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Prickly pear byproduct (*Opuntia* spp.) silage inclusion in dairy sheep diet: Effects on chemical, nutritional, and microbiological characteristics of milk and cheese

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ABSTRACT

Bioactive compounds often remain in agro-industrial byproducts. Feeding dairy sheep with prickly pear byproduct silage may enhance the chemical composition and bioactive content of milk and cheese. This study investigated these effects on the chemical and nutritional composition and bioactive compounds content of both milk and cheese. A total of 12 Valle del Belice breed ewes, ~90 DIM, balanced for parity, BW, and daily milk yield (1.038 ± 0.144 kg/d; ±SD), were selected and assigned to 3 experimental diets following a Latin square design with each period lasting 14 d. The diets with the same CP and NDF were (1) control (CTR) diet with hay and concentrate; (2) prickly pear peels (PPP) diet with PPP silage, hay, and concentrate; and (3) pulp, peels, and seeds (PPS) diet with PPS silage, hay and concentrate. Both bulk milk and cheese samples were analyzed for their chemical and nutritional composition and microbiological characteristics, including microbiota profiling via 16S rRNA analysis. Results showed no significant changes in the chemical composition of milk and cheese. However, supplementation with prickly pear byproducts increased their total phenolic content and antioxidant capacity. Cheeses produced from the experimental diets (PPP and PPS) exhibited higher concentrations of eucomic and piscidic acids. Cheese fatty acid composition and microbiological characteristics were largely unaffected by the dietary treatments. Sensory evaluation revealed significant differences among samples, but these did not affect overall consumer acceptance. The byproducts of prickly pear fruits can therefore be advantageously used in the feeding of dairy sheep, improving the nutritional characteristics of cheeses.

Key words: prickly pear byproducts, silage, ewe diet, polyphenol compounds, cheese microbiota

INTRODUCTION

In the Mediterranean region, the inclusion of agro-industrial byproducts in the diets of small ruminants, particularly dairy sheep, represents a well-established and traditional practice. In Sicily, the abundant availability of locally sourced byproducts, such as citrus (*Citrus lemon* and *Citrus sinensis*) fruits wastes, olive cake, grape marc, and tomato peels, offers a promising strategy to reduce competition with human food chains. Moreover, the incorporation of these byproducts into animal feeding systems provides an opportunity to mitigate the environmental footprint of livestock production, lower feed costs, and enhance both the quality and sustainability of animal-derived products (Foti et al., 2003; Natalello et al., 2020; Vastolo et al., 2022).

Many of these byproducts are characterized by a high content of bioactive compounds, particularly polyphenols, which contribute to their nutritional and functional value. Recently, prickly pear peels have gained attention for the recovery of valuable components, including phenolic antioxidants, fatty acids, and vitamins (Amaya-Cruz et al., 2019; Özcan et al., 2023). The high phenolic content of prickly pear peel residue makes it an ideal source of natural antioxidants, promoting the exploitation of its extracts in the food, pharmaceutical, and cosmetic sectors (Ioannou et al., 2025). Moreover, the inclusion of polyphenols in the diets of small dairy ruminants has been associated with multiple beneficial effects, including improved ruminal metabolism, enhanced animal health status, and superior quality of dairy products (Correddu et al., 2020).

The growing scientific interest in bioactive compounds, such as polyphenols, within ruminant nutrition (Min et al., 2003; Vasta et al., 2019) has consequently

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stimulated the use of polyphenol-rich byproducts as feed ingredients for ruminants (Castrica et al., 2019), although their impact on milk yield and gross composition remains relatively limited (Correddu et al., 2020).

Among the agro-industrial byproducts employed in ruminant nutrition within Sicily, those derived from the prickly pear fruit (*Opuntia ficus-indica* L. Mill.) represent a noteworthy and underexploited feed resource (Todaro et al., 2020). These byproducts originate from the Opuntia cactus fruits, a resilient and highly versatile species extensively cultivated across arid and semi-arid regions worldwide for its nutritionally valuable fruit and a wide array of commercial applications. Italy is the world's third-largest producer of Opuntia fruit, after Mexico and the United States, with production overwhelmingly concentrated in Sicily, which accounts for 97.54% of the national output. As a result, substantial quantities of prickly pear byproducts are generated, offering significant potential for their incorporation into ruminant feeding systems (Todaro et al., 2020).

In the juice extraction industry, the whole fruit is typically processed, including the peel, resulting in a residual byproduct composed of pulp, peels, and seeds. To enhance juice quality, the implementation of automated peeling technologies is increasingly adopted, allowing for the efficient separation of the rind, seeds, subsequently exploited for oil extraction, and the liquid or pulp fraction (Gannuscio, 2025). This industrial process yields a substantial quantity of prickly pear byproducts which, when appropriately preserved through ensiling, may constitute a valuable dietary resource for small ruminants (Vastolo et al., 2020; Nayel et al., 2023; Gannuscio et al., 2024a,b), particularly during periods of limited fresh forage availability.

When prickly pear byproduct (**PPB**) silages were included in the diet of lactating ewes at a level of 1.5 kg/head per day, a reduction in voluntary feed intake was observed, which consequently led to a decrease in daily milk yield. However, milk fat and protein concentrations, as well as milk yield standardized for fat and energy content, were not significantly affected. Furthermore, ewes receiving the PPB-based diet produced milk characterized by a higher casein content and a lower urea concentration, suggesting an improvement in nitrogen utilization efficiency (Hassan et al., 2025).

The objective of the present study, which builds upon the work of Hassan et al. (2025), was to evaluate the effects of including 2 types of prickly pear byproduct silages—peels and pulp, peels and seeds—in the diets of lactating ewes on the nutritional composition of bulk milk and cheese, with particular emphasis on total phenolic content (**TPC**) and its metabolites, antioxidant capacity (**AOC**), and fatty acid (**FA**) profile.

MATERIALS AND METHODS

Experimental Design, Animals, and Diets

The experimental trial was carried out on a commercial livestock farm located in Menfi, Agrigento Province, Sicily (Italy). The farm managed a flock of ~500 Valle del Belice ewes reared under a semi-extensive production system. All experimental procedures were conducted in accordance with the ethical standards for animal experimentation approved by the Animal Welfare Committee of the University of Palermo (protocol number: UNPA-CLE 201954 – 12/12/2023).

Prickly pear peels (**PPP**) and prickly pear pulp, peels and seeds (**PPS**), both mixed with 12% of wheat bran by raw weight, were ensiled separately for 50 d (Gannuscio et al., 2024a) and incorporated in diets for lactating ewes (Hassan et al., 2025). In summary, 12 Valle del Belice ewes, ~90 DIM, were assigned to 3 experimental groups. The groups were balanced for parity (ranging from the third to the sixth lambing), BW (53.66 ± 6.57 kg), and daily milk yield (1.038 ± 0.144 kg/d). Fourteen days before the commencement of the trial, all ewes received antiparasitic treatment against both internal and external parasites, consisting of a 2-mL administration of ivermectin (Ivomec, Merial, France).

The ewes were housed individually in straw-bedded pens equipped with feeders and drinkers. They first underwent a 2-wk adaptation period to the new housing and diet, during which they were fed ad libitum sulla hay and 800 g/head per day of commercial concentrate for dairy ewes to meet nutritional requirements (INRA, 2018).

After an adaptation period in which all 3 groups received the same diet consisting of hay and concentrate, each ewe group was randomly assigned to one of 3 experimental diets using a Latin square design (3 × 3) with 3 phases, each of which lasted for 14 d, 9 d for adaptation to diets and 5 d for sampling. Table 1 reports the chemical composition, TPC, and antioxidant capacity of the experimental feeds, which were analyzed as detailed in Gannuscio et al. (2024a).

For each ewe, offered feeds and orts were weighed daily during each experimental phase to determine feed intake, and the results are given below (g of DM/d/head):

- Control (**CTR**) group: 1,499, 0, and 795 for hay, silage and concentrate respectively;
- Prickly pear peels (PPP) group: 1,269, 307 and 442 for hay, silage and concentrate respectively;
- Prickly pear pulp, peels, and seeds (PPS) group: 1,403, 410, and 442 for hay, silage and concentrate respectively.

Table 1. Chemical composition of the experimental feeds, adapted from Gannuscio et al. (2024a) and Hassan et al. (2025)[1]

Item	Unit	Concentrate	Hay	PPP silage	PPS silage
DM	g/kg FM	882.9	900.9	200.3	413.7
CP	g/kg DM	247.4	71.5	120.2	95.5
EE	g/kg DM	40.2	14.4	61.5	37.8
NDF	g/kg DM	265.8	796.8	253.1	666.6
ADF	g/kg DM	97.0	523.0	113.8	500.3
ADL	g/kg DM	15.9	83.6	21.8	267.7
NFC	g/kg DM	369.8	49.2	450.1	119.2
Ash	g/kg DM	76.8	68.2	115.2	80.8
TPC	mg GAE/ g DM	13.52	2.72	30.24	24.22
DPPH	mmol TEAC/100 g DM	4.99	2.70	15.75	10.06
ABTS	mmol TEAC/100 g DM	12.17	6.49	26.46	21.58

[1]PPP silage = prickly pears peels + 12% wheat bran; PPS silage = prickly pears pulp, peels and seeds + 12% wheat bran. FM = fresh matter; EE = ether extract; NFC = 100 − (CP + ether extract + ash + NDF); TPC = total phenolic content; DPPH = 2,2-diphenyl-1-picrylhydrazyl; ABTS = 2,2′-azino-bis 3-ethylbenzothiazolino-6-sulphonic acid; GAE = gallic acid equivalents; TEAC = trolox equivalent antioxidant capacity.

Experimental Cheesemaking

During the study, individual milk production at the morning and at the evening milking was recorded. At the conclusion of each experimental phase, bulk milk collected over 48 h from each group during each experimental phase was processed under controlled conditions in the farm's dairy plant, following the traditional protocol for the manufacture of fresh Pecorino Siciliano cheese (Todaro et al., 2024).

Cheesemaking was carried out in an artisanal dairy facility located in Santa Margherita di Belice (Agrigento, Italy). Briefly, the bulk milk from each group was heated to 38°C and gently stirred manually for ~5 min before the addition of lamb rennet paste (36 g/100 L of milk; Rennet Regional Consortium, Poggioreale, Italy). Coagulation occurred within 40 to 50 min. Subsequently, hot water at 74°C was added to the intact curd to promote syneresis, and the curd was then cut with a ladle until small, rice-sized grains (3–7 mm in diameter) were obtained. Following whey drainage, the curd was hand-pressed into 1-kg molds, producing a total of 9 cheeses, 3 for each experimental diet (CTR, PPP, and PPS) within each of the 3 phases (1, 2, and 3). The cheeses were subsequently cooked in hot whey at 72°C for 3 h. Twenty-four hours after cooking, all cheeses were salted in saturated brine for 24 h and then ripened for 48 h in a storage chamber maintained at 16°C and 85% relative humidity.

Milk and Cheese Physical-Chemical Analysis

The bulk milk samples were analyzed for lactose, fat, protein, casein, urea, and SCC by infrared method (Combi-foss 6000, Foss Electric, Hillerød, Denmark), pH by HI 9025 pH-meter (Hanna Instruments, Ann Arbor, MI), and titratable acidity by Soxhlet–Henkel method expressed in °SH/50 mL. Total nitrogen (**TN**), non-casein nitrogen (**NCN**), and NPN were determined by standard International Dairy Federation procedures (IDF, 1964c, 1993) to calculate total protein (TN × 6.38), casein [TN − (NCN × 0.994) × 6.38] and whey protein [(NCN − NPN) × 6.38]. Bulk milk samples were further assessed for coagulation properties using a Formagraph apparatus (Foss Electric, Hillerød, Denmark). The parameters recorded included coagulation time (r, min), curd-firming time (k_{20}, min), curd firmness at 30 min (a_{30}, mm), and curd firmness at twice the coagulation time (a_{2r}, mm). Analyses were performed on 10 mL of milk at 35°C following the addition of 0.2 mL of a diluted rennet solution (1.6:100, vol/vol; rennet strength 1:15,000; Chr. Hansen, Parma, Italy).

Cheese samples were collected 96 h after cheesemaking for chemical, colorimetric, and rheological analyses. Two subsamples were obtained from each cheese by cutting into the inner portion using a sharp blade. The samples were promptly analyzed for surface color and texture profile measurements. Surface color was measured in duplicate using a Minolta Chroma Meter CR300 (Minolta, Osaka, Japan) using the illuminant C; results are expressed as lightness (L*, from 0 = black, to 100 = white), redness (a*, from red = +a, to green = −a), and yellowness (b*, from yellow = +b, to blue = −b), according to the CIE L* a* b* system (Hunt and Pointer, 2011). Cheese hardness was evaluated with an Instron 5564 tester (Instron, Trezzano sul Naviglio, Milan, Italy) measuring the maximum resistance to compression (compressive stress, N/mm^2) of samples (2 cm × 2 cm × 2 cm) kept at room temperature (22°C). Water activity was determined at 23°C at the surface of each sample slice by using an activity-meter instrument (Rotronic Int.).

