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Comprehensive analysis of *Moringa oleifera* leaves' antioxidant properties in ovine cheese

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ABSTRACT

This study aimed to enhance "Pecorino" type ovine cheese by adding *Moringa oleifera* leaves powder (MOLP). Cheese-making trials, conducted at industrial level, used raw ewes' milk and two selected *Lactococcus lactis* strains. The experimental plan included a control production (CTR), and two experimental productions with 1% or 2% MOLP addition (1-MOLP and 2-MOLP, respectively). MOLP did not hinder starters development, which reached about 8.0 Log CFU/g in 2-month ripened cheeses. Illumina results highlighted lactococci dominance in all trials [45.98%–62.48% of relative abundance (RA)]. Physicochemical analysis showed that MOLP-enriched cheeses had higher protein content and lower secondary lipid oxidation. The addition of MOLP increased total phenolic compounds in cheese, reaching 3.64 mg GAE/g in the 2-MOLP sample. MOLP-enriched cheeses showed significantly higher radical scavenging activity than CTR production ($p < 0.0001$). Ultrahigh-performance liquid chromatography-electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS) revealed increased levels of chlorogenic acid, protocatechuic acid, caffeic acid, and ferulic acid due to MOLP enrichment. In the presence of MOLP, cheese volatile organic compounds were affected by compounds like 2-octanone, 3-hexen-2-one, heptane, nonanol, and linalool. 1-MOLP cheese was comparable to CTR production in overall satisfaction (sensory evaluation). Including MOLP in cheese production offers exciting opportunities for functional Sicilian ewes' milk products.

1. Introduction

Lately, academic research institutes and food industry R&D units have actively explored natural alternatives to enhance food properties, aligning with the European Green Deal principles (Tóth, 2019). Using plant-derived products, such as leaf or seed extracts, crude oils, and essential oils, provides a successful approach to boost safety and functionality in processed foods (Gaglio et al., 2023; Garofalo et al., 2023). These products contain bioactive molecules that positively impact human health, particularly in oxidative stress-related conditions (Salehi et al., 2020). Additionally, they mitigate microbial contamination due to their phytochemical constituents (Barbieri et al., 2017).

Moringa oleifera Lam., commonly known as the "miracle plant" or the "tree of life", belongs to the Moringaceae family. It has gained attention for its medicinal properties based on local folk knowledge (Alegbeleye, 2018; Matic et al., 2018). Several studies have explored the nutritional composition and bioactive compounds in different parts of the plant, including the root, bark, leaf, flower, pod, and seeds (Dalei et al., 2016;

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Falowo et al., 2018; Lin et al., 2018; Mbikay, 2012). Notably, *M. oleifera* exhibits high nutritional, antioxidant, antimicrobial, and health-promoting properties. Its leaves, in particular, contain more minerals, vitamins, and essential phytochemicals compared to other parts of the plant (Gopalakrishnan et al., 2016). Fresh and dried *M. oleifera* leaves are incorporated into various food products, including cereal-based, meat, and dairy items, to improve nutritional value, oxidative stability, shelf life, and sensory properties (Oyeyinka & Oyeyinka, 2018). Although *M. oleifera* leaves have been added to cow and buffalo milk products to enhance their nutritional and health value (Lisak Jakopović et al., 2022; Salem et al., 2013), there are no published studies on incorporating these leaves in ovine cheeses. In Sicily, where sheep breeding is prominent (Sitzia et al., 2015; Todaro et al., 2023) traditional factory-processed ewe's cheeses are often considered nutritionally unbalanced (Gaglio, Restivo, et al., 2021). To address this, dairy producers are actively exploring ways to enrich ewe's milk cheeses with plant-derived products, emphasizing the impact of bioactive compounds on human health.

The aim of this study was to develop a novel functional ewes' milk cheese by incorporating *M. oleifera* leaves in powder (MOLP) form along with selected *Lactococcus lactis* starter cultures. The resulting cheeses underwent evaluation for microbiological, physicochemical, and sensory characteristics.

