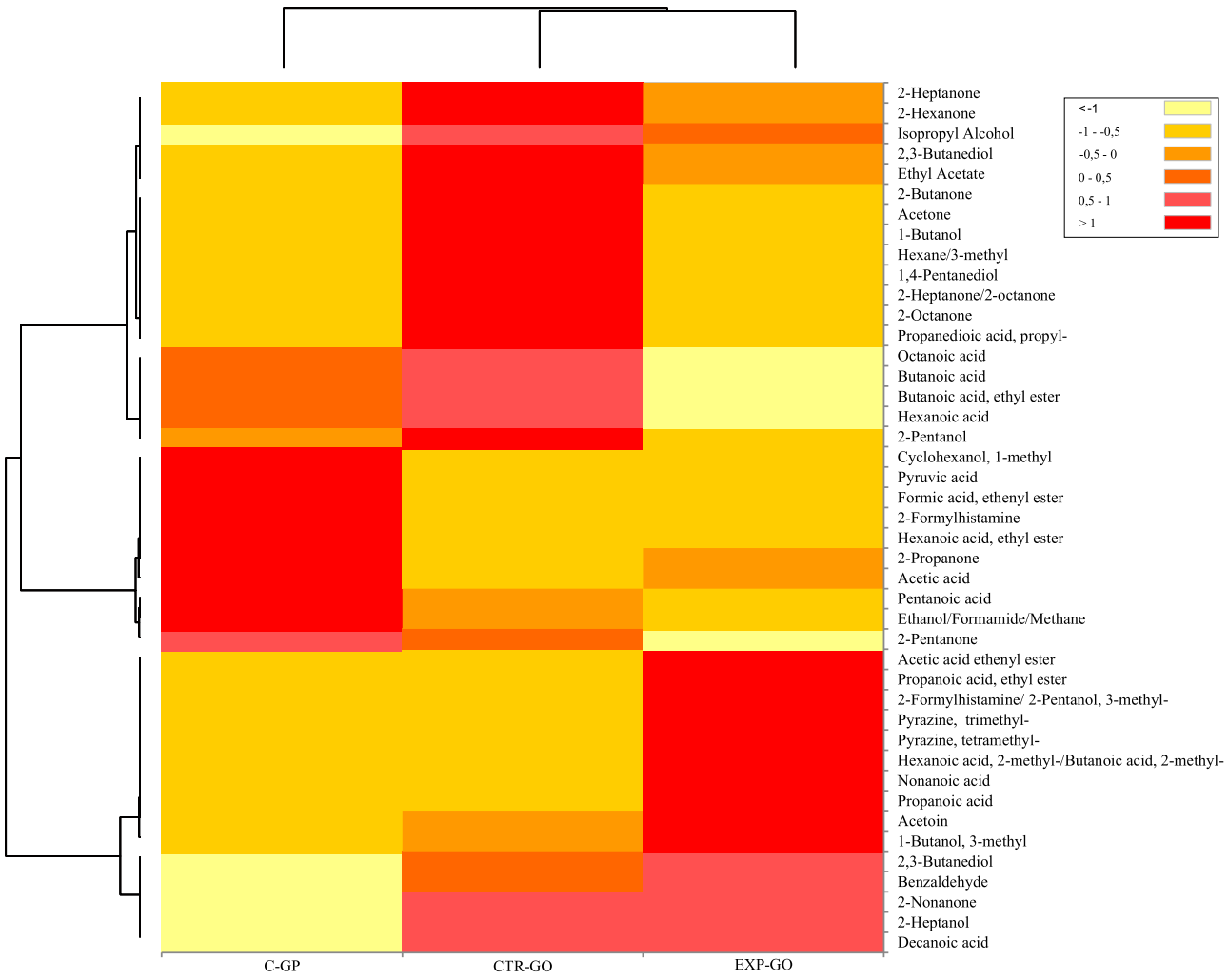

Fig. 4. Heat map of VOCs in Gran Ovino samples and commercial Grana Padano.