Cheese samples, after freeze-dried, were analyzed for DM, fat, CP (N × 6.38), and ash content according to the International Dairy Federation standards (4A [IDF, 1982], 5B [IDF, 1986], 25 [IDF, 1964a], and 27 [IDF, 1964b], respectively). Cheese samples soluble N was

determined on an aqueous filtrate using the Kjeldahl method (MAF, 1986).

Antioxidant Capacity and Total Phenolic Content of Milk and Cheese

A methanolic extraction was prepared according to the method of Rashidinejad et al. (2013) with slight modifications. Five hundred milligrams of each milk and cheese sample were mixed with 5 mL of distilled water containing 1% HCl, and 5 mL of hexane. The mixtures were centrifugated at 2,700 × *g* at 4°C. After centrifugation, the upper fat layer was removed, taking care not to remove the aqueous layer. Then, 10 mL of methanol (100%) was added, and the samples were sonicated in an ultrasonic bath for 40 min at 50°C. The supernatants were filtered through Whatman no. 541 filter paper (Whatman International Ltd., Maidstone, UK). The filtered solutions were stored at −20°C until analysis.

The measurement of antioxidant capacity was carried out according to previously established procedures (Sciacca et al., 2023; Viola et al., 2023). Two free radicals that are 2,2-diphenyl-1-picrylhydrazyl (**DPPH**) and 2,2′-azino-bis(3-ethylbenzothiazolino-6-sulfonic acid) (**ABTS**) were employed to assess antioxidant capacity by observing the reaction of the sample with DPPH and ABTS, which results in a discoloration of the solution proportional to the antioxidant content in the sample. The extracts (100 µL) were combined with 3 mL of DPPH (60 µ*M*) and incubated in the dark at 25°C for 30 min. Scavenging activity was measured at 515 nm using a UV-visible spectrophotometer (VWR UV-1600PC; VWR, Radnor, PA), with methanol serving as the blank. For the ABTS assay, absorbance was measured 5 min after adding 3 mL of diluted $ABTS^{*+}$ to 100 µL of the extracted sample. The decrease in absorbance at 734 nm, compared with methanol, indicated the $ABTS^{*+}$ free radical scavenging ability. A calibration curve was constructed using Trolox across a concentration range of 1 to 75 µ*M*. The results were reported as Trolox equivalent antioxidant activity (**TEAC**) and expressed as millimoles of Trolox equivalent per 100 g of dried sample.

The TPC was measured using the Folin-Ciocâlteu method (Di Stefano et al., 2022; Roppolo et al., 2024). An aliquot of the extracts (125 µL) was mixed with 120 µL of a 7% Na_2CO_3 solution and 625 µL of Folin-Ciocâlteu reagent (diluted 1:5) and incubated in the dark at 25°C for 60 min. Absorbance was assessed at 765 nm using a UV-visible spectrophotometer (VWR UV-1600PC), with methanol as the blank. Gallic acid (**GA**) was used to create a standard curve for calibration (0.01 to 0.5 mg/mL). Total phenolic content was reported as milligrams of gallic acid equivalents per gram of the freeze-dried sample.

Cheese Fatty Acid Composition

Fatty acids in lyophilized cheese samples (100 mg) were directly methylated in 1 mL of hexane with 2 mL of 0.5 *M* $NaOCH_3$ at 50°C for 15 min, followed by treatment with 1 mL of 5% HCl in methanol at 50°C for another 15 min, according to the bimethylation procedure described by Lee and Tweed (2008). Fatty acid methyl esters (**FAME**) were extracted into 1.5 mL of hexane. Using an autosampler, 1 µL of each sample was injected into an HP 6890 gas chromatography system equipped with a flame-ionization detector (Agilent Technologies, Santa Clara, CA).

Fatty acid methyl esters separation was achieved on a CP-Sil 88 capillary column (100 m length, 0.25 mm i.d., 0.25 µm film thickness; Chrompack, Middelburg, the Netherlands). The injector temperature was maintained at 255°C, and the detector temperature at 250°C, with hydrogen flow at 40 mL/min, air flow at 400 mL/min, and constant helium flow at 45 mL/min. The oven temperature program started at 70°C (held for 1 min), increased by 5°C/min to 100°C (held for 2 min), then by 10°C/min to 175°C (held for 40 min), and finally by 5°C/min to 225°C (held for 45 min). Helium served as the carrier gas at a pressure of 158.6 kPa and a flow rate of 0.7 mL/min (linear velocity of 14 cm/s).

To identify individual FA, a FAME mix in hexane (Nu-Check-Prep, Elysian, MN) was used. Conjugated linoleic acid isomers were identified using a standard mixture of C18:2 *cis*-9,*trans*-11 (rumenic acid, **RA**) and C18:2 *cis*-10,*trans*-12 methyl esters (Sigma-Aldrich, Milan, Italy), along with published isomeric profiles (Kramer et al., 2004; Luna et al., 2005). Total FA quantification was performed using C23:0 (Sigma-Aldrich) as an internal standard (4 mg/g lyophilized cheese).

Based on the FA profile, 2 indices reflecting the health-related properties of cheese fat were calculated as follows:

$$\text{Thrombogenic index} = (\text{C14:0} + \text{C16:0} + \text{C18:0})/(0.5 \times \text{MUFA} + 0.5 \times \text{n-6 PUFA} + 3 \times \text{n-3 PUFA} + \text{n-3/n-6})$$

(Ulbricht and Southgate, 1991), and

$$\text{Health-promoting index} = (\text{n-3 PUFA} + \text{n-6 PUFA} + \text{MUFA})/(\text{C12:0} + 4 \times \text{C14:0} + \text{C16:0})$$

(Chen et al., 2004).

Extraction of Polyphenol Metabolites in Milk and Cheese Samples

In this work, phenolic metabolites were extracted according to the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method with some modifications (Boti et al., 2021). Briefly, 3 g of freeze-dried sample (milk or cheese) were mixed with 5 mL of water and 10 mL of methanol acidified with 1% vol/vol formic acid. The mixture was vortexed vigorously for 2 min. Subsequently, 4 g of magnesium sulfate and 1 g of sodium chloride were added, and the samples were vortexed again for 2 min. The mixtures were then centrifuged at 474 × *g* at 4°C for 10 min. The supernatants were transferred into 15 mL centrifuge tubes containing 1,200 mg of magnesium sulfate, 400 mg of primary secondary amine (**PSA**), and 400 mg of C18 sorbent. Each tube was mixed for 1 min and centrifuged again for 10 min at 474 × g at 4°C. The resulting supernatants were evaporated to dryness using a rotary evaporator at 45°C. The residues were redissolved in 1 mL of methanol, filtered through 0.45-µm syringe filters, and transferred into ultra-high-performance liquid chromatography (**UHPLC**) vials for subsequent analysis.

Profiling of Phenolic Compounds and Metabolites

The identification of polyphenols metabolites on milk and cheese samples was carried out using UHPLC-electrospray ionization (**ESI**)-MS analysis with slight modifications (Agulló et al., 2024), with a Q-Exactive LCq/Orbitrap mass spectrometer (Thermo Fisher Scientific), coupled to an Ultimate 3000 RS UHPLC system (Dionex) and equipped with a heated electrospray ionization source. Chromatographic separation was performed on a Luna C18(2) column (150 mm × 2.0 mm, 5 µm particle size) with a pre-column installed. The column temperature was maintained at 30 °C, and the flow rate was set to 400 µL/min. The mobile phase consisted of solvent A (water containing 0.1% formic acid) and solvent B (methanol with 0.1% formic acid). The gradient program was as follows: 0 to 2 min, 95% A; 2 to 18 min, linear change from 95% to 5% A; 18 to 20 min, 5% A; 20 to 40 min, linear return from 5% to 95% A. An equilibration step at the initial conditions (95% A) was maintained for 3 min before each run. A 1-µL aliquot of each sample was injected, and the total run time was 40 min. The ESI source parameters were set as follows: spray voltage ± 3,000 V, capillary temperature ± 300°C, sheath gas flow rate ± 30, auxiliary gas flow rate ± 15, and S-Lens RF level 50. The analysis was performed in full-scan mode under negative ionization conditions, with a scan range from *m*/*z* 80 to 1,000.

Microbiological Analysis

All samples collected throughout the fresh Pecorino Siciliano cheese production process, from raw ewe's milk to the final cheese product, were subjected to comprehensive microbiological analyses. For liquid samples (raw milk), 1 mL was serially diluted (1:10) in Ringer's solution (Sigma-Aldrich, Milan, Italy), followed by the preparation of subsequent decimal dilutions (up to 10^{-5}) for microbiological enumeration. For solid samples (curd and cheese), 25 g was placed into sterile stomacher bags (BagFilter P, Interscience, Saint Nom, France) and homogenized with 225 mL of 2% (wt/vol) sodium citrate solution using a BagMixer 400 stomacher (Interscience) at maximum speed for 2 min. Decimal dilutions of the resulting homogenates (up to 10^{-7}) were then prepared before plating. From each sample, appropriate dilutions were plated on selective agar media to quantify and characterize specific microbial groups: total mesophilic microorganisms (**TMM**), spread-plated on skim milk agar and incubated at 30°C for 72 h; mesophilic lactic acid bacteria (**LAB**) cocci, pour-plated on medium 17 agar supplemented with 5 g/L lactose, incubated at 30°C for 48 h; mesophilic LAB rods, pour-plated on de Man, Rogosa, Sharpe agar, acidified to pH 5.4 with 5 *M* lactic acid, incubated at 30°C for 48 h; enterococci, on kanamycin esculin azide agar, incubated aerobically at 37°C for 24 h; pseudomonads, on *Pseudomonas* agar base supplemented with cephaloridine, sodium fusidate, and cetrimide, incubated aerobically at 25°C for 48 h; yeasts, on yeast peptone dextrose agar containing chloramphenicol (0.1 mg/mL), incubated aerobically at 25 °C for 48 h; *Enterobacteriaceae*, on violet red bile glucose agar, incubated under microaerobic conditions at 37 °C for 24 h, following the methods reported by Garofalo et al. (2023). Additionally, all samples were screened for the presence of key pathogenic microorganisms: *Escherichia coli* and *Salmonella* spp. were plated on hektoen enteric agar and incubated for 24 h at 37°C; coagulase-positive staphylococci on Baird Parker agar with rabbit plasma fibrinogen, incubated for 48 h at 37°C; *Listeria monocytogenes* was investigated by plating on *Listeria* selective agar base with SR0140E supplement, following the methods reported by Gaglio et al. (2021). All microbiological counts were carried out in triplicate.

Culture-Independent Analysis of Total Bacterial Community

Total genomic DNA was extracted from 3 different samples of fresh pecorino cheese using the DNeasy PowerFood Microbial Kit (Qiagen, Hilden, Germany) ac-

cording to the kit instructions. The yield and purity of the extracted DNA were determined by the Nanodrop8800 fluorospectrometer (Thermo Fisher Scientific, Waltham, MA).

Amplicon library preparation, the determination of the quality and quantification of pooled libraries, and pair-end sequencing using the Illumina MiSeq system (Illumina, San Diego, CA) were performed at the Sequencing Platform, Fondazione Edmund Mach (FEM, San Michele a/Adige, Italy).

The PCR amplification was performed by targeting 16S rRNA gene V3-V4 variable regions (Baker et al., 2003; Claesson et al., 2010), with the bacterial primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GACTACNVGGGTWTCTAATCC-3′). Polymerase chain reaction was performed by the GeneAmp PCR System 9700 (Thermo Fisher Scientific). All PCR reactions were carried out using a Verity 96-well Thermal Cycler, according to the following protocol: 95°C for 5 min and 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 40 s, with a final elongation step of 72°C for 5 min. The PCR products were checked by gel electrophoresis and cleaned using an Agencourt AMPure XP system (Beckman Coulter, Brea, CA), following the manufacturer's instructions. After 7 PCR cycles (16S metataxonomic Sequencing Library Preparation, Illumina), Illumina adaptors were attached (Illumina Nextera XT Index Primer). Libraries were purified using Agencourt AMPure XP (Beckman Coulter, Brea, CA), and then sequenced on an Illumina MiSeq (Run Chemistry: 2 × 300 PE) platform (MiSeq Control Software 2.0.5 and Real-Time Analysis software 1.16.18, Illumina, San Diego, CA). Raw paired-end FASTQ files were demultiplexed using idemp and imported into Qiime2 (version 2018.2; https://github.com/qiime2/q2-feature-classifier). Sequences underwent quality filtering, trimming, de-noising, and merging using the DADA2 algorithm implemented within the R environment through the QIIME 2 dada2 plugin. Chimeric sequences were identified and removed using the consensus method within DADA2 (Callahan et al., 2016). Representative bacterial sequences were aligned using MAFFT and used for phylogenetic reconstruction in FastTree, utilizing the alignment and phylogeny plugins (Price et al., 2009; Katoh and Standley, 2013). Taxonomic and compositional analyses were conducted using the feature-classifier plugin. A pretrained Naive Bayes classifier, based on the Greengenes 13_8 99% operational taxonomic units (**OTU**) database. The data generated by Illumina MiSeq sequencing have been deposited in the National Center for Biotechnology Information Sequence Read Archive and are available under the Accession Number PRJNA1337445.

