Description of "Ovino Belmontese", a new semisoft sheep's milk cheese processed using "Italico" cheese technology

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ABSTRACT

The objective of this study was to create a new semisoft sheep's milk cheese called "Ovino Belmontese" cheese (OBCh) by applying the "Italico" cheese-making technology. The cheese production took place under industrial conditions, with the addition of a commercial starter formulation containing *Streptococcus thermophilus*. The microbiological, physicochemical, and sensory characteristics of OBCh were assessed and compared to those of a commercially available cow's Italico cheese (CICh). *Streptococcus thermophilus* dominated the microbial community during the cheese-making process, reaching levels of approximately 9.0 Log CFU/g in both OBCh and CICh. Among physical characteristics, no statistically significant difference ($p \geq 0.05$) was registered in terms of lightness, redness, yellowness, and hardness between the two cheeses. OBCh exhibited a twofold higher short-chain fatty acid content compared to CICh. Both cheeses displayed similar classes of volatile organic compounds, although their relative percentages differed. The application of Italico cheese technology to process sheep's milk did not negatively affect sensory attributes. This study highlighted that utilizing a cheese-making technology not commonly used for processing sheep's milk represents a promising strategy to diversify Sicilian dairy productions.

***Keywords*:** Sheep's milk; *Streptococcus thermophilus*; Novel cheeses; Physicochemical properties; Fatty acids; Sensory evaluation

1. Introduction

Cheese, a culinary staple with a rich global history, has been produced for centuries (Praça et al., 2023). Archaeological evidence, including cave paintings, traces cheese making back to the Palaeolithic era (Harboe et al., 2010). Over time, cheese production techniques have evolved due to various factors, including population growth, lifestyle changes, and the integration of cheese as a fundamental ingredient in the food service industry (Szafrańska & Sołowiej, 2020).

Sicily, strategically positioned in the Mediterranean Sea, has significantly influenced European cheese history (Dalby, 2009). Here, sheep farming prevails over cattle breeding due to the arid climate and rugged soil conditions (Sitzia et al., 2015). Ovine breeding plays a crucial role in the regional economy (Todaro et al., 2023). Sicilian ewe's cheeses are intrinsically tied to their specific production areas and remain niche products due to their ancient and traditional methods (Scintu & Piredda, 2007). Among these cheeses, Pecorino Siciliano, Piacentinu Ennese, and Vastedda della valle Belìce have earned the prestigious protected denomination of origin (PDO) status. While Vastedda della valle Belìce thanks to its stretching phase can be enjoyed soon after production (Mucchetti et al., 2008), the other two cheeses, made from raw milk as well, require a minimum ripening period of four months (Giammanco et al., 2011). During this ripening period, the cheeses develop a robust and enduring aromatic profile, which may not be fully appreciated by all consumers, especially those with post-modern tastes (McSweeney & Sousa, 2000). To address this, the Sicilian sheep dairy industry is actively exploring innovative approaches. Developing ewe's milk products that can be marketed shortly after production while satisfying modern consumer preferences is a priority.

Traditionally, the production of typical cheeses has limited opportunities for innovation within the sheep's milk sector. However, diversifying dairy products remains a crucial competitive strategy to adapt the ever-changing market dynamics (Fusté-Forné & Mundet i Cerdan, 2021). Recently advancements have explored the application of Crescenza cheese technology, commonly used for cows' milk, to create a novel Sicilian ewes' cheese (Garofalo et al., 2021). This innovative approach has yielded quality characteristics that resonate well with consumers. Beyond product diversification, this initiative also serves a broader purpose: revitalizing sheep breeding in rural marginal areas marked by significant land abandonment (O'Rourke, 2019). By embracing new cheese making techniques, Sicily aims to encourage sustainable sheep farming practices.

This research represents an initial endeavour to produce innovative ewe dairy products, drawing inspiration from the well-established and beloved Italico-cheese, a soft-rind, short-ripened cows' cheese (Mucchetti & Neviani, 2006).

Cheese making trials were performed on an industrial scale using commercial *Streptococcus thermophilus* starter cultures. The focus was on creating a new semisoft ewe's milk cheese "Ovino Belmontese" (OBCh), hailing from the homonymous municipality in Palermo province (Belmonte Mezzagno, Palermo, Italy), which was evaluated for its microbiological, physicochemical, and sensory characteristics. This research is part of a broader project aimed at promoting the value of Sicilian ewes' milk by developing innovative dairy products.

2. Materials and methods

2.1. Milk and milk starter culture preparation

The bulk milk used for cheese production came from several farms within Palermo province (Sicily, Italy). These farms raised sheep of the Valle del Belìce and Comisana breeds. Collected milk was transported in a refrigerated road tanker (4–6 °C) to the "Il Caciocavallo" industrial dairy factory in Belmonte Mezzagno (Italy). The whole milk underwent pasteurization at 75 °C for 15 s using a Comat PS 15351 system (Bellizzi, Italy), previously sanitized with a UNIPLUS solution (Sydex S.p.A., Cercola, Italy). The characteristics of pasteurized milk (average data of the bulks used in this study) were: pH 6.62 ± 0.02, lactose 4.03% ± 0.29%, fat 6.26% ± 0.21%, protein 5.09% ± 0.23%, casein 3.86% ± 0.25%, and urea 33.91 ± 1.21 mg/dL. Freeze-dried cheese lactic acid bacteria (LAB) starter culture LYOBAC-D (Alce International s.r.l., Quistello, Italy) was employed to start the fermentation process. This starter culture consisted of various strains of *Streptococcus* *thermophilus*. Specifically, a package containing 5 units of freeze-dried starter preparation was reactivated in 2 L of pasteurized milk. After incubation at 44 °C for 50 min, this mixture became the Milk Starter Culture (MSC), the essential fermenting agent for cheese production.