with a more uniform industrial production. Forty-five VOCs, including 12 ketones, 10 carboxylic acids, 9 alcohols, 8 esters, 2 aldehydes, 3 pyrazines and 1 hydrocarbon were detected in the other samples, indicating a complex volatilome. The class of carboxylic acids represented an important percentage of VOCs in all samples (between 45.88 % to 67.31 % relative abundance), followed by ketones (23.66 % to 41.77 %) and alcohol (5.33 % to 7.98 %). Although this coherent trend, each class's compounds differed considerably. CTR-GO cheese was characterized by a high content of 2-Pentanone (15.4 %) and 2-Heptanone (20.74 %), ketones associated with fruity and floral notes (Spinnler et al., 2025). In addition, high concentrations of butanoic acid (29.96 %) and hexanoic acid (10.15 %) were detected, these compounds are responsible for distinctive pungent and animal notes typical of aged ovine cheeses (Sádecká et al., 2016). However, the same compounds in the EXP-GO sample showed significantly lower concentrations than CTR-GO (13.10 % and 5.56 %, respectively). This result could represent an advantage in terms of acceptance of this product by younger consumers, who generally dislike strong ovine notes (Vargas-Bello-Pérez et al., 2022). In contrast, traditional industrial cheeses often retain higher levels of these compounds, which may limit their appeal to a broader consumer base.

EXP-GO additionally shows a marked presence of acetic acid (13.99 %) and propanoic acid (33.35 %), which contribute to sweetish and pungent notes (Gasser et al., 2023).

Meanwhile, the presence of pyrazines (trimethyl- and tetramethyl-), uncommon compounds generated by complex fermentations or heat treatments provide toasted and nutty notes (El-Shamy & Farag, 2022) that balance the flavor profile of EXP-GO cheese.

3.7. Sensory perception

Fig. 5 reports a spider plot illustrating the sensory profiles obtained from the analysis of the cheese samples. This figure represents differences in attribute appreciation among the cheeses, as measured through the hedonic scale. The sensory attributes of the CTR-GO and EXP-GO productions were compared to those of the C-GP and sensory differences supported by statistical significance ($p < 0.05$) were interpreted. The use of commercial and selected strains during Gran Ovino production did not particularly influence the sensory attributes of the final cheeses. Indeed, the sensory properties of cheese are generally influenced by the microbiological quality of milk, animal diet and farm management practices (Kilcawley et al., 2018; Martin et al., 2005; Todaro et al., 2014). In addition, several studies, such as that of Fox et al. (2004), highlight that the sensory characteristics of cheese result from a complex combination of physicochemical and microbiological factors, closely linked to the raw material and production technology. Nonetheless, certain differences were observed between the cheeses produced in this study and the C-GP cheese, particularly in terms of color, odor appreciation, and spiciness appreciation, which were more pronounced in CTR-GO and EXP-GO. These variations are likely attributable to the use of ewe's milk, which imparts greater complexity to the final products compared to cheeses made from cow's milk (Ryffel et al., 2008).