Sensory Analysis

The sensory evaluation of the fresh cheeses was performed with a triangle test in accordance with International Organization for Standardization (**ISO**) standard 4120 (ISO, 2004). The test was conducted in a sensory analysis room equipped with 10 individual booths, maintained under controlled temperature and humidity conditions, and free from extraneous odors and noise. The panel consisted of students and staff members from the SAAF department, all possessing modest to good experience in sensory evaluation. Ten assessors participated in a single session comprising 3 consecutive triangle tests comparing 2 cheeses. The sensory evaluation was carried out 3 times, once for each cheese.

For each test, 3 cheese samples (10 g each), 2 originating from the same batch, were presented in 50-mL disposable plastic cups with gas-tight lids. The samples were coded with random 3-digit numbers. Assessors were instructed to open each cup sequentially, inhale the headspace aroma deeply, and reclose it before proceeding to the next sample. Their task was to identify, through olfactory and gustatory evaluation, the sample perceived as different from the other 2. The tasting order was randomized according to a complete block design. No information regarding sample origin was provided to the assessors. The results of the sensory evaluation were analyzed following the procedures outlined in ISO standard 4120 (ISO, 2004), and were statistically evaluated at the 0.05 and 0.01 significance levels.

Statistical Analysis

The composition of the raw materials, silages, bulk milk, and cheese was analyzed using a one-way ANOVA, with diet included as a fixed factor, after verifying the assumptions of normality and homoscedasticity of variances. When a significant effect was detected ($P \leq 0.05$), means were compared using the Tukey–Kramer multiple comparison test. All statistical analyses were conducted using SAS software (version 9.1; SAS Institute Inc., 2010) with the GLM procedure.

RESULTS AND DISCUSSION

Milk and Cheese Composition

The physicochemical parameters of the bulk milk used for cheesemaking are presented in Table 2. The results concerning milk composition are consistent with those previously reported by Todaro et al. (2023) for Valle del Belìce sheep milk.

The dietary treatment did not exert a significant effect on any of the qualitative parameters assessed, with the

Table 2. Physical, chemical, and technological parameters of bulk milk used for cheesemaking[1]

Item	Diet			SEM	*P*-value
	CTR	PPP	PPS		
Lactose, %	4.39	4.28	4.33	0.111	0.778
Fat, %	7.21	7.80	7.63	0.524	0.704
Protein, %	5.73	6.03	5.88	0.193	0.590
Casein, %	4.46	5.04	4.58	0.356	0.509
Whey protein, %	1.60	1.56	1.59	0.262	0.993
Non-caseinic nitrogen (NCN), %	0.21	0.16	0.21	0.031	0.467
Urea, mg/dL	45.78^{A}	31.87^{B}	33.48^{B}	1.152	0.001
SCC, $\log_{10}$ n/mL	5.32	5.56	5.30	0.254	0.738
Total bacterial count, $\log_{10}$ cfu/mL	4.79	4.99	4.69	0.215	0.634
Coagulation time (r), min	20.51	22.53	21.38	1.758	0.729
Curd firming rime (k_{20}), min	1.75	2.13	1.66	0.337	0.600
Curd firmness (a_{30}), mm	43.61	45.69	46.32	3.961	0.882
Curd firmness (a_{2r}), mm	39.44	39.33	42.57	2.573	0.625
pH	5.93	6.47	6.40	0.382	0.589
Titratable acidity, °SH/50 mL	6.53	6.61	6.61	0.038	0.305

^{A,B}Values with different superscript letters within a row are significant ($P \leq 0.01$).

[1]CTR = control diet; PPP = diet with the inclusion of prickly pear peels silage; PPS = diet with inclusion of prickly pear pulp, peels and seeds silage.

exception of milk urea concentration. Specifically, milk urea concentration, which was markedly lower ($P < 0.001$) in milk from ewes receiving diets supplemented with prickly pear byproducts (31.87 and 33.48 mg/dL for PPP and PPS, respectively) compared with the CTR diet (45.78 mg/dL). This decrease could be explained by the improved ruminal NDF-to-CP ratio observed in the PPP (6.68) and PPS (6.89) diets compared with the CTR diet (6.07). It is well established that polyphenol-rich feeds can influence rumen microbiota by altering protein degradability, thereby reducing milk urea concentration (Toral et al., 2011; Correddu et al., 2020; Maniaci et al., 2024). Moreover, the higher TPC detected in PPP and PPS silages (Table 1) may explain the reduced urea concentrations observed in PPP and PPS milk. The higher TPC in PPP- and PPS-based diets, in addition to modulating the ruminal degradability of dietary proteins, may also affect the qualitative characteristics of the milk produced by sheep consuming these rations. Polyphenols are plant secondary metabolites known for their health-promoting properties, which are primarily linked to their antioxidant and anti-inflammatory activities (Balasundram et al., 2006). These compounds represent a noteworthy class of plant-derived bioactive molecules with promising applications in animal nutrition (Vasta and Luciano, 2011).

The physicochemical composition of cheeses obtained from the different dietary treatments is presented in Table 3. No significant differences were detected among the evaluated parameters; nevertheless, the characteristics observed in the present study fall within the normal range reported for comparable sheep milk cheeses (Ponte et al., 2022; Todaro et al., 2024).

Table 3. Physico-chemical parameters of fresh Pecorino cheese[1]

Item	Diet			SEM	*P*-value
	CTR	PPP	PPS		
DM, %	61.87	61.82	65.03	1.144	0.154
Fat, % DM	52.32	51.24	51.42	0.533	0.368
Protein, % DM	42.56	42.47	41.79	0.445	0.452
Ash, % DM	5.46	5.85	5.50	0.260	0.542
pH	5.17	5.33	5.26	0.057	0.240
Water activity	0.984	0.985	0.980	0.003	0.543
Hardness (N/mm^2)	0.39	0.34	0.38	0.078	0.906
L*, lightness	80.79	78.70	78.51	0.944	0.248
a*, redness	−3.02	−2.68	−2.59	0.561	0.852
b*, yellowness	11.93	11.24	10.29	1.615	0.781

[1]CTR = control diet; PPP = diet with the inclusion of prickly pear peels silage; PPS = diet with inclusion of prickly pear pulp, peels and seeds silage.

Total Phenolic Content and Antioxidant Capacity of Milk and Cheese

The TPC and AOC of milk and cheeses obtained from different bulk milk groups were positively influenced by the animals' diet (Table 4).

The inclusion of prickly pear byproducts silage in the sheep diet resulted in milk and cheese with significantly higher TPC and AOC values ($P < 0.001$), with the PPP group showing the greatest levels.

The increased TPC observed in milk and cheese from the PPP-fed ewes can be attributed to the PPP silage supplementation, which contained a higher concentration of polyphenols compared with PPS silage and the CTR diet.

Several studies have highlighted a positive correlation between the intake of total phenolic compounds and the AOC of milk (De Feo et al., 2006; Maniaci et al., 2024). Nevertheless, it is important to note that phenolic compounds derived from prickly pear byproducts can undergo degradation in the rumen and further metabolism along the intestinal tract, leading to structural modifications. As a result, lower molecular weight metabolites are formed, absorbed into the bloodstream, and subsequently incorporated into milk (Olagaray and Bradford, 2019).

With regard to AOC, as assessed by the DPPH and ABTS assays, the CTR group exhibited lower values compared with both the PPP and PPS groups in milk and cheese samples. In particular, the DPPH assay revealed the highest antioxidant capacity in the PPP group, followed by PPS, whereas the CTR group showed the lowest levels.

As a result, the antioxidant capacity patterns observed in milk and cheese samples were consistent. Comparable findings were reported by Branciari et al. (2015), who demonstrated that supplementing sheep diets with rosemary leaves enhanced total phenolic content and improved the antioxidant characteristics of Pecorino cheese.

Phenolic Profile of Feeds

The polyphenolic composition of the feed samples exhibited pronounced variability in the concentrations of individual bioactive constituents (Table 5).

The phenolic compounds present in higher amounts in prickly pear peels were eucomic and piscidic acids, in accordance with other studies (Alexandre et al., 2021; da Silveira Agostini-Costa, 2022; El-Beltagi et al., 2023). Eucomic acid was found at markedly elevated concentrations in PPP and PPP silage, where it represented the predominant phenolic compound compared with the other matrices, thus making a major contribution to the overall polyphenolic composition. Piscidic acid ranked as the second most abundant phenolic acid and likewise reached its highest levels in PPP-based samples, further emphasizing the phenolic richness of this byproduct. The simultaneous abundance of eucomic and piscidic acids in PPP silages indicates their preservation during the ensiling process and supports their potential relevance for functional applications. These phenolic substances are more effective as antioxidants that can postpone protein, DNA, and lipid prooxidative effects by producing stable radicals (Yeddes et al., 2013). Moreover, piscidic and eucomic acids have been cited among antioxidants from plants that protect against skin photoaging (Petruk et al., 2018) and piscidic acid derivatives exhibited neuroprotective effects in vitro (Lv et al., 2017).

Notably, the PPS byproduct also contained lower yet appreciable levels of these phenolic acids, which remained stable during the ensiling process. This comparatively reduced abundance suggests that PPS is less suitable for applications primarily focused on antioxidant activity. These results further highlight the high functional potential of the peel matrix relative to other fractions, owing to its more diverse and abundant polyphenolic composition. The present findings are consistent with previous studies. Albergamo et al. (2022) reported higher concentrations

Table 4. Total phenolic content (TPC) and antioxidant capacity (AOC) of milk and cheese[1]

	Diet				
Item	CTR	PPP	PPS	SEM	*P*-value
Milk					
TPC (mg GAE/g DM)	1.48^{C}	2.44^{A}	2.09^{B}	0.14	0.001
DPPH (mmol Trolox/100 g DM)	0.59^{C}	3.29^{A}	2.14^{B}	0.40	0.001
ABTS (mmol Trolox/100 g DM)	0.48^{B}	4.12^{A}	3.27^{A}	0.56	0.001
Cheese					
TPC (mg GAE/ g DM)	1.64^{B}	3.29^{A}	2.81^{A}	0.26	0.001
DPPH (mmol Trolox/100 g DM)	1.63^{C}	5.46^{A}	3.13^{B}	0.57	0.001
ABTS (mmol Trolox/100 g DM)	0.64^{C}	6.72^{A}	4.10^{B}	0.91	0.001

$^{A–C}$Values with different superscript letters within a row are significant ($P \leq 0.01$).

[1]CTR = control diet; PPP = diet with the inclusion of prickly pear peels silage; PPS = diet with inclusion of prickly pear pulp, peels and seeds silage; TPC = total phenolic content; GAE = gallic acid equivalents; DPPH = 2,2-diphenyl-1-picrylhydrazyl; ABTS = 2,2′-azino-bis 3-ethylbenzothiazolino-6-sulphonic acid.