2.2. Cheese production and sample collection

The production of Ovino Belmontese cheese (OBCh) followed the principles of the "Italico" semisoft cheese technology (Fig. 1). Five hundred liters of pasteurized ewe's milk were transferred to a multi-purpose cheese vat (Comat mod. POL15P12, Bellizzi, Italy). The milk was cooled to 42 °C and then gently stirred (20 rpm) for 10 min while inoculating it with the MSC. Coagulation was initiated by adding 225 mL of Astro Chymosin 200 liquid rennet (Calza Clemente s.r.l., Acquanegra Cremonese, Italy). After 20 min, the coagulum was manually crosscut using a stainless-steel rod, called "lira". An additional 20 min of mechanical agitation broke the curd into nut-size grains. Partial whey was drained, and the curd was promptly transferred into rectangular perforated plastic containers (20 cm × 13 cm × 11 cm) purchased from GR s.r.l. (Trapani, Italy). The curds underwent an initial 30 min steam stewing at 45 °C. They were then inverted in the molds and stewed for an additional 30 min. After 24 h of stewing, all cheeses were immersed in 18 °Bé brine for 20 min. The cheeses were then stored for 10 d at 6 °C and 90% relative humidity (RH) in a seasoning cabinet model 701 Glass (Everlasting s.r.l., Suzzara, Italy). Experimental cheese production was performed in triplicate over three consecutive months (three independent experimental replicates). Samples were collected at various stages: pasteurized milk, freeze-dried starter preparation, inoculated milk with MSC, curd, and final cheese after 10 d of storage. Three commercial cow's Italico cheese (CICh), with the same maturation period of OBCh, produced by Lactalis Galbani (Milan, Italy) and purchased from a retail store were used as control cheeses.

2.3. Microbiological analyses of cheeses

All samples collected throughout the production chain of OBCh were subjected to the serial decimal dilution procedure (Garofalo et al., 2021). Cell suspensions at decreasing cell densities were plated on: Plate Count Agar (PCA) incubated aerobically for 3 d at 30 °C for the enumeration of total mesophilic microorganisms (TMM); Sucrose Peptone Yeast pH 9.3 (SPY9.3) agar incubated anaerobically for 2 d at 42 °C for *S. thermophilus* (Shani et al., 2021); Kanamycin esculin Azide Agar (KAA) incubated aerobically for 1 d at 37 °C for enterococci; Coliforms Chromogenic Medium (CHROM) agar incubated aerobically for 1 d at 37 °C for *Escherichia coli*; *Listeria* Selective Agar Base (LSAB) added with SR0140E supplement, incubated aerobically for 1 d at 37 °C for *Listeria monocytogenes*; Baird Parker (BP) agar with rabbit plasma fibrinogen (RPF) supplement, incubated aerobically for 2 d at 37 °C for coagulase-positive staphylococci (CPS); Xylose Lysine Deoxycholate (XLD) agar incubated aerobically for 1 d at 37 °C for *Salmonella* spp.. Detection of *L. monocytogenes*, and *Salmonella* spp. was carried out on 25 mL of milk samples or 25 g of curd and cheese samples after enrichment on selective broth media as reported by Scatassa et al. (2015). All media, except for CHROM (provided by Condalab, Madrid, Spain) were purchased from Oxoid (Basingstoke, United Kingdom). Analyses were performed in duplicates for all samples.

2.4. Isolation, typing and identification of thermoduric milk LAB

All presumptive *S. thermophilus* and enterococci developed on SPY9.3 and KAA, respectively, inoculated with the cell suspensions of pasteurized milk were purified and subjected to Gram reaction and catalase activity tests (Barbaccia et al., 2021). Differentiation of the collected isolates was carried out using random amplification of polymorphic DNA (RAPD)-PCR analysis as described by Garofalo et al. (2023). Genotypic identification of the distinct strains was performed at the AGRIVET Centre (Palermo, Italy), following the approach reported by Gaglio et al. (2016).

2.5. Monitoring of commercial starter culture

The dominance of commercial *S. thermophilus* starter culture over LAB resistant to pasteurization was carried out by RAPD-PCR analysis. Specifically, RAPD profiles obtained from bacteria isolated from SPY9.3 at the various stages of the OBCh production chain were compared with a pure cultures of the *S. thermophilus* strains originating from the freeze-dried starter preparation.

2.6. Physicochemical analyses of cheeses

The colorimetric parameters of the cheese samples were determined using a tristimulus chromometer Minolta CR-400 (Minolta, Osaka, Japan), measuring the values of L* (lightness), a* (redness/greenness), and b* (yellowness/blueness), according to the Commission Internationale de l'Éclairage standard (CIE, 1986).

The pH was measured by immersing a portable Hanna HI98161 pH meter (Hanna Instruments, Woonsocket, RI, USA) into homogenized cheese sample. Hardness analysis was carried out using a TA.XTplus Texture Analyser (Stable Micro Systems, Godalming, UK). The cheeses were cut into cubes (3 cm × 3 cm × 3 cm) using a sharp knife and then compressed at a constant crosshead speed of 2 mm/s. The centesimal chemical composition of the samples was analyzed, and the dry matter (DM), fat, protein, and ash content were determined according to AOAC International methods (AOAC, 2012a; AOAC, 2012b; AOAC, 2012c; AOAC, 2012d). Physicochemical determinations were performed in duplicate.

2.7. Determination of cheeses fatty acids

The fatty acid composition of the cheeses was analysed using Gas Chromatography-Mass Spectrometry (7890B GC - 7010B MS/MS, Agilent Technologies Inc., Santa Clara, CA, USA). Grated cheese samples weighing 10 g underwent fatty acid esterification following the method outlined by De Jong and Badings (1990) with modifications. Specifically, a 1 µL aliquot of the sample with a split ratio of 1:40 was injected into a GC-MS/MS system. Separation of the fatty acids was conducted using a capillary DB-WAX column (60 m x 0.25 µm x 0.25 µm, J&W

Scientific, Folsom, CA, USA) with helium as the carrier gas flowing at a rate of 1 mL/min. The oven temperature program started at 50 °C for 1 min, then increased to 200 °C at a rate of 25 °C/min, held for 10 min, further increased to 230 °C at a rate of 3 °C/min, and maintained at this temperature for 26 min. The inlet temperature and detector were set to 250 °C and 300 °C, respectively. Identification of fatty acids was confirmed by comparing the retention times of sample peaks with those of reference standards (Supelco 37 Component FAME Mix, Sigma-Aldrich, St. Louis, MO, USA).