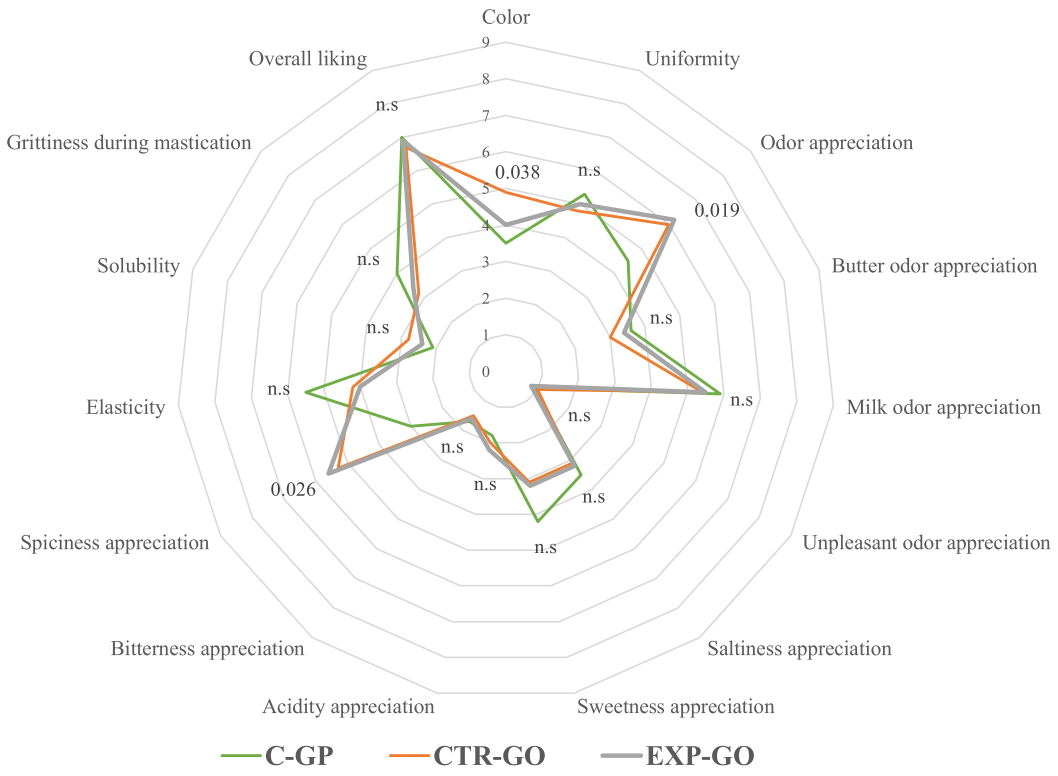

Fig. 5. Spider plot of descriptive sensory analysis of CTR-GO, EXP-GO and C-GP cheeses.

The use of a 22-member trained panel represents a limitation in terms of generalizing hedonic acceptability to a broad consumer population. Nevertheless, this panel size and methodological approach are appropriate for preliminary comparative evaluations of attribute appreciation under controlled conditions. The use of a hedonic scale was intentional, as the aim of the study was to assess attribute liking rather than to develop a full descriptive sensory profile. Future studies incorporating descriptive sensory profiling and consumer testing will be required to validate and expand upon these findings.

3.8. *Industrial applicability and technology transfer considerations*

From an industrial perspective, the use of DWSC based on selected strains of *S. thermophilus* represents a technologically feasible and scalable strategy for the production of Gran Ovino cheese. *S. thermophilus* is widely employed in industrial dairy fermentations due to its technological robustness, predictable acidification performance, and long history of safe use, which facilitates compliance with European food safety and regulatory frameworks (Ghailan et al., 2025; Mukherjee et al., 2022). Compared to commercial starter cultures, the production of DWSC may involve additional operational costs associated with strain maintenance and culture propagation (Holzapfel, 2002). However, these costs are potentially offset by improved process control, reproducibility, and the valorisation of autochthonous microbial resources linked to territorial identity (Mills et al., 2010). The consistency of physicochemical and microbiological parameters observed across independent production trials supports the reproducibility of the proposed process under industrial conditions. In terms of scalability, DWSC production relies on standardized fermentation and preservation steps already implemented in the dairy industry, suggesting limited technological barriers to large-scale adoption.

Overall, the approach shows promising potential for technology transfer, enabling the industrial production of an ovine cheese that preserves its local identity while meeting the requirements of modern dairy processing. Nevertheless, further studies addressing full-scale industrial validation, detailed techno-economic analyses, and long-term process standardization are necessary to support widespread commercial implementation.

4. Conclusions

This study assessed the industrial scalability of Gran Ovino cheese produced from pasteurized milk by comparing an autochthonous defined whey starter culture (DWSC) with a commercial starter under identical conditions. After 12 months of ripening, the two systems yielded cheeses with distinct compositional, textural, lipid, and aromatic profiles, confirming the influence of the starter culture on maturation dynamics. Differences in FFA profile, namely lower short-chain FFAs and higher long-chain saturated fatty acids and oleic acid, suggested potential implications for texture, oxidative stability, and nutritional traits. However, these differences did not translate into a clear overall improvement in compositional, nutritional, or sensory quality for the DWSC cheese. Sensory variations between products were limited and appeared to be more closely related to milk characteristics than to the starter culture used. Future studies should integrate metabolomic and proteomic approaches to elucidate more deeply the biochemical pathways underlying starter-induced differences during ripening and to complement the qualitative information provided by metataxonomic analysis on pooled samples. The comparison with C-GP was used solely as an external descriptive reference, without the possibility of drawing technological conclusions due to the uncontrolled differences between the products.

Overall, the DWSC proved compatible with industrial production, contributing to specific biochemical and sensory features while supporting the preservation of territorial identity. However, Nonetheless, further production trials and expanded sensory evaluations are needed to better define its technological and sensory impact. Finally, although the use of defined starter cultures appears technically feasible, additional studies on large-scale validation, economic sustainability, and regulatory aspects are required to support broader industrial adoption.

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Ethical statement

The present work did not involve human subjects for medical purposes.