Table 5. Phenolics composition of feed samples[1] (mg/100 g FM, mean ± SD)

Item	Concentrate	Raw material		Silage	
		PPP	PPS	PPP	PPS
DM (g/kg FM)	882.9	141.4	381.4	200.3	413.7
Phenolic acids and derivatives					
Sinapic acid	0.016 ± 0.005	0.278 ± 0.026	0.073 ± 0.008	0.219 ± 0.020	0.067 ± 0.008
Protocatechuic acid	ND	ND	0.022 ± 0.003	ND	0.042 ± 0.003
p-OH-benzoic acid	0.021 ± 0.004	0.007 ± 0.002	0.037 ± 0.008	0.111 ± 0.010	0.121 ± 0.010
Caffeic acid	0.126 ± 0.010	0.016 ± 0.002	0.011 ± 0.002	0.023 ± 0.003	ND
Coumaric acid	0.016 ± 0.002	0.002 ± 0.001	0.010 ± 0.002	0.022 ± 0.002	0.006 ± 0.001
Eucomic acid	0.043 ± 0.007	19.445 ± 0.350	3.190 ± 0.212	17.769 ± 0.275	1.441 ± 0.128
Piscidic acid	0.028 ± 0.008	14.779 ± 0.452	3.027 ± 0.205	16.694 ± 0.432	2.256 ± 0.143
Piscidic acid isomer	0.014 ± 0.004	0.239 ± 0.034	1.239 ± 0.102	0.292 ± 0.037	1.435 ± 0.126
Chlorogenic acid	7.288 ± 0.307	ND	ND	ND	ND
Flavonols					
Quercetin	ND	0.062 ± 0.003	0.186 ± 0.015	0.400 ± 0.020	0.355 ± 0.015
Quercetin-3-O-glucoside	ND	0.008 ± 0.002	0.006 ± 0.001	ND	ND
Rutin	0.005 ± 0.001	0.312 ± 0.020	0.188 ± 0.013	0.097 ± 0.008	0.011 ± 0.002
Kaempferol	ND	0.016 ± 0.002	0.053 ± 0.004	0.110 ± 0.010	0.121 ± 0.010
isorhamnetin	0.022 ± 0.003	1.246 ± 0.023	1.046 ± 0.051	1.807 ± 0.101	2.226 ± 0.110
Flavanones					
Naringenin	ND	0.018 ± 0.002	0.016 ± 0.002	0.020 ± 0.002	0.018 ± 0.002
Other bioactive compounds					
Indicaxanthin	ND	1.937 ± 0.127	0.025 ± 0.003	ND	ND
Pinellic acid	8.402 ± 0.279	0.965 ± 0.103	1.853 ± 0.142	2.196 ± 0.130	1.424 ± 0.075
Quinic acid	0.217 ± 0.015	0.234 ± 0.030	0.192 ± 0.020	0.721 ± 0.066	0.047 ± 0.008
Succinic acid	0.187 ± 0.015	0.335 ± 0.015	0.570 ± 0.030	0.885 ± 0.035	2.967 ± 0.153

[1]PPP = prickly pears peels; PPS = prickly pears peels, pulp, and seeds; ND = not detected.

of phenolic compounds in prickly pear peel extracts compared with pulp and seed fractions, which represent the main constituents of PPS. Similarly, Mena et al. (2018) observed comparable patterns, supporting the hypothesis of a selective distribution of phenolic biosynthesis among plant tissues. Such distribution may be linked to ecological functions, particularly the plant's defensive mechanisms against environmental stressors.

Among the flavonol compounds, isorhamnetin was detected at the highest concentration. In addition, isorhamnetin (3′-O-methylquercetin) derivatives, together with quercetin and kaempferol, have been widely reported in various tissues of *Opuntia* species (da Silveira Agostini-Costa, 2022).

Among other phytochemicals, indicaxanthin represents the most relevant bioactive compound identified in prickly pear byproducts, particularly in the peel fraction. However, this pigment was exclusively detected in the raw materials of PPP and PPS, whereas it was completely absent after the ensiling process, indicating its potential degradation or biochemical transformation during fermentation. Indicaxanthin and betanin are the most representative compounds belonging to the betalain family (Belhadj Slimen et al., 2017). Many studies have highlighted the health effects of these molecules as promising antioxidant, anti-inflammatory, hepatoprotective, antitumor, antidiabetic, hypolipidemic, antimicrobial, radioprotective, and antiproliferative agents (Kaur et al., 2018).

The obtained results corroborate the hypothesis that prickly pear byproducts, especially the peel fraction, constitute promising sources of antioxidant compounds. Furthermore, the majority of these bioactives seem to persist after the ensiling process, suggesting a good preservation of their functional properties.

Phenolic Metabolites on Milk and Cheese Samples

A total of 18 phenolic compounds, corresponding to microbial and hepatic phase II metabolites of dietary polyphenols, were detected by HPLC-ESI-MS analysis in milk and cheese samples obtained from ewes receiving prickly pear byproduct silage in their diet (Table 6).

The metabolites detected in the milk and cheese of ewes receiving PPP- and PPS-based silage were substantially more numerous than those observed in the CTR group, thereby underscoring the strong influence of dietary composition. This outcome is likely attributable to the higher TPC of prickly pear byproducts, which, following microbial biotransformation in the gut and phase II hepatic metabolism, exhibit increased solubility and enhanced excretion into ewe milk.

In milk samples from the CTR group, the only phenolic compounds detected were hippuric acid, 2-methylhippuric acid, and 4-hydroxyhippuric acid. Notably, metabolites derived from flavonols, such as quercetin, rhamnetin, rutin, and kaempferol, were identified, these compounds being primarily localized in the fruit peel. Specifically,

Table 6. Phenolic metabolites identified by HPLC-ESI-MS in milk and cheese samples from ewes fed prickly pear byproduct silage[1]

			Milk									Cheese								
			CTR			PPP			PPS			CTR			PPP			PPS		
Compound	Formula	[M-H]⁻	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Vanillic acid	$C_8H_8O_4$	167,0344							x								x	X	x	x
Hippuric acid	$C_9H_9NO_3$	178,0504	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x
2-Methyl-hyppuric acid	$C_{10}H_{11}NO_3$	193,0738	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	X	x	x
4-Hydroxy-hippuric acid	$C_9H_9NO_4$	194,0453				x	x	x	x	x	x				x	x	x	X	x	x
5-(3′,5′-Dihydroxyphenyl)-gamma-valerolactone	$C_{11}H_{12}O_4$	207,0653				x	x	x	x	x	x		x	x	x	x	x	X	x	x
Piceatannol	$C_{14}H_{12}O_4$	243,0657				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
O-desmethyl angolensin	$C_{15}H_{14}O_4$	257,0813				x	x	x	x	x	x				x	x	x	x	x	x
Urolithin A sulfate	$C_{13}H_8O_7S$	306,9910				x	x	x							x	x	x	x	x	x
Equol-4-sulfate	$C_{15}H_{14}O_6S$	321,0433					x	x	x	x	x					x	x	x	x	x
Hydroxy-equol sulfate	$C_{15}H_{15}O_7S$	337,0382	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x
Apigenin-7 sulfate	$C_{15}H_{10}O_8S$	350,0096					x		x	x	x					x	x	x	x	x
Ferulic acid 4-O- glucuronide	$C_{16}H_{18}O_{10}$	369,0821							x						x		x	x		x
Urolithin B glucuronide	$C_{19}H_{16}O_9$	387,0720				x	x	x			x				x	x	x			
Quercetin 3-sulfate	$C_{15}H_{10}O_{10}S$	381,9994																		x
Dihydrodaidzein-7-O-glucuronide	$C_{21}H_{20}O_{10}$	432,1056				x		x							x	x	x			
Apigenin-7- glucuronide	$C_{21}H_{18}O_{11}$	446,0849					x									x				
Enterolactone 3′-glucuronide	$C_{24}H_{26}O_{10}$	473,1447			x	x	x		x	x					x	x		x	x	x
Quercetin 3-O-xylosyl-glucuronide	$C_{26}H_{26}O_{17}$	609,1091														x	x			

[1]CTR = control; PPP = prickly pears peels; PPS = prickly pears peels, pulp, and seeds.

PPP milk contained apigenin-7-glucuronide, urolithin B glucuronide, apigenin-7-sulfate, and urolithin A sulfate. With respect to phenolic acids, metabolites including piceatannol, 5-(3′,5′-dihydroxyphenyl)-γ-valerolactone, as well as derivatives of caffeic and p-coumaric acid, were found in PPP milk; ferulic acid 4-O-glucuronide was instead detected in PPS milk. The same metabolites identified in milk were also found in cheeses from both PPP- and PPS-fed ewes.

Urolithins are microbial catabolites derived from ellagitannins and ellagic acid, compounds abundantly present in a variety of ruminant feeds (Raya-Morquecho et al., 2025). In the present study, 2 such metabolites, urolithin-B-glucuronide and urolithin-A-sulfate, were detected. Previous research indicates that these compounds exert multiple beneficial biological activities, including anti-inflammatory, antioxidant, anticancer, neuroprotective, anti-atherogenic, antidiabetic, and anti-obesity effects (Trapali and Lagouri, 2025; Romo-Vaquero et al., 2019).

The PPP diet yielded a more diverse spectrum of polyphenol-derived metabolites compared with both the CTR and PPS diets. Therefore, the consumption of cheese derived from ewes fed polyphenol-rich byproducts may represent a promising strategy to enhance the intake of bioactive phenolic metabolites, complementing those commonly obtained from plant-based foods. Phenolic compounds are widely recognized for their antioxidant properties and their beneficial effects on human health (Li et al., 2023).

More broadly, the targeted inclusion of key antioxidant compounds in the diet may serve as a strategic approach to sustaining the health of dairy sheep and enhancing the nutritional value of the milk and dairy products obtained.

Bioactive phenolic compounds in ewe milk largely originate from diet-borne precursors metabolized by the gut microbiota (Figure 1; Landete et al., 2016). Feeding polyphenol-rich forages supports animal health, can enhance milk production and quality (Serra et al., 2021), and influences the presence and metabolic profile of polyphenols in milk, despite partial degradation in the rumen and subsequent absorption, conjugation, and hepatic metabolism before excretion or secretion into milk (Tretola et al., 2023). The extent of metabolite transfer and the specific polyphenols profile present in milk are highly variable, reflecting a complex interplay of factors (Forte et al., 2026). Beyond modulating the polyphenol metabolite profile, dietary supplementation with polyphenol-rich sources can also lead to additional improvements in milk quality, notably by increasing its overall antioxidant capacity. Consequently, milk and dairy products may serve as secondary dietary sources of phenolic metabolites for humans (Roura et al., 2008), potentially reducing inter-individual variability in intestinal cholic metabolite production and providing a more

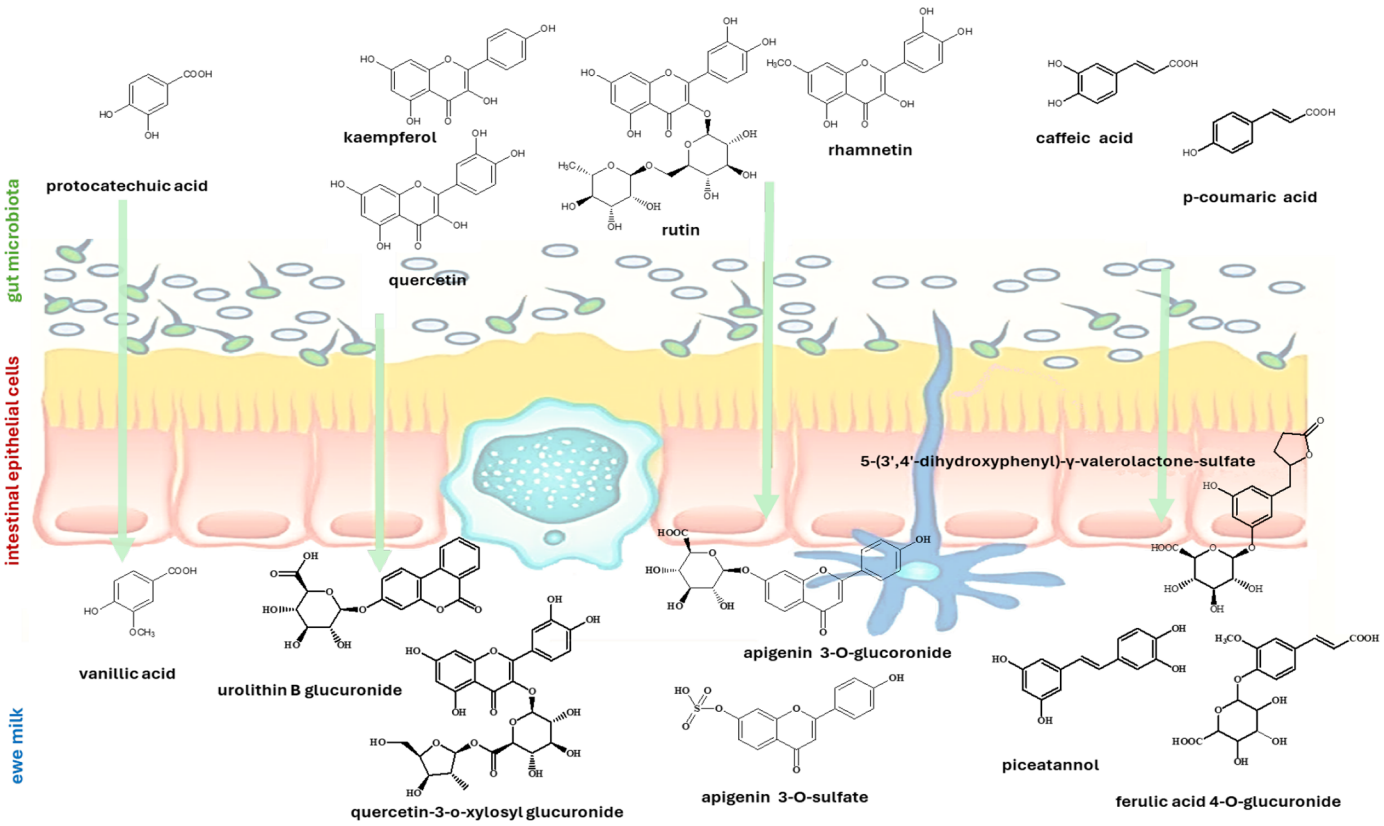

Figure 1. Origin of polyphenol metabolites found in milk and cheese.

consistent supply of compounds with health-promoting effects.