2.8. Analysis of volatile organic compounds emitted from cheeses

The volatile organic compounds (VOCs) of cheeses were determined using the headspace solid-phase microextraction method (HS-SPME) and analysed via Gas Chromatography (Agilent 7890B GC, Agilent Technologies Inc.) coupled with mass spectrometry (7010B MS, Agilent Technologies Inc.). Initially, the samples were heated to 30 °C for 15 min, allowing the volatile compounds to be adsorbed onto a coated fiber (Carboxen TM/PDMS StableFlexTM) for 30 min. Subsequently, the samples were desorbed for 5 min through a splitless GC injector and injected into a capillary column (60 m x 0.25 mm i.dx 0.25µm, J&W Scientific).

The column temperature was programmed to increase gradually from 40 °C to 90 °C at a rate of 3 °C per min, followed by maintaining an isothermal hold at 130 °C for 4 min with a ramp of 4 °C per min. Afterwards, the temperature was further raised to 240 °C at a rate of 5 °C per min and held for 8 min. Helium served as the carrier gas at a flow rate of 1 mL/min. The acquisition was conducted under scanning conditions within a mass range spanning from 40 to 600 m/z. The partition ratio was 1:10.

Identification of volatile compounds was accomplished using the NIST 05 library, and the results were expressed as percentages of the peak area relative to the total area of significant peaks.

2.9. Sensory evaluation of cheeses

A group of 13 judges (comprising six women and seven men, aged between 27–62 years) assessed the sensory characteristics of OBCh and CICh cheeses. The evaluation followed EN ISO 22935–2:2023 guidelines. These evaluators were chosen based on their familiarity with cheese consumption and were unaware of the experimental setup. The cheeses, cut into 2 cm cubes, were allowed to acclimate at room temperature (approximately 20–22 °C) for 1 h. They were then served in a random order on white plastic plates, each labeled with a unique digit code unrelated to the experimental batches. The sensory evaluation took place in individual chambers illuminated by white light. An iPad connected to the Smart Sensory Box software (Smart Sensory Solutions S.r.l., Sassari, Italy) facilitated the assessment. The judges evaluated the following sensory traits of the cheeses: colour, uniformity, intensity of odour, odour of milk, odour of butter, unpleasant odour, salty, sweet, acid, bitter, spicy, chewiness, solubility, grittiness, unpleasant aroma, taste persistency and overall acceptability. Their scores were recorded using a line scale ranging from 1 to 9 cm, as previously described by Garofalo et al. (2021).

2.10. Statistical analyses

Microbiological, physicochemical, and sensory characteristics were analysed using One-Way Variance Analysis (ANOVA) and pairwise comparisons with Tukey's test at a significance level of $p \leq 0.05$. Heat map cluster analysis was used to identify the distribution of VOCs emitted from OBCh and CICh. All analyses were conducted using XLSTAT software version 2020.3.1 (Addinsoft, New York, NY, USA) evaluating only the effect of cheese (OBCh and CICh).

3. Results and discussion

3.1. Evolution of microbiological parameters during cheese production

The results of the microbiological investigation carried out throughout the production chain of OBCh cheese, from ewes' milk to curd samples, are reported in Table 1. The targeted search for *E. coli*, CPS, *L. monocytogenes*, and *Salmonella* spp., which are relevant for monitoring food hygiene and safety standards (EFSA, 2005), yielded no colonies in any of the analyzed samples. Notably, the commercial dried starter culture was predominantly composed of *S. thermophilus* (10.36 Log CFU/mL). The levels of TMM, streptococci and enterococci of pasteurized milk were 3.34, 3.05 and 2.07 Log CFU/mL, respectively. This aligns with the typical microbial levels found in pasteurized ewes' milk used for cheese production (Barbaccia et al., 2022; Salmerón et al., 2022). The occurrence of TMM and LAB primarily results from the inability of the pasteurization process to completely inhibit the growth of thermoduric milk microbiota (Grappin & Beuvier, 1997). The analysis of inoculated milk with MSC showed an increase in *S. thermophilus* up to 6.91 Log CFU/mL. Blaiotta et al. (2017) observed the same behavior by analysing bovine milk inoculated with the same starter culture used to produce Italico-type cheese. Following curdling, the cell densities of these microorganisms reached approximately 8.0 Log CFU/g. The observed increase in curd samples is an anticipated phenomenon attributed to whey drainage (Settanni et al., 2013). Interestingly, no statistically significant differences ($p \geq 0.05$) were detected in the levels of TMM and *S. thermophilus* between CICh and OBCh samples (Fig. 2). The results of the CPS, *E. coli*, *L. monocytogenes*, and *Salmonella* spp. are not included in Fig. 2, because no CICh and OBCh samples were scored positive for their presence. Both cheeses exhibited *S. thermophilus* levels of approximately 9.0 Log CFU/g, consistent with the patterns commonly observed in pressed ovine and bovine cheeses (Bonanno et al., 2019; Gaglio et al., 2021).

3.2. Identification of thermoduric milk LAB

After enumeration, all presumptive *S. thermophilus* isolates from pasteurized ewes' milk underwent strain typing using RAPD-PCR. The identification via 16S rRNA gene sequencing revealed that the LAB community isolated from pasteurized ewes' milk consisted of six distinct strains belonged to the to the species *Enterococcus faecium* (Ac. No. PP789677-PP789678) and *S. thermophilus* (Ac. No. PP621851-PP621854) (Fig. 3). These LAB species are characteristic of sheep milk microbiota (Quigley et al., 2013) and are part of the common dairy starter and non-starter LAB cultures

(Grujović et al., 2022). Despite their typical association with sheep milk, the presence of *En. faecium* and *S. thermophilus* in pasteurized milk primarily stems from its remarkable ability to withstand the conventional heat pasteurization process (Delgado et al., 2013; McAuley et al., 2012).