CRediT authorship contribution statement

Giuliana Garofalo: Writing – original draft, Formal analysis, Data curation. **Tansu Taspinar:** Formal analysis, Data curation. **Gabriele Busetta:** Writing – original draft, Formal analysis, Data curation. **Elena Franciosi:** Formal analysis, Data curation. **Maria Teresa Sardina:** Resources, Project administration, Funding acquisition. **Giancarlo Moschetti:** Supervision. **Nicola Francesca:** Methodology. **Huseyin Erten:** Writing – review & editing, Methodology. **Raimondo Gaglio:** Writing – review & editing, Validation, Supervision, Conceptualization. **Luca Settanni:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Alessandria, V., Ferrocino, I., De Filippis, F., Fontana, M., Rantsiou, K., Ercolini, D., & Cocolin, L. (2016). Microbiota of an Italian Grana-like cheese during manufacture and ripening, unraveled by 16S rRNA-based approaches. *Applied and Environmental Microbiology, 82*, 3988–3995. https://doi.org/10.1128/AEM.00999-16

AOAC. (2012a). Loss on drying (moisture) in cheese, method no. 926.08. In G. W. Latimer (Ed.), *Pages in official methods of analysis of AOAC international. (19a edition)*. Gaithersburg, MD: AOAC International. Jr.

AOAC. (2012b). Fat content of raw and pasteurized whole milk, Gerber method by weight, method no. 2000.18. In G. W. Latimer (Ed.), *Pages in official methods of analysis of AOAC international. (19a edition)*. Gaithersburg, MD: AOAC International. Jr.

AOAC. (2012c). Determination of nitrogen (total) in cheese, Kjeldahl method. In G. W. Latimer (Ed.), *Pages in official methods of analysis of AOAC international* (19a ed.). Gaithersburg, MD: AOAC International. Jr.

AOAC. (2012d). Ash of milk, gravimetric method, method no. 945.46. Pages in Official Methods of Analysis of AOAC International. (19a edition). G. W. Latimer, Jr., ed. AOAC International, Gaithersburg, MD.

Bansal, V., & Veena, N. (2024). Understanding the role of pH in cheese manufacturing: General aspects of cheese quality and safety. *Journal of Food Science and Technology, 61*, 16–26. https://doi.org/10.1007/s13197-022-05631-w

Baker, G. C., Smith, J. J., & Cowan, D. A. (2003). Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods, 55*, 541–555. https://doi.org/10.1016/j.mimet.2003.08.009

Barbaccia, P., Busetta, G., Matraxia, M., Sutera, A. M., Craparo, V., Moschetti, G., Francesca, N., Settanni, L., & Gaglio, R. (2021). Monitoring commercial starter culture development in presence of red grape pomace powder to produce polyphenol-enriched fresh ovine cheeses at industrial scale level. *Fermentation, 7*, 35. https://doi.org/10.3390/fermentation7010035

Bassi, D., Puglisi, E., & Cocconcelli, P. S. (2015). Understanding the bacterial communities of hard cheese with blowing defect. *Food Microbiology, 52*, 106–118. https://doi.org/10.1016/j.fm.2015.07.004

Beltrán Sanahuja, A., Pesci de Almeida, R., Igler Marí, K. A., Lamadrid, M. C., Valdés García, A., & Nadal, E. S. (2023). Sensory attributes and instrumental chemical parameters of commercial Spanish cured ewes' milk cheeses: Insights into cheese quality figures. *Foods, 13*, 127. https://doi.org/10.3390/foods13010127

Bemer, H. L. (2017). *Analysis of oil stability in oleogel cream cheese product (Master's thesis)*. Columbus, OH: The Ohio State University.

Bergamini, C. V., Wolf, I. V., Perotti, M. C., & Zalazar, C. A. (2010). Characterisation of biochemical changes during ripening in Argentinean sheep cheeses. *Small Ruminant Research, 94*, 79–89. https://doi.org/10.1016/j.smallrumres.2010.07.004

Białek, A., Białek, M., Lepionka, T., Czerwonka, M., & Czauderna, M. (2020). Chemometric analysis of fatty acids profile of ripening cheeses. *Molecules, 25*, 1814. https://doi.org/10.3390/molecules25081814

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., & Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology, 37*, 852–857. https://doi.org/10.1038/s41587-019-0209-9

Bücher, C., Burtscher, J., Zitz, U., & Domig, K. J. (2024). One-year monitoring of prevalence and diversity of dairy propionic acid bacteria in raw milk by means of culture-dependent and culture-independent methods. *Foods, 13*, 1921. https://doi.org/10.3390/foods13121921

Busetta, G., Garofalo, G., Taspinar, T., Guarcello, R., Franciosi, E., Sardina, M. T., Moschetti, G., Settanni, L., & Gaglio, R. (2025). Enhancing "Ewiss" cheese production using autochthonous lactic acid bacteria and *Propioniciclava flava* as an alternative to commercial propionic acid bacteria. *International Dairy Journal.* , Article 106473. https://doi.org/10.1016/j.idairyj.2025.106473

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*, 581–583. https://doi.org/10.1038/nmeth.3869

CIE (Commission Internationale de l'Éclairage). (1986). *Colorimetry* (2nd ed). Vienna, Austria: Central Bureau of the Commission Internationale de l'Éclairage.