2. Materials and methods

2.1. Raw material and natural milk starter culture preparation

Commercially available *M. oleifera* leaf powder branded as "NaturaBio" (International Food Europe srl, Monterotondo, Italy) was used to produce the new ewes' milk cheese. The labeled nutritional values per 100 g were: 23.8 g of proteins, 23.0 g of carbohydrates, 6.9 g of fats (including 3.7 g of saturated fats), 30.5 g of fibres, 668 μg of vitamin A, 39 mg of iron, and 1815 mg of calcium. Raw ewes' milk from cross-breeds between "Sarda" × "Valle del Belice" sheep, reared in an artisanal dairy farm (Azienda Agricola Basile, Ventimiglia di Sicilia, Italy), was used for cheese production. The characteristics of milk were as follows: pH 6.62 ± 0.02, casein 3.72% ± 0.10%, fat 5.81% ± 0.15%, lactose 4.03% ± 0.29%, protein 5.00% ± 0.12%, and urea 29.90 ± 2.26 mg/dL. The Natural Milk Starter Culture (NMSC) used in this study included two strains of *Lc. lactis* PON36 (Ac. No. KC545886) and CAG4 (Ac. No. KC351901). These strains, selected for their high dairy traits such as acidification capacity, antibacterial activity, autolysis kinetics, and diacetyl production belonged to the bacterial culture collection of the Department of Agricultural, Food and Forest Sciences (University of Palermo, Italy) and were previously isolated from Protected Designation of Origin (PDO) Sicilian cheeses (Gaglio et al., 2014; Settanni et al., 2013). Briefly, both strains were grown overnight at 30 °C in Medium 17 (M17) broth (Oxoid, Basingstoke, UK). After centrifugation and washing in Ringer's solution (Oxoid), they were inoculated (1%, v/v) into pasteurized ewe's milk (heated to 75 °C for 15 s) following the method described by Gaglio, Barbaccia, et al. (2021). After fermentation at 30 °C for 24 h, this milk became the NMSC used as a fermenting agent in cheese production.

2.2. Description of dairy plant

Cheese production took place in an industrial setting at the dairy factory "Azienda Agricola Basile" in Ventimiglia di Sicilia (Palermo, Italy). The dairy plant (Fig. 1) had a working capacity of 200 L and was equipped with a high-efficiency wet-bottom condensing steam generator. Additionally, the plant featured a milk pasteurization and coagulation tank, temperature-monitoring probes, a gearmotor for curd cutting, and a perforated steel table for curd molding.

The TFRE series comprises a wet-bottom condensing steam generator with flame reversal. It can produce steam at a rate of 150 to 1300 kg/h, operating at a pressure range of 5–12 bar. The generator is CE-marked and capable of yielding 90,000 to 780,000 kcal/h with horizontal smoke. The generator features a fully inspectable cavity front door with water recirculation. Notably, it employs three smoke rounds (the first and second in the firebox, the third in the pipes) to enhance efficiency. The large-diameter shell is equipped with inspection doors and a stainless-steel pre-heater tested at 20 bar. By directing feed water against the current, it raises the temperature by over 40 °C and reduces flue gas temperature to 120 °C, achieving an impressive 95% efficiency. Furthermore, the generator also includes an automatic electro-pneumatic stainless-steel drain, time-adjustable via PLC with manual functions.

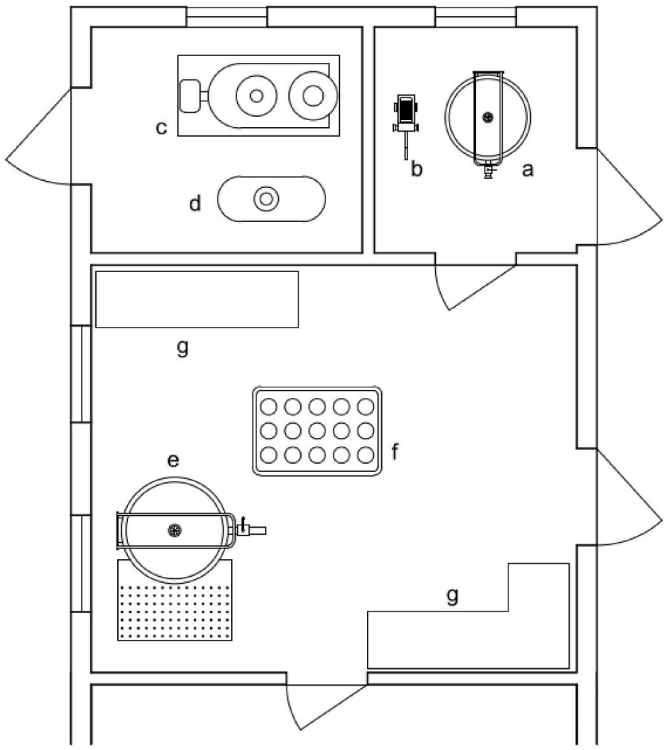

Fig. 1. Schematic view of dairy plant. (a) cooling vat; (b) milk movement pump; (c) steam generator; (d) condensate recovery and return system; (e) multipurpose coagulation vat; (f) perforated steel table; (g) packaging workstation.