Cheese Fatty Acid Composition

The FA composition of the cheese samples, expressed as the relative percentage of identified FA, is reported in Table 7. Most FA belonged to the SFA (>63%), consistent with values previously described for fresh Pecorino cheese (Gannuscio et al., 2025). Among SFA, palmitic and myristic acids were the most abundant, whereas oleic acid predominated among UFA, in agreement with earlier findings (Ponte et al., 2022; Gannuscio et al., 2025).

The inclusion of prickly pear silages in the ewe diet induced moderate modifications in the FA profile of the resulting cheeses; nonetheless, significant differences emerged exclusively between the PPP and PPS treatments. Cheeses derived from ewes fed the PPP diet contained greater proportions of short-chain FA relative to those from the PPS group. Among FA with fewer than 15 carbon atoms (short FA), lauric acid differed significantly, displaying higher concentrations in the PPP samples. It is well established that dietary input is a major determinant of the FA composition of sheep milk (Bocquier and Caja, 2001), and consequently of cheese. In particular, the PPP diet, which is richer in digestible fiber (Hassan et al., 2025), enhances ruminal acetate production, thereby stimulating de novo FA synthesis in the mammary gland. Indeed, these FA are synthesized de novo using acetate and BHB generated during ruminal fermentation (Vlaeminck et al., 2006).

Significant differences in cheese long-chain FA were also observed between the 2 prickly pear–based diets. Cheeses produced from the PPS diet contained higher concentrations of CLA and trans-vaccenic acid compared with those derived from the PPP diet. These differences may be attributed to the higher PUFA content (61.84% vs. 56.95% for PPS and PPP, respectively) and to the effects of ruminal biohydrogenation of dietary PUFA (Wąsowska et al., 2006). During this process, linoleic and α-linolenic acids undergo incomplete hydrogenation by rumen microbiota, resulting in the accumulation of trans-vaccenic acid (**TVA**) and CLA, which are subsequently absorbed and incorporated into milk fat. Moreover, a portion of TVA is endogenously converted into CLA (*cis*-9,*trans*-11) within the mammary gland through the action of Δ9-desaturase. Dietary modulation of ruminal fermentation, particularly shifts in microbial activity

Table 7. Effect of diet on fatty acid (FA) composition (g/100 g FA) and health index of cheese fat

	Diet[1]				
Fatty acid[2]	CTR	PPP	PPS	SEM	*P*-value
Total FA, % DM	47.03	45.09	45.25	0.858	0.282
C4:0	2.64	2.90	2.42	0.202	0.315
C6:0	2.38	2.82	1.93	0.274	0.151
C8:0	2.15	2.59	1.63	0.254	0.096
C10:0	5.75	6.83	4.42	0.586	0.071
C10:1	0.34	0.41	0.27	0.039	0.118
C12:0	3.39^{AB}	3.79^{A}	2.79^{B}	0.139	0.006
C12:1	0.14	0.15	0.12	0.007	0.105
C14:0	10.79	11.05	10.10	0.230	0.063
C14:0 *iso*	0.16	0.17	0.19	0.014	0.437
C14:1	0.94	0.90	1.04	0.070	0.409
C15:0	1.04	1.06	1.20	0.058	0.201
C15:0 *iso*	0.39	0.35	0.44	0.032	0.212
C15:1	0.27	0.29	0.27	0.031	0.903
C16:0	27.34	27.33	27.29	0.509	0.997
C16:1	2.21	2.16	2.36	0.116	0.498
C17:0	0.74	0.76	0.78	0.027	0.532
C17:1	0.24	0.26	0.26	0.024	0.668
C18:0	8.89	7.77	9.47	0.620	0.225
C18:1 *trans*-11, TVA	2.38^{ab}	2.03^{b}	2.69^{a}	0.146	0.046
C18:1 *cis*-9	20.28	18.74	21.88	0.819	0.091
C18:1 others	2.11	2.09	2.45	0.095	0.062
C18:2 n 6, LA	2.26	2.25	2.20	0.153	0.960
C18:2 *cis*-9,*trans*-11 CLA	1.10^{ab}	0.96^{b}	1.38^{a}	0.091	0.041
C18:3 n-6	0.03	0.04	0.03	0.004	0.275
C18:3 n-3, ALA	0.24	0.27	0.27	0.026	0.727
C19:0	0.22	0.20	0.24	0.013	0.205
C20:0	0.49	0.50	0.54	0.029	0.470
C20:4 n-6, ARA	0.12	0.15	0.13	0.009	0.255
C20:5 n-3, EPA	0.09	0.10	0.10	0.006	0.369
C21:0	0.10	0.10	0.10	0.006	0.955
C22:0	0.21	0.22	0.23	0.011	0.533
SFA	66.65	68.43	63.75	1.330	0.115
MUFA	29.33	27.62	31.98	1.087	0.076
PUFA	4.02	3.95	4.27	0.254	0.665
n-6 PUFA	2.49	2.52	2.44	0.163	0.940
n-3 PUFA	0.34	0.38	0.37	0.030	0.631
n-6/n-3	7.42	6.75	6.60	0.301	0.202
∑ Short chain FA	30.36^{ab}	33.29^{a}	26.80^{b}	1.324	0.037
Thrombogenic index	2.77	2.83	2.54	0.129	0.319
HPI	0.45^{ab}	0.42^{b}	0.52^{a}	0.024	0.050

^{a,b}Different superscript letters within a row indicate significant differences ($P < 0.05$).

^{A,B}Different superscript letters within a row indicate significant differences ($P < 0.01$).

[1]CTR = control; PPP = prickly pear peels; PPS = prickly pear pulp, peels and seeds.

[2]TVA = *trans* vaccenic acid; LA = linoleic acid; ALA = α-linolenic acid. ARA = arachidonic acid; EPA = eicosapentaenoic acid. ∑ Short chain FA = C4:0 + C6:0 + C8:0 + C10:0 + C10:1 + C12:0 + C12:1 + C14:0 + C14:0 iso + C14:1 + C15:0 + C15:0 iso + C15:1; Thrombogenic index = (C14:0 + C16:0 + C18:0) / (0.5 × MUFA + 0.5 × n-6 PUFA + 3 × n-3 PUFA + n-3/n-6) (Ulbricht and Southgate, 1991). HPI = health-promoting index = (n-3 PUFA + n-6 PUFA + MUFA)/(C12:0 + 4 × C14:0 + C16:0) (Chen et al., 2004).

and hydrogenation pathways, therefore promotes the increased synthesis and secretion of these FA intermediates in milk (Palmquist and Jenkins, 1980).

Lastly, cheeses obtained from ewes fed the PPS diet exhibited a significantly more favorable health-promoting index compared with those produced from the PPP group. In dairy sheep, nutritional regimen constitutes the primary determinant of both the FA profile and the nutritional quality of cheese lipids, and targeted dietary strategies can markedly improve the proportion of health-beneficial FA in cheese (Correddu et al., 2020). Notably, the cheesemaking process exerts minimal influence on the FA composition, resulting in a final product whose lipid profile closely mirrors that of the original raw milk (Nudda et al., 2005).

Microbial Dynamics

The quantitative results of viable microbial groups monitored throughout the fresh Pecorino cheese pro-

Table 8. Effect of diet on microbial levels in samples collected during fresh Pecorino Siciliano cheesemaking process[1]

	Bacterial count						
Sample	TMM	Mesophilic cocci	Mesophilic rod	Enterococci	*Pseudomonas*	Yeasts	*Enterobacteriaceae*
Milk							
M-CTR	5.19	4.76	4.32	3.29	4.13	3.68	2.53
M-PPP	5.26	4.67	4.33	3.27	3.84	3.57	2.39
M-PPS	5.11	4.71	4.19	3.36	3.88	3.44	2.24
SEM	0.051	0.030	0.036	0.040	0.041	0.027	0.034
P-value	0.892	0.909	0.781	0.929	0.478	0.312	0.352
Curd							
C-CTR	5.80	5.26	4.99	4.30	3.80	3.15	2.99
C-PPP	5.68	5.42	4.63	4.44	3.99	3.10	2.77
C-PPS	5.63	5.48	5.12	4.12	3.98	3.19	2.62
SEM	0.041	0.031	0.050	0.037	0.033	0.023	0.038
P-value	0.828	0.531	0.150	0.295	0.627	0.817	0.222
Fresh Pecorino Siciliano cheese							
PS-CTR	6.67	6.62	6.15	4.22	3.51	3.01	2.82
PS-PPP	6.34	6.23	6.31	4.37	3.31	3.21	2.66
PS-PPS	6.38	6.18	6.11	4.07	3.60	3.11	2.74
SEM	0.042	0.049	0.036	0.034	0.054	0.033	0.038
P-value	0.392	0.241	0.612	0.278	0.655	0.636	0.822

[1]Units are log cfu/mL for milk samples and log cfu/g for curd and cheese samples. Results indicate the mean values ± SD of 4 plate counts (carried out in triplicate for 3 independent productions). TMM = total mesophilic microorganisms; M-CTR = milk control production; M-PPP = milk prickly pear peels production; M-PPS = milk prickly pear pulp, peels and seeds; C-CTR = curd control production; C-PPP = curd prickly pear peels production; C-PPS = curd prickly pear pulp, peels and seeds; PS-CTR = fresh Pecorino Siciliano cheese control production; PS-PPP = fresh Pecorino Siciliano cheese prickly pear peels production; PS-PPS = fresh Pecorino Siciliano cheese prickly pear pulp, peels and seeds.

duction process are presented in Table 8. Statistical analysis revealed no significant differences among the 3 dietary treatments, CTR, PPP, and PPS, at any stage of production. This indicates that the inclusion of prickly pear silage in the ewes' diet did not exert a measurable influence on the microbial composition of the resulting cheeses. Raw ewe milk samples exhibited consistent levels of TMM and mesophilic coccus LAB, averaging ~5.0 log cfu/mL. These values are typical for raw milk from the Valle del Belìce breed, traditionally used in sicilian cheesemaking (Garofalo et al., 2024; Todaro et al., 2024). Enterococci were detected at slightly above 3 log cfu/mL in raw milk and increased by ~1 log cycle during cheese production, confirming their resilience and ability to proliferate under the environmental conditions encountered during processing (Bonanno et al., 2019; Terzić-Vidojević et al., 2021).

Pseudomonads were present in raw milk at levels of 4 log cfu/mL, but their abundance declined significantly in all cheese samples, regardless of dietary treatment. This reduction likely reflects the inhibitory effects of the cheesemaking environment, which imposes stress conditions unfavorable to the growth of these psychrotrophic bacteria (Tirloni et al., 2021). Enterobacteriaceae were detected at low levels in raw milk and remained limited throughout the production process. Following curdling, a general increase in most microbial groups was observed. Specifically, TMM and all LAB groups increased by ~1 log cycle in curd samples across all dietary treatments. This trend is consistent with expected microbial concentration following whey drainage (Cardamone et al., 2018).

The final cheeses exhibited similar microbiological profiles across all production batches. Both TMM and LAB groups reached levels of ~7.0 log cfu/g, whereas yeasts were no longer detectable. The absence of yeasts in the finished product may be attributed to the short ripening period and limited oxygen availability, both characteristic of fresh cheeses. This observation aligns with previous studies indicating that fungal growth is strongly influenced by maturation time and surface exposure (Geronikou et al., 2020). Additionally, the competitive exclusion exerted by LAB, through the production of organic acids, hydrogen peroxide, and bacteriocins, is a well-documented mechanism for suppressing spoilage fungi in dairy environments (Cabañas et al., 2025).

Importantly, no pathogenic microorganisms of concern in dairy products, such as *L. monocytogenes*, *Salmonella* spp., and coagulase-positive staphylococci (**CPS**), were detected in any of the 25-g samples analyzed. Consequently, these results are not included in Table 8. Overall, the absence of significant differences in microbial dynamics among dietary treatments indicates that the inclusion of prickly pear silage does not interfere with the fermentation process or microbial succession typical of fresh Pecorino cheese, thereby preserving technological performance and process reproducibility. Moreover, the low levels of hygiene indicator microorganisms and

the absence of major pathogens confirm that this dietary strategy does not compromise the microbiological safety of the final product. Although no direct effects on human health were assessed, the maintenance of a LAB-dominated microbiota and the absence of pathogenic microorganisms suggest that the dietary treatment does not negatively affect consumer health, while preserving the microbiological quality expected of fresh Pecorino cheese.

DNA-Based Profiling of Bacterial Communities in Cheese Samples

A comprehensive analysis of the bacterial communities present in cheese samples was performed using Illumina-based DNA sequencing technology. The results, illustrated in Figure 2, display a bar chart representing the relative abundance (>0.1%) of OTU identified through Illumina MiSeq sequencing.