Claesson, M. J., Wang, Q., O'Sullivan, O., Greene-Diniz, R., Cole, J. R., Ross, R. P., & O'Toole, P. W. (2010). Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic Acids Research, 38*, e200. https://doi.org/10.1093/nar/gkq873

De Jong, C., & Badings, H. T. (1990). Determination of free fatty acids in milk and cheese: Procedures for extraction, clean up, and capillary gas chromatographic analysis. *Journal of High Resolution Chromatography, 13*, 94–98. https://doi.org/10.1002/jhrc.1240130204

De Paula, A. C. L., Medeiros, J. D., Fernandes, G. R., da Silva, V. L., & Diniz, C. G. (2021). Microbiome of industrialized Minas Frescal cheese reveals high prevalence of putative bacteria: A concern in the one Health context. *LWT, 139*, Article 110791. https://doi.org/10.1016/j.lwt.2020.110791

Di Gregorio, D., Bognanno, M., Laganà, V. R., & Nicolosi, A. (2022). Local proximity cheeses: Choices that guiding consumers and orienting producers—Case studies. *Sustainability, 15*, 740. https://doi.org/10.3390/su15010740

EFSA (European Food Safety Authority). (2010). Scientific opinion on trans fatty acids. *EFSA Journal, 8*, 1461.

El-Shamy, S., & Farag, M. A. (2022). Volatiles profiling in heated cheese as analyzed using headspace solid-phase microextraction coupled to gas chromatography coupled to mass spectrometry. *EFood, 3*, e2. https://doi.org/10.1002/efd2.2

Fox, P. F., McSweeney, P. L., Cogan, T. M., & Guinee, T. P. (2004). *Cheese: Chemistry, physics and microbiology. volume 1: General aspects*. Elsevier Academic Press.

Gadanakis, Y. (2024). Advancing farm entrepreneurship and agribusiness management for sustainable agriculture. *Agriculture, 14*, 1288. https://doi.org/10.3390/agriculture14081288

Gaglio, R., Francesca, N., Di Gerlando, R., Cruciata, M., Guarcello, R., Portolano, B., Moschetti, G., & Settanni, L. (2014). Identification, typing and investigation of the dairy characteristics of lactic acid bacteria isolated from "Vastedda della valle del Belìce" cheeses. *Dairy Science & Technology, 94*, 157–180. https://doi.org/10.1007/s13594-013-0150-5

Gaglio, R., Garofalo, G., Taspinar, T., Busetta, G., Barbera, M., Sutera, A. M., Alfonzo, A., Sardina, M. T., Erten, H., & Settanni, L. (2024). Description of "Ovino Belmontese", a new semisoft sheep's milk cheese processed using "Italico" cheese technology. *Food Bioscience, 60*, Article 104450. https://doi.org/10.1016/j.fbio.2024.104450

Gaglio, R., Todaro, M., Scatassa, M. L., Franciosi, E., Corona, O., Mancuso, I., Di Gerlando, R., Cardamone, C., & Settanni, L. (2019). Transformation of raw ewes' milk applying "Grana" type pressed cheese technology: Development of extra-hard "Gran Ovino" cheese. *International Journal of Food Microbiology, 307*, Article 108277. https://doi.org/10.1016/j.ijfoodmicro.2019.108277

Gargano, G., Licciardo, F., Verrascina, M., & Zanetti, B. (2021). The agroecological approach as a model for multifunctional agriculture and farming towards the European Green Deal 2030–Some evidence from the Italian experience. *Sustainability, 13*, 2215. https://doi.org/10.3390/su13042215

Garofalo, G., Busetta, G., Maniaci, G., Sardina, M. T., Portolano, B., Badalamenti, N., Maggio, A., Bruno, M., Gaglio, R., & Settanni, L. (2021). Development of "Quadrello di Ovino", a novel fresh ewe's cheese. *Foods (Basel, Switzerland), 11*, 25. https://doi.org/10.3390/foods11010025

Garofalo, G., Taspinar, T., Busetta, G., Mastrangelo, S., Portolano, B., Sardina, M. T., Gaglio, R., Erten, H., & Settanni, L. (2024). Description of Ewiss cheese, a new ewe milk cheese processed by Swiss cheese manufacturing techniques: Microbiological, physicochemical, and sensory aspects. *Journal of Dairy Science, 107*, 6614–6628. https://doi.org/10.3168/jds.2024-24711

Gasser, B. G., Fuchsmann, P., & Fröhlich-Wyder, M. T. (2023). Sensory characteristics of Swiss-type cheese varieties. In J. J. Tuohy (Ed.), *Sensory profiling of dairy products* (pp. 195–224). John Wiley & Sons.