The dairy facility incorporates an energy recovery system that enhances energy efficiency by recycling condensate back to the thermal power plant. The Condensate Recovery and Return System is designed for longevity and effective condensate recovery, resulting in cost savings and increased efficiency. By capturing and returning hot condensate to the boiler, the system reduces make-up water expenses, minimizes fresh water requirements, lowers chemical costs, and decreases blowdown. This approach not only prevents energy loss from the boiler but also improves stability and prolongs the lifespan of equipment by removing condensate from heat exchange and process machinery.

2.3. Cheese productions and sample collection

Cheese-making trials were carried out applying the semi-ripened Pecorino cheese technology. The experimental plan, depicted in Fig. 2, encompasses three distinct trials: CTR, control cheese was prepared from raw ewes' milk inoculated with the NMSC without MOLP addition; 1-MOLP, experimental cheese prepared from raw ewes' milk inoculated with the NMSC and enriched with 1% (w/w of curd) MOLP; and 2-MOLP experimental cheese prepared from raw ewes' milk inoculated with the NMSC and enriched with 2% (w/w of curd) MOLP. Each trial was

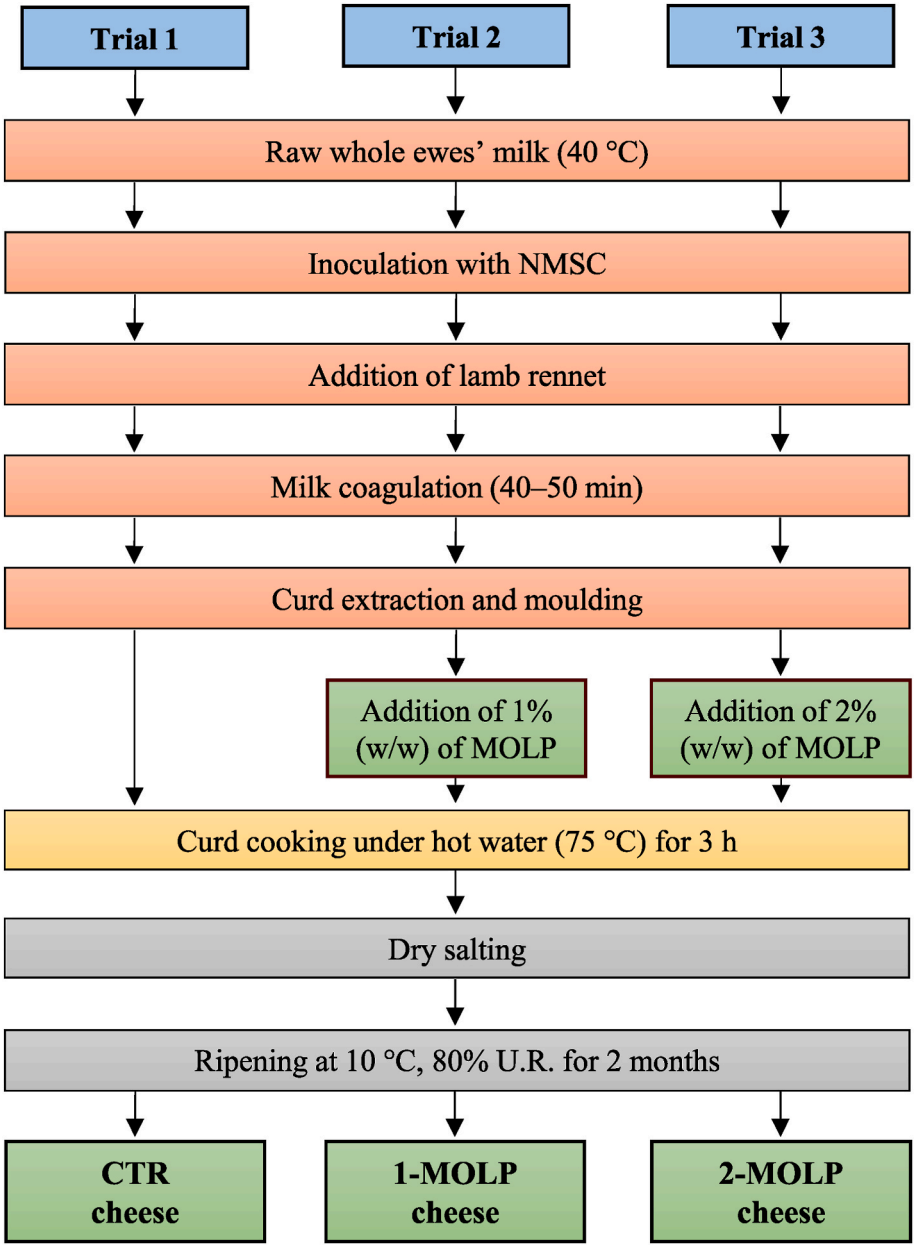

Fig. 2. Flow diagrams of experimental cheese production. Abbreviations: CTR, control cheese prepared without *Moringa oleifera* leaves in powder form (MOLP) addition; 1-MOLP, experimental cheese enriched with 1% (w/w) of MOLP; 2-MOLP, experimental cheese enriched with 2% (w/w) of MOLP. Abbreviation: NMSC, natural milk starter culture.

performed at a dairy plant (Sfoggia & C. SAS, Montebelluna, Italy) using 200 L of whole ewes' milk. In brief, the process involved heating bulk milk to 40 °C, inoculating it with 2 L of NMSC, and gently stirring for 10 min before adding kid rennet paste (90 g, Micromilk Srl, Cremosano, Italy). After 40–50 min, the coagulum was mechanically broken into 3–7 mm diameter grains. Following whey drainage, the control curd was promptly placed in perforated plastic molds, while the experimental curds were blended with 1% and 2% (w/w) MOLP before transferring them to similar molds. All curds underwent immediate cooking in hot water, dry salting, and ripening for 2 months. These trials were conducted in duplicate over two consecutive months (two independent experimental replicates), and samples were collected at various stages: MOLP, raw milk, inoculated milk with NMSC, curd, cheeses soon after production, and after 2 months of ripening.