Taxonomic classification revealed the presence of 4 phyla, 1 order, 6 families, 10 genera, and 1 species across all samples. Notably, the microbial profiles were dominated by undesirable gram-negative genera, including *Acinetobacter*, *Pseudomonas*, and *Stenotrophomonas*. These genera were consistently detected at relatively high levels in all samples, with *Acinetobacter* reaching 26.96% and *Pseudomonas* 13.89% in the CTR group, and *Pseudomonas* peaking at 15.45% in the PPS group. These bacteria, although nonpathogenic, are known for their ability to contaminate food products and processing environments. Their persistence is likely due to their psychrotrophic nature, minimal nutritional requirements, and high metabolic adaptability (Møretrø and Langsrud, 2017). Their presence in dairy products, particularly fresh cheeses, has been previously documented (Damaceno et al., 2015; Stellato et al., 2015). Additionally, *Chryseobacterium* and *Sphingobacterium* were consistently found across all cheese samples. *Chryseobacterium* was particularly dominant in the PPP group, accounting for 32.40% of the total microbial community, whereas *Sphingobacterium* showed relatively stable abundances in CTR (4.70%) and PPS (4.59%). These findings are in line with previous reports of their presence in raw milk and dairy products (Lauková et al., 2021; Mohamed et al., 2022).

Although present at lower concentrations, LAB such as *Lactococcus* and *Streptococcus* were more abundant in the PPS samples, with *Streptococcus* reaching 6.09% and *Lactococcus* 4.20%. These genera are essential for lactic fermentation and contribute significantly to the development of cheese aroma and flavor (Settanni and Moschetti, 2010). The discrepancy between culture-dependent and DNA-based results may be attributed to several factors: LAB strains may have been grouped within unassigned OTU, may have occurred at relative abundances below the 0.1% detection threshold, or their DNA may have

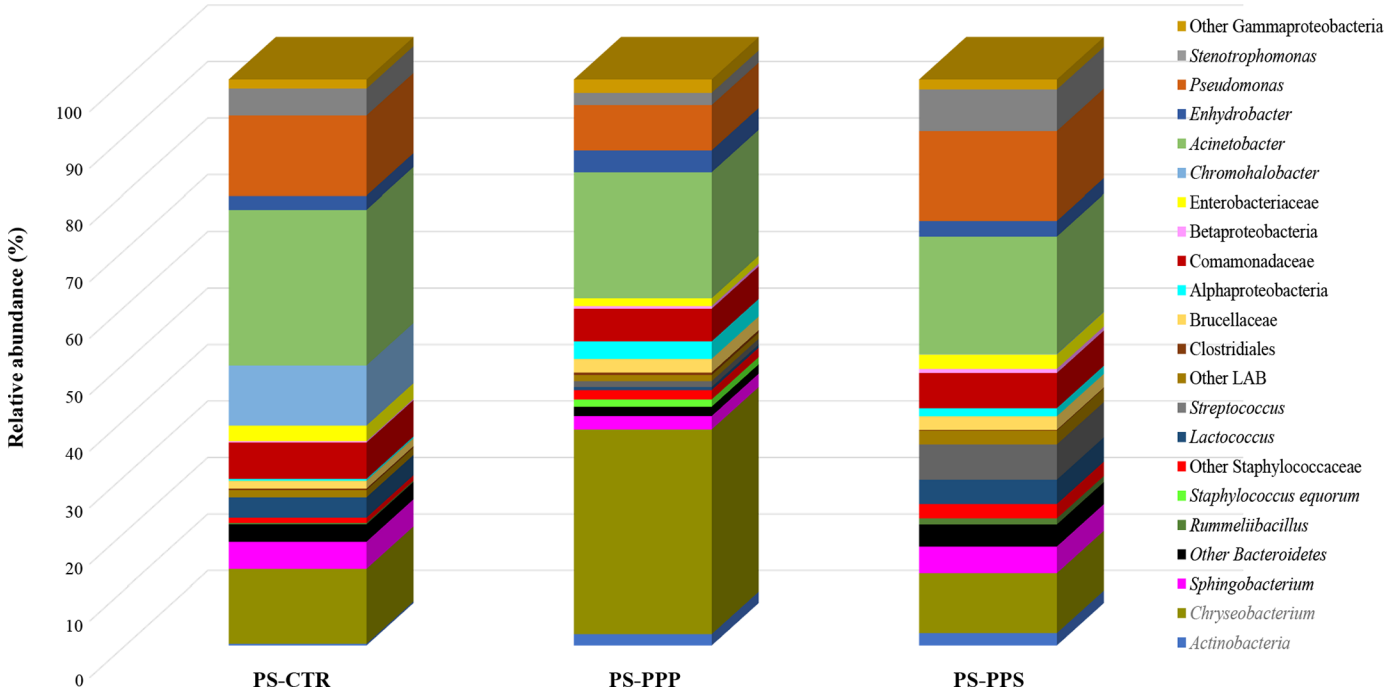

Figure 2. Relative abundances (%) of bacteria identified by IlluminaMiSeq. PS-CTR = fresh pecorino cheese control production; PS-PPP = fresh pecorino cheese prickly pear peels silage production; PS-PPS = fresh pecorino cheese prickly pear pulp, peels and seeds silage production.

been degraded by nucleases, rendering it undetectable (Xue et al., 2018).

Importantly, no DNA corresponding to major dairy pathogens, *E. coli*, *Salmonella* spp., *L. monocytogenes*, or CPS, was detected in any cheese sample, corroborating the findings obtained through culture-based microbiological assays.

Sensory Analysis: Triangle Test

According to the statistical reference criteria provided in ISO standard 4120 (ISO, 2004), at a 0.01 significance level, a minimum of 42 correct answers out of 90 is required for the panel to detect a significant difference between 2 samples. In our sensory assessment, a significant distinction among the 3 experimental cheeses was confirmed, as the panel recorded 42 correct identifications and 48 incorrect ones. These findings are consistent with those reported by Gannuscio et al. (2025), who employed a neuromarketing methodology. In this study, the panelists identified distinct sensory profiles among fresh pecorino cheeses (control vs. experimental), although sweetness remained the dominant attribute across the experimental conditions, likely due to the incorporation of prickly pear silage into the sheep diet. The inclusion of various agro-industrial byproducts in sheep feeding strategies has been shown to influence the sensory characteristics of Pecorino cheeses, ultimately enhancing consumer acceptability of the final product (Caccamo et al., 2019; Bolletta et al., 2022; Bennato et al., 2023).

CONCLUSIONS

The results indicated that the chemical composition of both milk and cheese remained unchanged across dietary treatments. However, the high phenolic content of prickly pear peels led to an increase in total phenolic content, specific phenolic compounds, and antioxidant capacity in both products. The fatty acid profile of the cheese was largely unaffected by the different diet. The data also confirmed that including prickly pear silage in the ewe's diet does not negatively affect the cheese's microbiological characteristics. Sensory evaluation highlighted significant differences among the cheeses, although consumer acceptance remained unaffected. Moreover, using this byproduct may help reduce feeding cost and waste disposal issues, making it a valuable strategy for improving the sustainability of dairy production.

NOTES

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Nonstandard abbreviations used: ABTS = 2,2′-azino-bis(3-ethylbenzothiazolino-6-sulfonic acid); AOC = antioxidant capacity; ARA = arachidonic acid; CPS = coagulase-positive staphylococci; CTR = control; DPPH = 2,2-diphenyl-1-picrylhydrazyl; EE = ether extract; ESI = electrospray ionization; FA = fatty acid; FAME = fatty acid methyl esters; FM = fresh matter; GAE = gallic acid equivalents; HPI = health-promoting index; LAB = lactic acid bacteria; NCN: noncasein nitrogen; OTU = operational taxonomic unit; PPB = prickly pear byproduct; PPP = prickly pear peels; PPS = prickly pear pulp, peels, and seeds; PSA = primary secondary amine; TEAC = Trolox equivalent antioxidant capacity; TMM = total mesophilic microorganisms; TN = total nitrogen; TPC = total phenolic content; TVA = *trans* vaccenic acid; UHPLC = ultra-high-performance liquid chromatography.

REFERENCES

Agulló, V., C. Favari, N. Pilla, L. Bresciani, F. A. Tomás-Barberán, A. Crozier, D. Del Rio, and P. Mena. 2024. Using targeted metabolomics to unravel phenolic metabolites of plant origin in animal milk. Int. J. Mol. Sci. 25:4536. https://doi.org/10.3390/ijms25084536.

Albergamo, A., A. G. Potortí, G. Di Bella, N. B. Amor, G. Lo Vecchio, V. Nava, R. Rando, H. Ben Mansour, and V. Lo Turco. 2022. Chemical characterization of different products from the Tunisian *Opuntia ficus-indica* (L.) Mill. Foods 11:155. https://doi.org/10.3390/foods11020155.

Alexandre, E. M., M. C. Coelho, K. Ozcan, C. A. Pinto, J. A. Teixeira, J. A. Saraiva, and M. Pintado. 2021. Emergent technologies for the extraction of antioxidants from prickly pear peel and their antimicrobial activity. Foods 10:570. https://doi.org/10.3390/foods10030570.

Amaya-Cruz, D. M., I. F. Pérez-Ramírez, J. Delgado-García, C. Mondragón-Jacobo, A. Dector-Espinoza, and R. Reynoso-Camacho. 2019. An integral profile of bioactive compounds and functional properties of prickly pear (*Opuntia ficus indica* L.) peel with different tonalities. Food Chem. 278:568–578. https://doi.org/10.1016/j.foodchem.2018.11.031.

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

Balasundram, N., K. Sundram, and S. Samman. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem. 99:191–203. https://doi.org/10.1016/j.foodchem.2005.07.042.

Belhadj Slimen, I., T. Najar, and M. Abderrabba. 2017. Correction to chemical and antioxidant properties of Betalains. J. Agric. Food Chem. 65:1466. https://doi.org/10.1021/acs.jafc.7b00512.

Bennato, F., A. Ianni, M. Bellocci, L. Grotta, G. Sacchetti, and G. Martino. 2023. Influence of dietary grape pomace supplementation on chemical and sensory properties of ewes' cheese. Int. Dairy J. 143:105671. https://doi.org/10.1016/j.idairyj.2023.105671.

Bocquier, F., and G. Caja. 2001. Production et composition du lait de brebis: Effets de l'alimentation. INRA Prod. Anim. 14:129–140. https://doi.org/10.20870/productions-animales.2001.14.2.3734.

Bolletta, V., M. Pauselli, C. Pomente, A. Natalello, L. Morbidini, G. Veneziani, V. Granese, and B. Valenti. 2022. Dietary olive leaves improve the quality and the consumer preferences of a model sheep cheese. Int. Dairy J. 134:105464. https://doi.org/10.1016/j.idairyj.2022.105464.

Bonanno, A., A. Di Grigoli, M. Todaro, M. Alabiso, F. Vitale, A. Di Trana, D. Giorgio, L. Settanni, R. Gaglio, B. Laddomada, and G. Di Miceli. 2019. Improvement of oxidative status, milk and cheese production, and food sustainability indexes by addition of durum wheat bran to dairy cows' diet. Animals 9:698. https://doi.org/10.3390/ani9090698.

Boti, V., V. Kobothekra, T. Albanis, and I. Konstantinou. 2021. QuEChERS-based methodology for the screening of alkylphenols and bisphenol A in dairy products using LC–LTQ/Orbitrap MS. Appl. Sci. (Basel) 11:9358. https://doi.org/10.3390/app11209358.

Branciari, R., D. Ranucci, M. Trabalza-Marinucci, M. Codini, M. Orru, R. Ortenzi, C. Forte, M. R. Ceccarini, and A. Valiani. 2015. Evaluation of the antioxidant properties and oxidative stability of Pecorino cheese made from the raw milk of ewes fed *Rosmarinus officinalis* L. *leaves*. Int. J. Food Sci. Technol. 50:558–565. https://doi.org/10.1111/ijfs.12712.

Cabañas, C. M., A. Hernández León, S. Ruiz-Moyano, A. V. Merchán, J. M. Martínez Torres, and A. Martín. 2025. Biocontrol of cheese spoilage moulds using native yeasts. Foods 14:2446. https://doi.org/10.3390/foods14142446.

Caccamo, M., G. Valenti, A. Luciano, T. Priolo, G. Rapisarda, V. M. Belvedere, S. Marino, A. Esposto, M. Taticchi, M. Servili, and M. Pauselli. 2019. Hazelnut as ingredient in dairy sheep diet: Effect on sensory and volatile profile of cheese. Front. Nutr. 6:125. https://doi.org/10.3389/fnut.2019.00125.