3.3. Dominance of S. thermophilus starter cultures

The prevalence of commercial starter cultures in relation to thermoduric milk LAB was monitored throughout the cheeses-making process. To achieve this, 107 isolates were collected and subjected to a comprehensive characterization using both microscopic inspection and RAPD-PCR analysis. This approach is commonly used to assess the dominance of added starter cultures in cheese productions (Fusco et al., 2019). Upon microscopic inspection, all isolates exhibited a characteristic arrangement: cells organized in long chains, a typical feature of streptococci (Barbaccia et al., 2020). The RAPD-PCR analysis conducted on isolates obtained from the commercial freeze-dried starter revealed the presence of three distinct *S. thermophilus* strains (Fig. 3). The strategic use of multiple-strain combinations of LAB is of paramount importance in mitigating phage-related challenges (Parente et al., 2017). Furthermore, a direct comparison of the polymorphic profiles of all LAB isolated along the OBCh production chain unequivocally demonstrated the dominance of the added *S. thermophilus* strains originating from freeze-dried commercial starter (Fig. 3). These strains effectively outcompeted the thermoduric milk LAB.

3.4. Physicochemical characterization of cheeses

The physicochemical characteristics of CICh and OBCh are summarized in Table 2. Notably, no statistically significant differences ($p \geq 0.05$) were observed between the two cheeses regarding color parameters (L*, a*, and b*) and hardness. These physical attributes play a defining role in determining visual acceptability and influencing consumer purchase decisions, especially for fresh cheeses (Comi et al., 2001). Our findings align with those reported by Mohamed et al. (2021) in fresh cheeses made from both sheep's and cow's milk. While the pH values exhibited variation between CICh and OBCh, they remained within the typical range of 5.06 to 5.52, commonly observed for rennet-curd cheeses (Filipczak-Fiuta et al., 2021). Regarding the chemical composition of the cheeses, significant differences ($p \leq 0.05$) were evident only in terms of dry matter and ash content. In particular, CICh showed higher values than those of OBCh, which can be attributed to the different milk types used in cheese production (Barłowska et al., 2011). Both cheeses shared an average fat content of 57.91% and a protein content of 21.81%. These results are consistent with previous findings reported by Gobbetti et al. (2018) for fresh cow's milk cheeses and by Garofalo et al. (2021) for sheep's milk cheeses.

3.5. Fatty acid composition of cheeses

The fatty acid composition of cheeses is influenced by various factors, and distinct characteristics emerge between the two productions (Table 3). Specifically, during OBCh production, significantly higher average percentages of short-chain fatty acids (SCFA) (17.35%) and medium-chain fatty acids (MCFA) (20.32%) were observed, while the average percentage of long-chain fatty acids (LCFA) was lower (62.30%) compared to CICh (SCFA = 7.78%; MCFA = 18.71%; LCFA = 73.93%). Comparable trends were observed in similar productions (Paszczyk & Łuczyńska, 2020; Prandini et al., 2011). Among the long-chain polyunsaturated fatty acids (PUFA), the isomer cis-9, trans-11 of linoleic acid (LA) (commonly known as rumenic acid) exhibited higher levels in OBCh production, corroborating existing literature from Contarini et al. (2009), Cruz-Hernandez et al. (2006), and Prandini et al. (2001). Notably, PUFA levels are not synthesized by ruminant tissues and strongly depend on animal feeding practices (Boland et al., 2001; Chilliard et al., 2000; Griinari & Bauman, 1999). Interestingly, previous studies indicate that among cows, goats, and sheep, the highest LA concentration is found in ewe's milk, even when these ruminant species are fed similar forages (Banni et al., 1996; Jahreis et al., 1999). This aspect holds significant health benefits, as rumenic acid is associated with anticarcinogenic, immunomodulatory, and anti-atherosclerotic properties (Kelley et al., 2007; Martin & Valeille, 2002). Additionally, both productions prominently featured the long-chain monounsaturated fatty acid oleic acid (C18:1 cis9). The presence of this compound is noteworthy due to its documented to possess anti-carcinogenic and anti-atherogenic properties, making it beneficial for inclusion in daily diets (Hanuš et al., 2018). In OBCh cheese, higher contents of short-chain fatty acids, such as caproic (C6:0), caprylic (C8:0), capric (C10:0), and lauric (C12:0) acids, were found compared to ClCh, following classic fatty acid profiles of sheep's milk cheeses (Hernández et al., 2005; Park et al., 2007). The increased presence of short-chain fatty acids not only improves the digestibility of the product but also contributes to the distinctive flavors found in cheeses from small ruminant animals.