2.4. Microbiological analysis by culture-dependent approach

All samples collected during both control and experimental cheese production using the decimal serial dilution procedure (Garofalo et al., 2021). The procedure involved plating cell suspensions at decreasing cell densities on specific media: Skim Milk Agar (SMA) incubated for 3 d at 30 °C to enumerate total mesophilic microorganisms (TMM); Medium 17 (M17) agar incubated for 2 d at 30 °C for mesophilic coccus lactic acid bacteria (LAB); *Pseudomonas* agar base (PAB) supplemented with cephaloridine sodium fusidate cetrimide (CFC) incubated for 2 d at 25 °C for *Pseudomonas* spp.; Violet Red Bile Agar (VRBA) incubated for 1 d at 37 °C to detect total coliforms; Baird Parker (BP) agar with rabbit plasma fibrinogen (RPF) supplement, incubated for 2 d at 37 °C to identify coagulase-positive staphylococci (CPS); Hektoen Enteric Agar (HEA) incubated for 1 d at 37 °C for *Escherichia coli*; *Listeria* Selective Agar Base (LSAB) added with SR0140E supplement, incubated for 1 d at

37 °C for *Listeria monocytogenes*; Xylose Lysine Deoxycholate (XLD) agar incubated for 1 d at 37 °C for *Salmonella* spp. These analyses were performed in triplicate using media purchased from Oxoid.

2.5. Culture-independent analysis of total bacterial community

Total genomic DNA was extracted using the DNeasy PowerFood Microbial Kit (QIAGEN, Hilden, Germany) following manufacturer's protocol. The Nanodrop 8800 Fluorospectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to quantify the DNAs. Subsequently, amplification, purification, quality assessments, and sequencing of the extracted DNA were carried out at the Illumina MiSeq Platform of Fondazione Edmund Mach (San Michele a/Adige, Italy) following the methodology reported by Gaglio et al. (2020). Sequencing analyses were performed using the Filter and Trim functions in DADA2. Taxonomic assignment and compositional analyses were carried out using the Greengenes 13_8 99% Operational Taxonomic Units (OTUs) database. Finally, the resulting FASTQ files were submitted to the NCBI Sequence Read Archive (SRA) with accession numbers PRJNA1126120.