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

Cardamone, C., A. M. Di Noto, I. Mancuso, S. Sciortino, and M. L. Scatassa. 2018. Behavior of *Escherichia coli* O157 during the manufacture and ripening of a traditional sicilian raw ewes' milk cheese. Carpath. J. Food Sci. Technol. 10:45–56.

Castrica, M., R. Rebucci, C. Giromini, M. Tretola, D. Cattaneo, and A. Baldi. 2019. Total phenolic content and antioxidant capacity of agri-food waste and by-products. Ital. J. Anim. Sci. 18:336–341. https://doi.org/10.1080/1828051X.2018.1529544.

Chen, S., G. Bobe, S. Zimmerman, E. G. Hammond, C. M. Luhman, T. D. Boylston, A. E. Freeman, and D. C. Beitz. 2004. Physical and sensory properties of dairy products from cows with various milk fatty acid compositions. J. Agric. Food Chem. 52:3422–3428. https://doi.org/10.1021/jf035193z.

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

Correddu, F., M. F. Lunesu, G. Buffa, A. S. Atzori, A. Nudda, G. Battacone, and G. Pulina. 2020. Can agro-industrial by-products rich in polyphenols be advantageously used in the feeding and nutrition of dairy small ruminants? Animals (Basel) 10:131. https://doi.org/10.3390/ani10010131.

da Silveira Agostini-Costa, T. 2022. Genetic and environment effects on bioactive compounds of *Opuntia* cacti—A review. J. Food Compos. Anal. 109:104514. https://doi.org/10.1016/j.jfca.2022.104514.

Damaceno, H. F. B., C. V. de Freitas Junior, I. L. Marinho, T. R. Cupertino, L. E. D. O. Costa, and J. D. S. Nascimento. 2015. Antibiotic resistance versus antimicrobial substances production by gram-negative foodborne pathogens isolated from minas frescal cheese: heads or tails? Foodborne Pathog. Dis. 12:297–301. https://doi.org/10.1089/fpd.2014.1876.

De Feo, V., E. Quaranta, V. Fedele, S. Claps, R. Rubino, and C. Pizza. 2006. Flavonoids and terpenoids in goat milk in relation to forage intake. Ital. J. Food Sci. 18:85–92.

Di Stefano, V., C. Buzzanca, M. G. Melilli, S. Indelicato, M. Mauro, M. Vazzana, V. Arizza, M. Lucarini, A. Durazzo, and D. Bongiorno. 2022. Polyphenol characterization and antioxidant activity of grape seeds and skins from Sicily: A preliminary study. Sustainability (Basel) 14:6702. https://doi.org/10.3390/su14116702.

El-Beltagi, H. S., A. R. Ahmed, H. I. Mohamed, H. H. Al-Otaibi, K. M. Ramadan, and H. O. Elkatry. 2023. Utilization of prickly pear peels flour as a natural source of minerals, dietary fiber and antioxidants: Effect on cakes production. Agronomy (Basel) 13:439. https://doi.org/10.3390/agronomy13020439.

Forte, L., N. Parabita, M. Santoro, F. Longobardi, G. Natrella, J. Quiñones, E. N. Ponnampalam, I. Tomasevic, P. De Palo, and A. Maggiolino. 2026. From rumen to milk: Dietary polyphenols in dairy cows—A critical review. Vet. Anim. Sci. 31:100569. https://doi.org/10.1016/j.vas.2026.100569.

Foti, F., P. Caparra, G. Giuffrida, M. Scerra, and L. Chies. 2003. Olive cake, citrus pulp, and wheat straw silage as an ingredient in lamb diets: 2. Effects on meat quality. Ital. J. Anim. Sci. 2:491–493. https://doi.org/10.4081/ijas.2003.11676053.

Gaglio, R., I. Restivo, M. Barbera, P. Barbaccia, M. Ponte, L. Tesoriere, A. Bonanno, A. Attanzio, A. Di Grigoli, N. Francesca, G. Moschetti, and L. Settanni. 2021. Effect on the antioxidant, lipoperoxyl radical scavenger capacity, nutritional, sensory, and microbiological traits of an ovine stretched cheese produced with grape pomace powder addition. Antioxidants 10:306. https://doi.org/10.3390/antiox10020306.

Gannuscio, R. 2025. Study of new agro-industrial by-products from prickly pear to increase the sustainability of livestock farming. PhD thesis, Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Italy.

Gannuscio, R., C. Cardamone, A. Vastolo, C. Lucia, A. D'Amico, G. Maniaci, and M. Todaro. 2024a. Ensiling as a conservation technique for *Opuntia ficus indica* (L.) By-Products: Peel and pastazzo. Animals (Basel) 14:3196. https://doi.org/10.3390/ani14223196.

Gannuscio, R., G. Gifuni, G. Maniaci, D. Bongiorno, S. Indelicato, C. Lino, M. Bilucaglia, A. Fici, M. Zito, V. Russo, M. Todaro, and G. Avellone. 2025. Fresh Pecorino cheese produced by ewes fed silage with prickly pear by-products: VOC, chemical, and sensory characteristics detected with a neuro-sensory approach combining EEG and TDS. Foods 14:3334. https://doi.org/10.3390/foods14193334.

Gannuscio, R., M. Ponte, A. Di Grigoli, G. Maniaci, A. Di Trana, M. Bacchi, M. Alabiso, A. Bonanno, and M. Todaro. 2022. Feeding dairy ewes with fresh or dehydrated sulla (*Sulla coronarium* L.) Forage. 1. Effects on feed utilization, milk production, and oxidative status. Animals (Basel) 12:2317. https://doi.org/10.3390/ani12182317.

Gannuscio, R., A. Vastolo, G. Maniaci, C. Lucia, S. Calabrò, M. Todaro, and M. I. Cutrignelli. 2024b. Improve nutritive value of silage based on prickly pear peel by-products. Ital. J. Anim. Sci. 23:492–503. https://doi.org/10.1080/1828051X.2024.2323629.

Garofalo, G., C. Buzzanca, M. Ponte, M. Barbera, A. D'Amico, C. Greco, M. M. Mammano, E. Franciosi, D. Piazzese, V. Guarrasi, S. Ciulla, S. Orlando, A. Di Grigoli, A. Bonanno, V. Di Stefano, L. Settanni, and R. Gaglio. 2024. Comprehensive analysis of *Moringa oleifera* leaves' antioxidant properties in ovine cheese. Food Biosci. 61:104974. https://doi.org/10.1016/j.fbio.2024.104974.

Garofalo, G., M. Ponte, C. Greco, M. Barbera, M. M. Mammano, G. Fascella, G. Greco, G. Salsi, S. Orlando, A. Alfonzo, A. Di Grigoli, D. Piazzese, A. Bonanno, L. Settanni, and R. Gaglio. 2023. Improvement of fresh ovine "Tuma" cheese quality characteristics by application of oregano essential oils. Antioxidants 12:1293. https://doi.org/10.3390/antiox12061293.

Geronikou, A., T. Srimahaeak, K. Rantsiou, G. Triantafillidis, N. Larsen, and L. Jespersen. 2020. Occurrence of yeasts in white-brined cheeses: Methodologies for identification, spoilage potential and good manufacturing practices. Front. Microbiol. 11:582778. https://doi.org/10.3389/fmicb.2020.582778.

Hassan, M. U., A. Vastolo, R. Gannuscio, G. Maniaci, I. Mancuso, S. Calabro, A. Gallo, M. Todaro, and M. I. Cutrignelli. 2025. Effects of feeding prickly pear by-product silage as a partial replacement of concentrate on dairy ewes: Milk characteristics, nutrient utilisation and in vitro ruminal fermentation. Anim. Feed Sci. Technol. 324:116330. https://doi.org/10.1016/j.anifeedsci.2025.116330.

Hunt, R. W. G., and M. R. Pointer. 2011. Measuring Colour. John Wiley & Sons Ltd., Chichester, UK.

IDF (International Dairy Federation). 1993. International Standard FIL-IDF 20B. Determination of Total Nitrogen in Milk. IDF, Brussels, Belgium.
IDF (International Dairy Federation). 1964a. International Standard FIL-IDF 25. Determination of the Protein Content of Processed Cheese Products. IDF, Brussels, Belgium.
IDF (International Dairy Federation). 1964b. International Standard FIL-IDF 27. Determination of the Ash Content of Processed Cheese Products. IDF, Brussels, Belgium.
IDF (International Dairy Federation). 1964c. International Standard FIL-IDF 29. Determination of the Casein Content of Milk. IDF, Brussels, Belgium.
IDF (International Dairy Federation). 1982. International Standard FIL-IDF 4A. Cheese and Processed Cheese Product. Determination of the Total Solids Content. IDF, Brussels, Belgium.
IDF (International Dairy Federation). 1986. International Standard FIL-IDF 5B. Cheese and Processed Cheese Product. Determination of Fat Content-Gravimetric Method (Reference Method). IDF, Brussels, Belgium.
INRA. 2018. INRA Feeding System for Ruminants. p. 640. Wageningen Academic Publishers, Wageningen, The Netherlands.
Ioannou, G. D., O. Christodoulou, A. Sofroniou, K. A. Ioannou, A. Christou, I. J. Stavrou, and C. P. Kapnissi-Christodoulou. 2025. Phenolic extraction from prickly pear peels using deep eutectic solvents with macrocyclic enhancers. Food Chem. 492:145275. https://doi.org/10.1016/j.foodchem.2025.145275.
ISO (International Organization for Standardization). 2004. ISO 4120:2004 Sensory Analysis-Methodology-Triangle Test. ISO, Geneva, Switzerland.
Istat (Istituto Nazionale di Statistica). 2025. IstatData. Accessed Nov. 2, 2025. https://esploradati.istat.it/databrowser/#/it/dw/categories/it1,z1000agr,1.0/agr_crp/dcsp_coltivazioni/it1,101_1015_df_dcsp_coltivazioni_1,1.0.
Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30:772–780. https://doi.org/10.1093/molbev/mst010.
Kaur, G., B. Thawkar, S. Dubey, and P. Jadhav. 2018. Pharmacological potentials of Betalains. J. Complement. Integr. Med. 15:20170063. https://doi.org/10.1515/jcim-2017-0063.
Kramer, J. K., C. Cruz-Hernandez, Z. Deng, J. Zhou, G. Jahreis, and M. E. Dugan. 2004. Analysis of conjugated linoleic acid and trans 18:1 isomers in synthetic and animal products. m. Am. J. Clin. Nutr. 79:1137S–1145S. https://doi.org/10.1093/ajcn/79.6.1137S.
Landete, J. M., J. Arqués, M. Medina, P. Gaya, B. De Las Rivas, and R. Muñoz. 2016. Bioactivation of phytoestrogens: Intestinal bacteria and health. Crit. Rev. Food Sci. Nutr. 56:1826–1843. https://doi.org/10.1080/10408398.2013.789823.
Lauková, A., L. Micenková, M. Pogány Simonová, V. Fockova, J. Ščerbová, M. Tomáška, and M. Kološta. 2021. Microbiome associated with Slovak traditional ewe's milk lump cheese. Processes (Basel) 9:1603. https://doi.org/10.3390/pr9091603.
Lee, M. R. F., and J. K. S. Tweed. 2008. Isomerisation of cis-9 trans-11 conjugated linoleic acid (CLA) to trans-9 trans-11 CLA during acidic methylation can be avoided by a rapid base catalysed methylation of milk fat. J. Dairy Res. 75:354–356. https://doi.org/10.1017/S0022029908003518.
Li, W., H. Chen, B. Xu, Y. Wang, C. Zhang, Y. Cao, and X. Xing. 2023. Research progress on classification, sources, and functions of dietary polyphenols for prevention and treatment of chronic diseases. J. Future Foods 3:289–305. https://doi.org/10.1016/j.jfutfo.2023.03.001.
Luna, P., M. A. de la Fuente, and M. Juárez. 2005. Conjugated linoleic acid in processed cheeses during the manufacturing stages. J. Agric. Food Chem. 53:2690–2695. https://doi.org/10.1021/jf048091x.
Lv, C., F. Yang, R. Qin, Z. Qi, W. Zhou, and J. Lu. 2017. Bioactivity-guided isolation of chemical constituents against H(2)O(2-)induced neurotoxicity on PC12 from Cimicifuga dahurica (Turcz.). Bioorg. Med. Chem. Lett. 27:3305–3309. https://doi.org/10.1016/j.bmcl.2017.06.020.
MAF (Ministero dell'Agricoltura e delle Foreste). 1986. Decreto ministeriale 21/4/1986. Approvazione dei metodi ufficiali di analisi per i formaggi (Italian Official Methods of Cheeses Analysis). Page 15 in Supplemento ordinario alla Gazzetta Ufficiale della Repubblica Italiana (GURI), n. 229.
Maniaci, G., M. Ponte, C. Giosuè, R. Gannuscio, M. Pipi, R. Gaglio, G. Busetta, A. Di Grigoli, A. Bonanno, and M. Alabiso. 2024. Cladodes of *Opuntia ficus-indica* (L.) as a source of bioactive compounds in dairy products. J. Dairy Sci. 107:1887–1902. https://doi.org/10.3168/jds.2023-23847.
Mena, P., M. Tassotti, L. Andreu, N. Nuncio-Jáuregui, P. Legua, D. Del Rio, and F. Hernández. 2018. Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSn metabolomics profiles and their chemometric analysis. Food Res. Int. 108:301–308. https://doi.org/10.1016/j.foodres.2018.03.062.
Min, B. R., T. N. Barry, G. T. Attwood, and W. C. McNabb. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. Anim. Feed Sci. Technol. 106:3–19. https://doi.org/10.1016/S0377-8401(03)00041-5.
Mohamed, A. S., L. M. El-Malt, K. G. Abdel Hameed, and M. A. El-Zamkan. 2022. Incidence of *Chryseobacterium* spp. in raw milk and some dairy products. SVU Int. J. Vet. Sci. 5:77–90. https://doi.org/10.21608/svu.2022.114720.1170.
Møretrø, T., and S. Langsrud. 2017. Residential bacteria on surfaces in the food industry and their implications for food safety and quality. Compr. Rev. Food Sci. Food Saf. 16:1022–1041. https://doi.org/10.1111/1541-4337.12283.
Natalello, A., A. Priolo, B. Valenti, M. Codini, S. Mattioli, M. Pauselli, M. Puccio, M. Lanza, M. Stergiadis, and G. Luciano. 2020. Dietary pomegranate by-product improves oxidative stability of lamb meat. Meat Sci. 162:108037. https://doi.org/10.1016/j.meatsci.2019.108037.
Nayel, U. A., H. A. El-Sheikh, F. M. Abo-Donia, and A. A. Marwan. 2023. Influence of co-ensiling rehydrated rice straw with prickly pear fruit peels feeding on degradability, digestibility, and nutritive value for sheep. Egypt. J. Nutr. Feeds 26:141–157. https://doi.org/10.21608/ejnf.2023.316696.
Nudda, A., M. A. McGuire, G. Battacone, and G. Pulina. 2005. Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and ricotta. J. Dairy Sci. 88:1311–1319. https://doi.org/10.3168/jds.S0022-0302(05)72797-1.
Olagaray, K. E., and B. J. Bradford. 2019. Plant flavonoids to improve productivity of ruminants–A review. Anim. Feed Sci. Technol. 251:21–36. https://doi.org/10.1016/j.anifeedsci.2019.02.004.
Özcan, M. M., N. Uslu, H. H. Kara, and M. M. Özcan. 2023. Variations in bioactive properties, phenolic compounds and fatty acid compositions of different parts of prickly pear (*Opuntia ficus-indica* spp.) fruits. Erwerbs-Obstbau 65:1163–1170. https://doi.org/10.1007/s10341-022-00766-8.
Ozturk, B., C. Parkinson, and M. Gonzalez-Miquel. 2018. Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents. Separ. Purif. Tech. 206:1–13. https://doi.org/10.1016/j.seppur.2018.05.052.
Palmquist, D. L., and T. C. Jenkins. 1980. Fat in lactation rations. J. Dairy Sci. 63:1–14. https://doi.org/10.3168/jds.S0022-0302(80)82881-5.
Petruk, G., R. Del Giudice, M. M. Rigano, and D. M. Monti. 2018. Antioxidants from plants protect against skin photoaging. Oxid. Med. Cell. Longev. 2018:1454936. https://doi.org/10.1155/2018/1454936.
Ponte, M., G. Maniaci, A. Di Grigoli, R. Gannuscio, M. R. Ashkezary, M. Addis, M. Pipi, M. Alabiso, M. Todaro, and A. Bonanno. 2022. Feeding dairy ewes with fresh or dehydrated Sulla (Sulla coronarium L.) forage. 2. Effects on cheese enrichment in bioactive molecules. Animals (Basel) 12:2462. https://doi.org/10.3390/ani12182462.
Price, M. N., P. S. Dehal, and A. P. Arkin. 2009. FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. Mol. Biol. Evol. 26:1641–1650. https://doi.org/10.1093/molbev/msp077.
Rashidinejad, A., E. J. Birch, D. Sun-Waterhouse, and D. W. Everett. 2013. Effects of catechin on the phenolic content and antioxidant