3.6. Volatile organic compounds profile of cheeses

Results of the analysis for the volatile organic profile of OBCh and ClCh are presented in Fig. 4. These VOCs encompass a variety of chemical classes, including acids, alcohols, esters, aldehydes, and ketones. Carboxylic acids constituted the primary class of VOCs in both ClCh (70.9%) and OBCh (39.1%). Alcohols followed in descending order, accounting for 16.7% in ClCh and 29.9% in OBCh. Ketones contributed 6.3% in ClCh and 20.8% in OBCh, aldehydes 4% in ClCh and 10% in OBCh, while esters 1.9% in ClCh and 0.2% in OBCh. Among the acids, hexanoic, butyric, and acetic acids were prominent volatile compounds in ClCh, and these same compounds were also detected in OBCh. Carboxylic acids significantly contribute to the overall flavor of cheese (Tomar et al., 2020). Specifically, hexanoic acid imparts a sour note, butanoic acid adds a cheesy flavor, and acetic acid contributes to vinegar and acidic notes (McSweeney & Sousa, 2000). However, while acids are important in cheese aroma, they also serve as precursors for other compounds, including ketones, alcohols, aldehydes, and esters (Collins et al., 2003; Thierry et al., 2017). Ketones, commonly found in dairy products, originate from the β-oxidation of fatty acids (Guillén et al., 2004). These compounds possess a distinctive odor and are detectable at low levels (Silva et al., 2023). Among the ketones, 2-butanone, 2-heptanone, and 2-nonanone were present in higher amounts (6.0%, 5.6%, and 4.6%, respectively, in the OBCh sample; and 0.3%, 3.2%, and 2.1% in the CICh sample). Similar findings have been observed in other PDO cheeses made from raw milk (Delgado et al., 2011), suggesting that these ketones play a crucial role in the final aroma of these cheeses. In particular, 2-butanone imparts a buttery odor, while 2-heptanone exhibits an herbaceous odor (Curioni & Bosset, 2002). Various methyl ketones, like nonanone, contribute fruity and floral notes, enhancing cheese flavor (Delgado et al., 2011). Despite the prevalence of carboxylic acids in all cheese samples, esters were poorly detected, likely due to the fresh nature of the investigated cheeses (Fernández-García et al., 2004; Todaro et al., 2018). In OBCh, additional odor-active compounds such as alcohols (1-butanol-3-methyl) and aldehydes (hexenal and heptanal) were also identified. Overall, the volatile composition in OBCh aligns with the profile observed in cheeses produced from sheep's milk in various studies (Busetta et al., 2022; Gaglio et al., 2021; Kırmacı et al., 2015).

3.7. Sensory traits of cheeses

The spider plot depicted in Fig. 5 illustrates the outcomes of the descriptive sensory evaluation conducted on OBCh and CICh. This evaluation is essential for assessing consumer satisfaction with new food products before their market launch (Świąder & Marczewska, 2021). While it is widely recognized that the sensory characteristics of dairy products are primarily influenced by factors such as the type of milk used, animal diet (Carpino et al., 2004), and raw milk characteristics (Martin et al., 2005), the comparison between OBCh and CICh did not reveal statistically significant differences ($p \geq 0.05$) for most of the evaluated attributes. However, some distinctions were observed: color, intensity of odor, spiciness, and taste persistency were higher for OBCh. These results are not surprising, since ovine milk imparts greater sensory complexity to the final products compared to cows' milk (Ryffel et al., 2008). However, the scores registered in this study are similar to those reported by Blaiotta et al. (2017) for bovine Italico cheese. Interestingly, unpleasant odors, a critical factor affecting consumers' acceptance of new products (Herz, 2006), were not detected in either of the evaluated cheeses. Overall, both OBCh and CICh received similar overall satisfaction scores, affirming that the transformation of sheep's milk using the Italico cheese technology does not adversely impact sensory characteristics.

4. Conclusion

In this comprehensive investigation, a novel Sicilian semisoft cheese made from sheep's milk underwent several analyses. The microbiological assessment confirmed the safety of the final cheeses and validated the use of a commercially available *S. thermophilus* formulation as a starter culture for OBCh production. Elevated levels of short-chain fatty acids were detected in OBCh, potentially enhancing product digestibility. OBCh exhibited higher values of the cis-9, trans-11 isomer of linoleic acid, known for its numerous health benefits. Despite varying proportions, both cheeses displayed comparable classes of VOCs, which did not significantly alter their aromatic profiles. Remarkably, the sensory analysis revealed that OBCh was on par with commercially available Italico cheese in terms of overall appreciation. This work has not only led to the creation of an unconventional dairy product in the Sicilian region but also holds promise for making sheep farming economically viable while preserving native breeds and mitigating land abandonment.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

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Table 1. Microbial counts of freeze-dried starter culture, milk, and curd samples

Microbial counts	Samples				SEM	*p* value
	DSC	PM	IM	C		
TMM	10.14 a	3.34 d	7.09 c	7.97 b	0.74	≤ 0.0001
S. thermophilus	10.36 a	3.05 d	6.91 c	7.83 b	0.79	≤ 0.0001
Enterococci	n.a.	2.07	n.a.	n.a.	n.e.	n.e.
CPS	<2	<1	<1	<2	n.e.	n.e.
E. coli	<2	<1	<1	<2	n.e.	n.e.
L. monocytogenes	<2	<1	<1	<2	n.e.	n.e.
Salmonella spp.	<2	<1	<1	<2	n.e.	n.e.

Units are CFU/g for freeze-dried starter culture and curd samples; CFU/mL for milk samples. Results indicate mean values of six plate counts (carried out in duplicate for three independent productions). Abbreviations: DSC, dried starter culture; PM, pasteurized milk; IM, inoculated milk; C, curd; SEM, standard error of the mean; TMM, total mesophilic microorganisms; CPS, coagulase-positive staphylococci; *E.*, *Escherichia*; *L.*, *Listeria*; n.a. not analysed; n.e., not evaluated. On the row: a, b, c, d = $p \leq 0.05$.

Table 2. Physicochemical analysis of cheeses

Parameters	Samples		SEM	*p* value
	CICh	OBCh		
Color				
Lightness L*	87.76	87.59	0.07	0.637
Redness a*	-3.61	-4.53	0.14	0.106
Yellowness b*	16.71	15.28	0.30	0.317
Hardness (N)	0.41	0.33	0.01	0.059
pH	5.12 b	5.21 a	0.01	0.012
Dry matter (%)	57.37 a	51.23 b	0.80	0.003
Fat in DM (%)	59.70	56.12	0.53	0.065
Protein (%)	22.00	21.61	0.08	0.319
Ash (%)	3.53 a	2.97 b	0.07	0.003

Results indicate mean values of six determinations (carried out in duplicate for three independent productions). Abbreviations: CICh, commercial cow's Italico cheese; OBCh, Ovino Belmontese cheese; SEM, standard error of the mean. On the row: a, b = $p \leq 0.05$.