Gatti, M., De Dea Lindner, J., De Lorentiis, A., Bottari, B., Santarelli, M., Bernini, V., & Neviani, E. (2008). Dynamics of whole and lysed bacterial cells during Parmigiano-Reggiano cheese production and ripening. *Applied and Environmental Microbiology, 74*, 6161–6167. https://doi.org/10.1128/AEM.00871-08

Gebereyowhans, S. (2024). Potential strategies to enhance conjugated linoleic acid content of milk and dairy products: A review. *Heliyon, 10*, Article e38844. https://doi.org/10.1016/j.heliyon.2024.e38844

Ghailan, A. Z., & Niamah, A. K. (2025). *Streptococcus thermophilus*: Metabolic properties, functional features, and useful applications. *Applied Microbiology, 5*, 101. https://doi.org/10.3390/applmicrobiol5040101

Gioacchini, A. M., De Santi, M., Guescini, M., Brandi, G., & Stocchi, V. (2010). Characterization of the volatile organic compounds of Italian "Fossa" cheese by solid-phase microextraction gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry, 24*, 3405–3412. https://doi.org/10.1002/rcm.4782

Grunert, K. G. (2015). The common ground between sensory and consumer science. *Current Opinion in Food Science, 3*, 19–22. https://doi.org/10.1016/j.cofs.2014.11.003

Güler, Z. (2005). Quantification of free fatty acids and flavor characteristics of Kasar cheeses. *Journal of Food Lipids, 12*, 209–221. https://doi.org/10.1111/j.1745-4522.2005.00018.x

Gutierres, D., Pacheco, R., & Reis, C. P. (2025). The role of omega-3 and omega-6 polyunsaturated fatty acid supplementation in human health. *Foods, 14*, 3299. https://doi.org/10.3390/foods14193299

Holzapfel, W. H. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology, 75*, 197–212. https://doi.org/10.1016/S0168-1605(01)00707-3

Hooi, R., Barbano, D. M., Bradley, R. L., Budde, D., Bulthaus, M., Chettiar, M., Lynch, J., Reddy, R., & Arnold, E. A. (2004). Chemical and physical methods. In H. M. Wehr, & J. F. Frank (Eds.), *Standard methods for the examination of dairy products* (pp. 385–460). American Public Health Association. https://doi.org/10.2105/9780875530024ch15.

IDF. (1993). *Milk determination of nitrogen content*. Brussels, Belgium: IDFInternational Dairy Federation. Standard 20B.

ISO. (2007). *8589:2007 Sensory analysis—Methodology—General guidance for establishing a sensory profile*. Geneva, Switzerland: ISO. Standard.

Iyer, R., Tomar, S. K., Maheswari, T. U., & Singh, R. (2010). *Streptococcus thermophilus* strains: Multifunctional lactic acid bacteria. *International Dairy Journal, 20*, 133–141. https://doi.org/10.1016/j.idairyj.2009.10.005

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution, 30*, 772–780. https://doi.org/10.1093/molbev/mst010

Kilcawley, K. N., Faulkner, H., Clarke, H. J., O'Sullivan, M. G., & Kerry, J. P. (2018). Factors influencing the flavour of bovine milk and cheese from grass-based versus non-grass-based milk production systems. *Foods (Basel, Switzerland), 7*, 37. https://doi.org/10.3390/foods7030037

Levante, A., De Filippis, F., La Storia, A., Gatti, M., Neviani, E., Ercolini, D., & Lazzi, C. (2017). Metabolic gene-targeted monitoring of non-starter lactic acid bacteria during cheese ripening. *International Journal of Food Microbiology, 257*, 276–284. https://doi.org/10.1016/j.ijfoodmicro.2017.07.002

Li, Y., Zhong, Y., Xing, F., Huang, A., & Wang, X. (2024). Comparative analysis of the quality of buffalo milk fresh cheese processed with *Moringa oleifera* seed milk coagulant and calf rennet. *Journal of Dairy Science, 107*, 10571–10583. https://doi.org/10.3168/jds.2024-25451