properties of low-fat cheese. Int. J. Food Sci. Technol. 48:2448–2455. https://doi.org/10.1111/ijfs.12234.
Raya-Morquecho, E. M., P. Aguilar-Zarate, L. Sepúlveda, M. R. Michel, A. Iliná, C. N. Aguilar, and J. A. Ascacio-Valdés. 2025. Ellagitannins and their derivatives: A review on the metabolization, absorption, and some benefits related to intestinal health. Microbiol. Res. (Pavia) 16:113. https://doi.org/10.3390/microbiolres16060113.
Romo-Vaquero, M., A. Cortés-Martín, V. Loria-Kohen, A. Ramírez-de-Molina, I. García-Mantrana, M. C. Collado, J. C. Espìn, and M. V. Selma. 2019. Deciphering the human gut microbiome of urolithin metabotypes: Association with enterotypes and potential cardiometabolic health implications. Mol. Nutr. Food Res. 63:1800958. https://doi.org/10.1002/mnfr.201800958.
Roppolo, P., C. Buzzanca, A. D'Amico, A. Culmone, I. Tinebra, R. Passafiume, S. Bonacci, V. Farina, and V. Di Stefano. 2024. Improvement of antioxidant activity and sensory properties of functional cookies by fortification with ultrasound-assisted hot-air-drying blackberry powders. Foods 13:2402. https://doi.org/10.3390/foods13152402.
Roura, E., C. Andrés-Lacueva, R. Estruch, M. Lourdes Mata Bilbao, M. Izquierdo-Pulido, and R. M. Lamuela-Raventós. 2008. The effects of milk as a food matrix for polyphenols on the excretion profile of cocoa (−)-epicatechin metabolites in healthy human subjects. Br. J. Nutr. 100:846–851. https://doi.org/10.1017/S0007114508922534.
SAS Institute Inc. 2010. 'SAS/STAT qualification tools user's guide (version 9.2); statistical analysis system. SAS Institute Inc., Cary, NC.
Sciacca, F., N. Virzì, N. Pecchioni, M. G. Melilli, C. Buzzanca, S. Bonacci, and V. Di Stefano. 2023. Functional end-use of hemp seed waste: Technological, qualitative, nutritional, and sensorial characterization of fortified bread. Sustainability (Basel) 15:12899. https://doi.org/10.3390/su151712899.
Serra, V., G. Salvatori, and G. Pastorelli. 2021. Dietary polyphenol supplementation in food producing animals: Effects on the quality of derived products. Animals (Basel) 11:401. https://doi.org/10.3390/ani11020401.
Settanni, L., and G. Moschetti. 2010. Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. Food Microbiol. 27:691–697. https://doi.org/10.1016/j.fm.2010.05.023.
Stellato, G., F. De Filippis, A. La Storia, and D. Ercolini. 2015. Coexistence of lactic acid bacteria and potential spoilage microbiota in a dairy processing environment. Appl. Environ. Microbiol. 81:7893–7904. https://doi.org/10.1128/AEM.02294-15.
Terzić-Vidojević, A., K. Veljović, N. Popović, M. Tolinački, and N. Golić. 2021. Enterococci from raw-milk cheeses: Current knowledge on safety, technological, and probiotic concerns. Foods 10:2753. https://doi.org/10.3390/foods10112753.
Tirloni, E., C. Bernardi, and S. Stella. 2021. Pseudomonas spp.: Are food grade organic acids efficient against these spoilage microorganisms in fresh cheeses? Foods 10:891. https://doi.org/10.3390/foods10040891.
Todaro, M., M. Alabiso, A. Di Grigoli, M. L. Scatassa, C. Cardamone, I. Mancuso, F. Mazza, and A. Bonanno. 2020. Prickly pear by-product in the feeding of livestock ruminants: Preliminary investigation. Animals (Basel) 10:949. https://doi.org/10.3390/ani10060949.
Todaro, M., R. Gannuscio, I. Mancuso, B. Ducato, and M. L. Scatassa. 2023. Relationships between chemical and physical parameters of bulk milk from Valle del Belice sheep. Ital. J. Anim. Sci. 22:953–958. https://doi.org/10.1080/1828051X.2023.2252846.
Todaro, M., G. Garofalo, G. Busetta, R. Gannuscio, A. R. Di Rosa, M. L. Scatassa, C. Cardamone, I. Mancuso, E. Franciosi, F. Rando, M. Agnolucci, V. Chiofalo, R. Gaglio, and L. Settanni. 2024. Reduction of PDO Pecorino Siciliano cheese making duration: Microbial dynamics and quality attributes deriving from replacing whey permeate with hot water during cooking. Int. J. Food Microbiol. 410:110481. https://doi.org/10.1016/j.ijfoodmicro.2023.110481.
Toral, P. G., G. Hervás, E. Bichi, Á. Belenguer, and P. Frutos. 2011. Tannins as feed additives to modulate ruminal biohydrogenation: Effects on animal performance, milk fatty acid composition and ruminal fermentation in dairy ewes fed a diet containing sunflower oil. Anim. Feed Sci. Technol. 164:199–206. https://doi.org/10.1016/j.anifeedsci.2011.01.011.
Trapali, M., and V. Lagouri. 2025. Urolithins—Gut microbial metabolites with potential health benefits. Open Med. Chem. J. 19:e18741045375825. https://doi.org/10.2174/0118741045375825250415073204.
Tretola, M., G. Bee, F. Dohme-Meier, and P. Silacci. 2023. Harmonised in vitro digestion and the Ussing chamber for investigating the effects of polyphenols on intestinal physiology in monogastrics and ruminants. Animal 17:100785. https://doi.org/10.1016/j.animal.2023.100785.
Ulbricht, T. L. V., and D. A. T. Southgate. 1991. Coronary heart disease: Seven dietary factors. Lancet 338:985–992. https://doi.org/10.1016/0140-6736(91)91846-M.
Vasta, V., M. Daghio, A. Cappucci, A. Buccioni, A. Serra, C. Viti, and M. Mele. 2019. Invited review: Plant polyphenols and rumen microbiota responsible for fatty acid biohydrogenation, fiber digestion, and methane emission: Experimental evidence and methodological approaches. J. Dairy Sci. 102:3781–3804. https://doi.org/10.3168/jds.2018-14985.
Vasta, V., and G. Luciano. 2011. The effects of dietary consumption of plants secondary compounds on small ruminants' products quality. Small Rumin. Res. 101:150–159. https://doi.org/10.1016/j.smallrumres.2011.09.035.
Vastolo, A., S. Calabrò, and M. I. Cutrignelli. 2022. A review on the use of agro-industrial CO-products in animals' diets. Ital. J. Anim. Sci. 21:577–594. https://doi.org/10.1080/1828051X.2022.2039562.
Vastolo, A., S. Calabrò, M. I. Cutrignelli, G. Raso, and M. Todaro. 2020. Silage of prickly pears (Opuntia spp.) juice by-products. Animals (Basel) 10:1716. https://doi.org/10.3390/ani10091716.
Viola, E., C. Buzzanca, I. Tinebra, L. Settanni, V. Farina, R. Gaglio, and V. Di Stefano. 2023. A functional end-use of avocado (cv. Hass) waste through traditional semolina sourdough bread production. Foods 12:3743. https://doi.org/10.3390/foods12203743.
Vlaeminck, B., V. Fievez, S. Tamminga, R. J. Dewhurst, A. van Vuuren, D. De Brabander, and D. Demeyer. 2006. Milk odd- and branched-chain fatty acids in relation to the rumen fermentation pattern. J. Dairy Sci. 89:3954–3964. https://doi.org/10.3168/jds.S0022-0302(06)72437-7.
Wąsowska, I., M. R. G. Maia, K. M. Niedźwiedzka, M. Czauderna, J. Ramalho Ribeiro, E. Devillard, K. J. Shingfield, and R. J. Wallace. 2006. Influence of fish oil on ruminal biohydrogenation of C18 unsaturated fatty acids. Br. J. Nutr. 95:1199–1211. https://doi.org/10.1079/BJN20061783.
Xue, Z., M. E. Kable, and M. L. Marco. 2018. Impact of DNA sequencing and analysis methods on 16S rRNA gene bacterial community analysis of dairy products. MSphere 3:e00410-18. https://doi.org/10.1128/mSphere.00410-18.
Yeddes, N., J. K. Chérif, S. Guyot, H. Sotin, and M. T. Ayadi. 2013. Comparative study of antioxidant power, polyphenols, flavonoids and betacyanins of the peel and pulp of three Tunisian Opuntia forms. Antioxidants 2:37–51. https://doi.org/10.3390/antiox2020037.

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