Table 3. Free fatty acid profile of cheeses

Fatty acids	Samples		SEM	*p* value
	CICh	OBCh		
Caproic acid (C6:0)	2.43 b	3.37 a	0.14	0.032
Caprylic acid (C8:0)	1.57 b	3.51 a	0.26	0.011
Capric acid (C10:0)	3.78 b	10.47 a	0.86	≤ 0.0001
Lauric acid (C12:0)	4.42 b	5.89 ± a	0.20	0.025
Myristic acid (C14:0)	12.91 ± 0.33	13.16 ± 0.36	0.08	0.542
Pentadecanoic acid (C15:0)	1.38	1.27	0.04	0.591
Palmitic acid (C16:0)	35.89 a	26.22 b	0.85	0.000
Palmitoleic acid (C16:1)	1.97 a	1.38 b	0.08	0.043
Stearic acid (C18:0)	9.75	8.84	0.13	0.058
Oleic acid (cis) (C18:1)	21.59 a	15.43 b	0.80	≤ 0.0001
Oleic acid (trans) (C18:1)	1.45 b	5.41 a	0.51	0.001
Linoleic acid (C18:2)	2.87 a	3.02 a	0.03	0.272
Linolenic acid (C18:3 n3)	0.41 b	2.02 a	0.21	0.003

Results indicate mean values of six determinations (carried out in duplicate for three independent productions). Abbreviations: CICh, commercial cow's Italico cheese; OBCh, Ovino Belmontese cheese; SEM, standard error of the mean. On the row: a, b = $p \leq 0.05$.

Legend to figures

Fig. 1. Flowsheet set up to produce "Ovino Belmontese" cheese.

Fig. 2. Microbiological loads of cheeses. Units are Log CFU/g. Results indicate mean values ± S.D. of six plate counts (carried out in duplicate for three independent productions). Abbreviations: CICh, commercial cow's Italico cheese; OBCh, Ovino Belmontese cheese; TMM, total mesophilic microorganisms; *S.*, *Streptococcus*.

Fig. 3. Dendrogram obtained from RAPD-PCR patterns of lactic acid bacteria strains isolated during cheese productions. Abbreviations: CSC, commercial starter culture; PM, pasteurized milk; IM, inoculated milk; C, Curd; OBCh, Ovino Belmontese cheese; *En.*, *Enterococcus*; *S.*, *Streptococcus*. The dendrogram shows only 12 of the 107 isolates analysed. The remaining 95 strains were excluded from Figure because they exhibited identical RAPD profiles as other cultures from the same sample.

Fig. 4. Distribution of volatile organic compounds among cheeses. The heat map plot depicts the relative concentration of each VOCs. Abbreviations: CICh, commercial cow's Italico cheese; OBCh, Ovino Belmontese cheese.

Fig. 5. Spider chart of descriptive sensory evaluation of cheeses. Abbreviations: CICh, commercial cow's Italico cheese; OBCh, Ovino Belmontese cheese; n.s., not significant.

Fig. 1.

pasteurized ewes' milk (75 °C for 15 s)

↓

cooling at 42 °C

↓

inoculation with milk starter culture

↓

addition of liquid rennet

↓

milk curdling (40 min)

↓

cross cut (20 min)

↓

curd breaking at nut size (20 min)

↓

curd extraction and moulding

↓

curd stewing at 45 °C (60 min)

↓

drying (24 h)

↓

salted in 18 °Bé brine (20 min)

↓

ripening (10 d) at 6 °C, > 90% relative humidity

↓

Ovino Belmontese cheese

Fig. 2.