Logares, R., Audic, S., Bass, D., Bittner, L., Boutte, C., Christen, R., Claverie, J. M., Decelle, J., Dolan, J. R., Dunthorn, M., Edvardsen, B., Gobet, A., Kooistra, W. H. C. F., Mahé, F., Not, F., Ogata, H., Pawlowski, J., Pernice, M. C., Romac, S., & Massana, R. (2014). Patterns of rare and abundant marine microbial eukaryotes. *Current Biology, 24*, 813–821. https://doi.org/10.1016/j.cub.2014.02.050

Madrau, M. A., Mangia, N. P., Murgia, M. A., Sanna, M. G., Garau, G., Leccis, L., Caredda, M., & Deiana, P. (2006). Employment of autochthonous microflora in Pecorino Sardo cheese manufacturing and evolution of physicochemical parameters during ripening. *International Dairy Journal, 16*, 876–885. https://doi.org/10.1016/j.idairyj.2005.08.005

Martin, B., Verdier-Metz, I., Buchin, S., Hurtaud, C., & Coulon, J. B. (2005). How do the nature of forages and pasture diversity influence the sensory quality of dairy livestock products? *Animal Science, 81*, 205–212. https://doi.org/10.1079/ASC50800205

Mills, S., O'Sullivan, O. R. L. A., Hill, C., Fitzgerald, G., & Ross, R. P. (2010). The changing face of dairy starter culture research: From genomics to economics. *International Journal of Dairy Technology, 63*, 149–170. https://doi.org/10.1111/j.1471-0307.2010.00563.x

Monfredini, L., Settanni, L., Poznanski, E., Cavazza, A., & Franciosi, E. (2012). The spatial distribution of bacteria in Grana cheese during ripening. *Systematic and Applied Microbiology, 35*, 54–63. https://doi.org/10.1016/j.syapm.2011.07.002

Mukherjee, A., Gómez-Sala, B., O'Connor, E. M., Kenny, J. G., & Cotter, P. D. (2022). Global regulatory frameworks for fermented foods: A review. *Frontiers in Nutrition, 9*, Article 902642. https://doi.org/10.3389/fnut.2022.902642

Öz, M., Ucak, İ., & Nayik, G. A. (2022). PUFA and MUFA. In J. Kour, & G. A. Nayik (Eds.), *Nutraceuticals and health care* (pp. 199–215). London, UK: Academic Press.

Pandohee, J., Kour, J., & Nayik, G. A. (2022). Alpha-linolenic acid. In J. Kour, & G. A. Nayik (Eds.), *Nutraceuticals and health care* (pp. 279–288). London, UK: Academic Press.

Perin, L. M., Belviso, S., Dal Bello, B., Nero, L. A., & Cocolin, L. (2017). Technological properties and biogenic amines production by bacteriocinogenic lactococci and enterococci strains isolated from raw goat's milk. *Journal of Food Protection, 80*, 151–157. https://doi.org/10.4315/0362-028X.JFP-16-267

Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution, 26*, 1641–1650. https://doi.org/10.1093/molbev/msp077

Ragazzi, D., Boccia, F., Covino, D., & Di Donato, S. (2008). Qualità e sicurezza nei sistemi agroalimentari: Innovazioni tecnologiche ed economiche. *Italian Journal of Agronomy, 3*, 173–190. https://doi.org/10.4081/ija.2008.1s.173

Rapporto Ismea Qualivita. (2024). *Tendenze e dinamiche recenti–ovicaprini* Accessed May 2024 https://www.ruminantia.it/wp-content/uploads/2024/05/Tendenze_ovino_1_2024.pdf.

Rinaldi, M., Chiavaro, E., & Massini, R. (2010). Pecorino of Appennino Reggiano cheese: Evaluation of ripening time using selected physical properties. *Italian Journal of Food Science, 22*, 54.