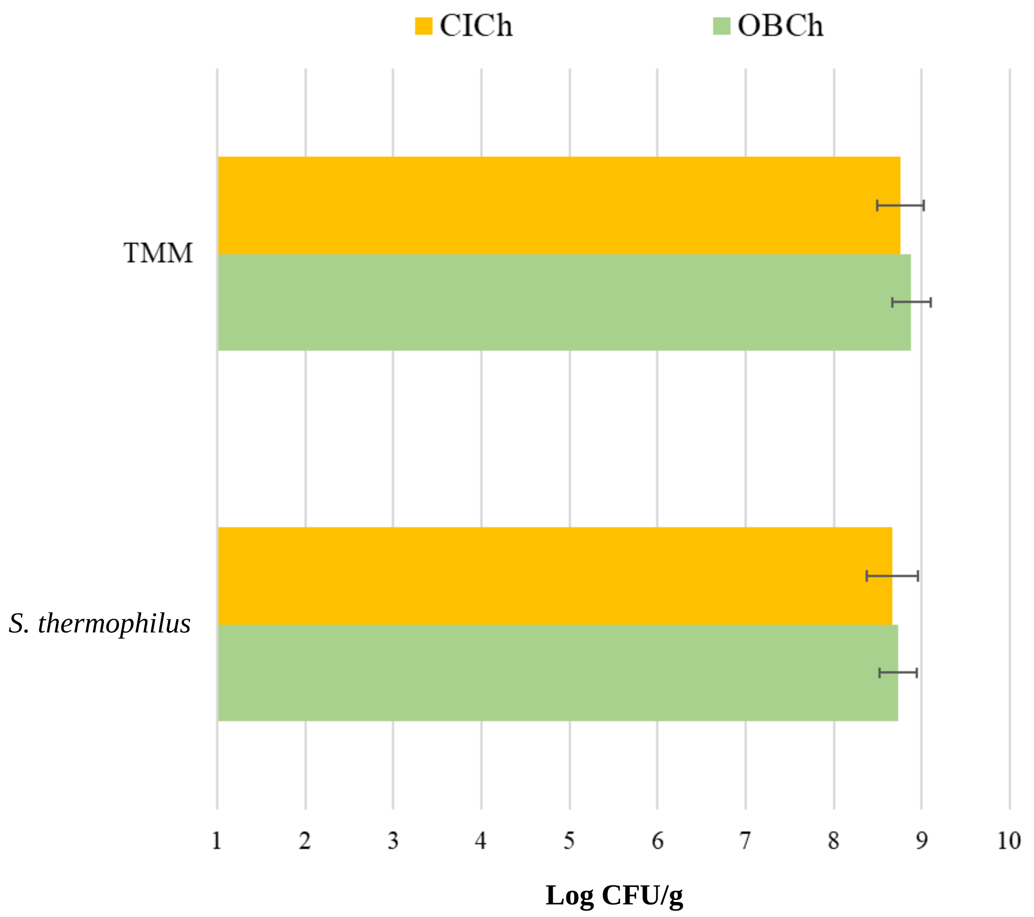
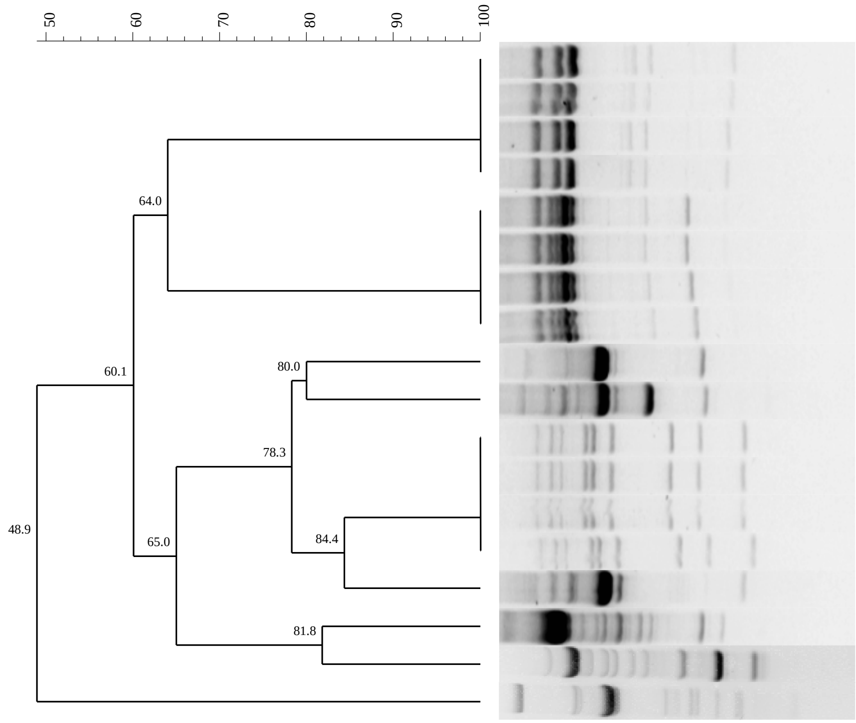
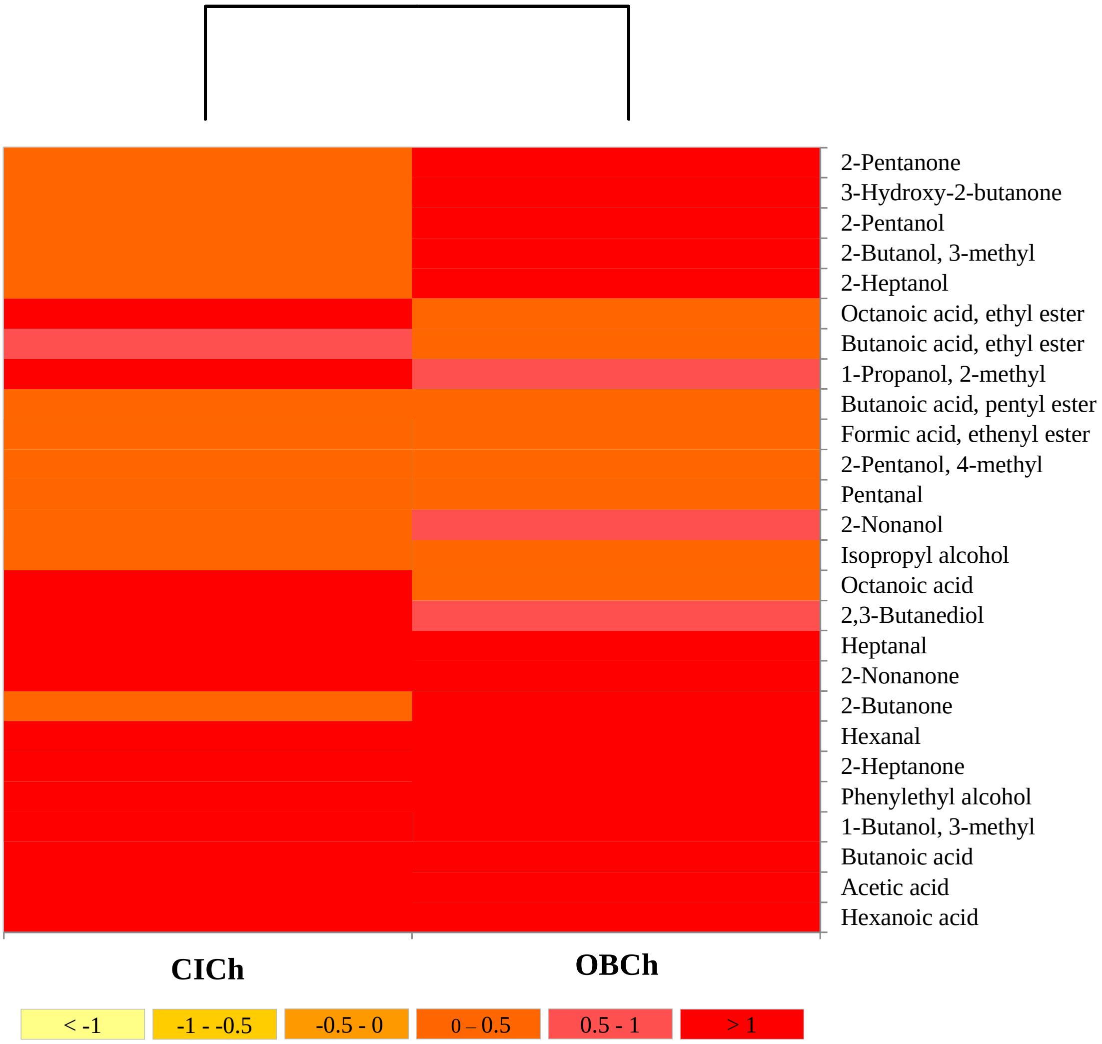


Fig. 3.

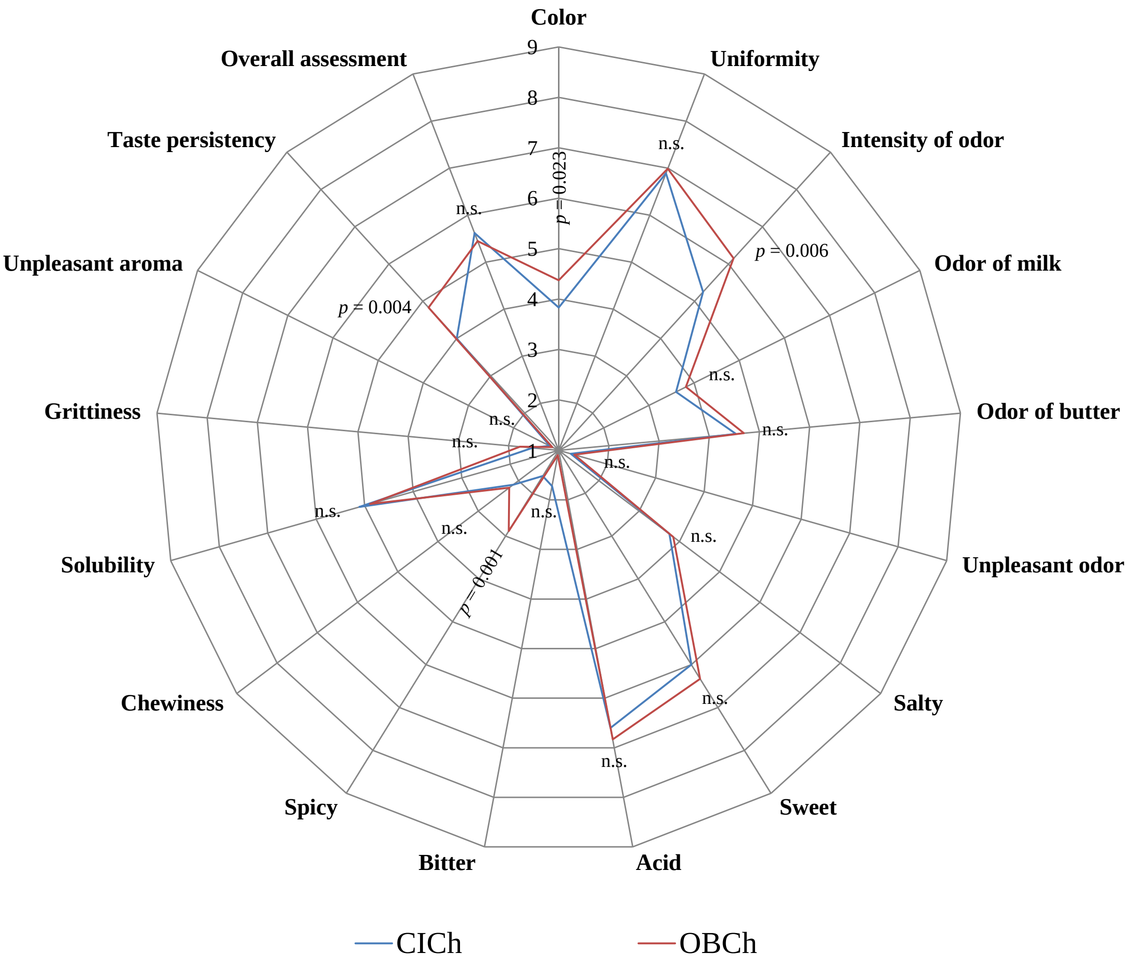


Strain	Sample	Species
OB2	CSC	*S. thermophilus*
OB52	IM	*S. thermophilus*
OB87	C	*S. thermophilus*
OB44	OBCh	*S. thermophilus*
OB12	CSC	*S. thermophilus*
OB70	OBCh	*S. thermophilus*
OB23	IM	*S. thermophilus*
OB62	C	*S. thermophilus*
POM5	PM	*S. thermophilus*
POM17	PM	*S. thermophilus*
OB7	CSC	*S. thermophilus*
OB33	C	*S. thermophilus*
OB104	OBCh	*S. thermophilus*
OB46	IM	*S. thermophilus*
POM23	PM	*S. thermophilus*
POM12	PM	*S. thermophilus*
POM43	PM	*En. faecium*
POM28	PM	*En. faecium*

Fig. 4.


2-Pentanone
3-Hydroxy-2-butanone
2-Pentanol
2-Butanol, 3-methyl
2-Heptanol
Octanoic acid, ethyl ester
Butanoic acid, ethyl ester
1-Propanol, 2-methyl
Butanoic acid, pentyl ester
Formic acid, ethenyl ester
2-Pentanol, 4-methyl
Pentanal
2-Nonanol
Isopropyl alcohol
Octanoic acid
2,3-Butanediol
Heptanal
2-Nonanone
2-Butanone
Hexanal
2-Heptanone
Phenylethyl alcohol
1-Butanol, 3-methyl
Butanoic acid
Acetic acid
Hexanoic acid
CICh
OBCh
< -1
-1 - -0.5
-0.5 - 0
0 – 0.5
0.5 - 1
> 1

653 **Fig. 4.**



Color
Uniformity
Intensity of odor
Odor of milk
Odor of butter
Unpleasant odor
Salty
Sweet
Acid
Bitter
Spicy
Chewiness
Solubility
Grittiness
Unpleasant aroma
Taste persistency
Overall assessment
9
8
7
6
5
4
3
2
1
p = 0.023
n.s.
p = 0.006
n.s.
n.s.
n.s.
n.s.
n.s.
n.s.
n.s.
p = 0.001
n.s.
n.s.
n.s.
n.s.
p = 0.004
n.s.
CICh
OBCh

654