Ritschard, J. S., & Schuppler, M. (2024). The microbial diversity on the surface of smear-ripened cheeses and its impact on cheese quality and safety. *Foods (Basel, Switzerland), 13*, 214. https://doi.org/10.3390/foods13020214

Ryffel, S., Piccinali, P., & Bütikofer, U. (2008). Sensory descriptive analysis and consumer acceptability of selected Swiss goat and sheep cheeses. *Small Ruminant Research, 79*, 80–86. https://doi.org/10.1016/j.smallrumres.2008.07.006

Sádecká, J., Šaková, N., Pangallo, D., Koreňová, J., Kolek, E., Puškárová, A., Bučková, M., Valík, L., & Kuchta, T. (2016). Microbial diversity and volatile odour-active compounds of barrelled ewes' cheese as an intermediate product that determines the quality of winter bryndza cheese. *LWT, 70*, 237–244. https://doi.org/10.1016/j.lwt.2016.02.048

Santarelli, M., Bottari, B., Lazzi, C., Neviani, E., & Gatti, M. (2013). Survey on the community and dynamics of lactic acid bacteria in Grana Padano cheese. *Systematic and Applied Microbiology, 36*, 593–600. https://doi.org/10.1016/j.syapm.2013.04.007

Serrapica, F., Masucci, F., Di Francia, A., Napolitano, F., Braghieri, A., Esposito, G., & Romano, R. (2020). Seasonal variation of chemical composition, fatty acid profile, and sensory properties of a mountain pecorino cheese. *Foods (Basel, Switzerland), 9*, 1091. https://doi.org/10.3390/foods9081091

Settanni, L., Di Grigoli, A., Tornambé, G., Bellina, V., Francesca, N., Moschetti, G., & Bonanno, A. (2012). Persistence of wild *Streptococcus thermophilus* strains on wooden vat and during the manufacture of a traditional Caciocavallo-type cheese. *International Journal of Food Microbiology, 155*, 73–81. https://doi.org/10.1016/j.ijfoodmicro.2012.01.022

Sgroi, F., & Modica, F. (2022). Localized agri-food systems: The case of Pecorino Siciliano PDO, a food product of the tradition of Mediterranean gastronomy. *International Journal of Gastronomy and Food Science, 27*, Article 100471. https://doi.org/10.1016/j.ijgfs.2022.100471

Spinnler, H. E. (2025). Generation of flavor compounds in cheese during ripening. In P. L. H. McSweeney, P. D. Cotter, D. W. Everett, & R. Govindasamy-Lucey (Eds.), *Cheese* (pp. 531–543). Academic Press.

Todaro, M., Bonanno, A., & Scatassa, M. L. (2014). The quality of Valle del Belice sheep's milk and cheese produced in the hot summer season in Sicily. *Dairy Science & Technology, 94*, 225–239. https://doi.org/10.1007/s13594-013-0155-0

Todaro, M., Garofalo, G., Busetta, G., Gannuscio, R., Di Rosa, A. R., Scatassa, M. L., Cardamone, C., Mancuso, I., Franciosi, E., Rando, F., Agnolucci, M., Chiofalo, V., Gaglio, R., & Settanni, L. (2024). Reduction of PDO Pecorino Siciliano cheese making duration: Microbial dynamics and quality attributes deriving from replacing whey permeate with hot water during cooking. *International Journal of Food Microbiology, 410*, Article 110481. https://doi.org/10.1016/j.ijfoodmicro.2023.110481

Topçu, A., & Saldamli, I. (2006). Proteolytical, chemical, textural and sensorial changes during the ripening of Turkish white cheese made of pasteurized cows' milk. *International Journal of Food Properties, 9*, 665–678. https://doi.org/10.1080/10942910500542238

Tregear, A., Arfini, F., Belletti, G., & Marescotti, A. (2007). Regional foods and rural development: The role of product qualification. *Journal of Rural Studies, 23*, 12–22. https://doi.org/10.1016/j.jrurstud.2006.09.010

Vargas-Bello-Pérez, E., Tajonar, K., Foggi, G., Mele, M., Simitzis, P., Mavrommatis, A., Tsiplakou, E., Habib, M. R., Gonzalez-Ronquillo, M., & Toro-Mujica, P. (2022). Consumer attitudes toward dairy products from sheep and goats: A cross-continental perspective. *Journal of Dairy Science, 105*, 8718–8733. https://doi.org/10.3168/jds.2022-21894

Watkinson, P., Coker, C., Crawford, R., Dodds, C., Johnston, K., McKenna, A., & White, N. (2001). Effect of cheese pH and ripening time on model cheese textural properties and proteolysis. *International Dairy Journal, 11*, 455–464. https://doi.org/10.1016/S0958-6946(01)00070-X

Yavuz, M., Kasavi, C., & Öner, E. T. (2021). Developments in effective use of volatile organic compound analysis to assess flavour formation during cheese ripening. *Journal of Dairy Research, 88*, 461–467. https://doi.org/10.1017/S0022029921000790