2.6. Physicochemical analyses of cheeses

All cheese samples were freeze-dried and analyzed for their composition following the method of International Dairy Federation (IDF): content of dry matter (DM) (IDF, 1982), ash (IDF, 1964a), protein (N x 6.38) (IDF, 1964b), and fat (IDF, 1986). The values of pH were measured by a pH meter HI 9025 (Hanna Instruments, Ann Arbor, MI, USA), while water activity (a_w) was assessed with the HygroPalm portable water activity meter (Rotronic, Bassersdorf, Germany) following EN ISO 21807:2004 guidelines. Moreover, the susceptibility of cheese fat to oxidation was computed determining, in duplicate, the index of primary lipid oxidation, named peroxide value (POV, mEq O_2/kg fat; IDF, 1991) and the index of secondary lipid oxidation named thiobarbituric acid-reactive substances (TBARs, μg malonylaldehyde (MDA)/kg DM) as reported by Bonanno et al. (2019).

To assess cheese strength, maximum resistance to compression (compressive stress, N/mm^2) was evaluated using an Instron 5564 tester (Instron, Trezzano sul Naviglio, Milan, Italy) 2 cm × 2 cm x 2 cm samples maintained at 22 °C.

Furthermore, cheeses were evaluated for color using a Minolta Chroma Meter CR300 (Minolta, Osaka, Japan) according to the Commission Internationale de l'Éclairage standard (CIE, 1986), L* a* b* system. The values represent lightness (L*, ranging from 0 to 100, where 0 is black and 100 is white), redness (a*, positive for red and ngative for green), and yellowness (b*, positive for yellow and negative for blue).

2.7. Chemical characterization of raw materials and cheese samples

2.7.1. Samples preparation

Raw materials (raw ewes' milk and MOLP) and cheese (CTR, 1-MOLP and 2-MOLP) samples were placed in especially glass bottles for freeze-drying and stored at −80 °C for 24 h. The frozen glass bottles were then freeze-dried at −50 °C for 48 h under a vacuum (0.05 mbar) using a freeze-dryer (Christ Gamma, Germany) according to the manufacturer's instructions. Freeze-dried samples were used for chemical characterization and antiradical activity.

2.7.2. Total polyphenol content

Total phenolic content (TPC) was determined using the optimized Folin-Ciocâlteu method (Viola et al., 2023), with slight modifications. Five hundred milligrams of each freeze-dried sample was mixed with 4 mL of methanol/water solution (80:20 v/v), sonicated for 45 min and filtered through Whatman 0.45 μm PTFE filters (Sigma-Aldrich, St. Louis, MO, USA). An aliquot of the filtrate (0.125 mL), 120 μL of a 7% Na_2CO_3 solution and 625 μL of Folin-Ciocâlteu reagent (Sigma-Aldrich) (1:5) were incubated in the dark at 25 °C for 60 min. The intensity of color is proportional to phenolic compounds concentration in the sample. The absorbance was evaluated at 765 nm using a UV/Vis spectrophotometer (UV–1600PC, VWR® International, Leuven, Belgium). Methanol was used as the blank and gallic acid (GA) was used for calibration of the standard curve (0.01–0.5 mg/mL). TPC was expressed as mg gallic acid equivalents per g (mg GAE/g) of the freeze-dried sample (d.m.). These analyses were performed in triplicate.

2.7.3. Antioxidant activity

The measurement of anti-radical activity follows procedures previously described (Di Stefano et al., 2023; Sciacca et al., 2023). DPPH and ABTS were used to determine the antioxidant power through the reaction of the sample with a solution of DPPH [2, 2-diphenyl-1-picrylhydrazyl] and ABTS [2,2′-azino-bis(3-ethylbenzothiazolino-6-Sulphonic acid], that cause a discoloration of the solution proportional to the antioxidant charge present in the sample. For the DPPH assay, 1 g of sample was extracted using 10 mL of methanol, sonicated and filtered through Whatman 0.45 μm PTFE filters. The filtrate (100 μL) was mixed with 3 mL of DPPH (60 μM) and incubated in the dark at 25 °C for 30 min. Scavenging activity was measured at 515 nm using a UV–Vis spectrophotometer (UV–1600PC, VWR® International), and methanol was used as the blank. For the ABTS assay, four mL of MeOH was added to 2 g of each sample, sonicated and filtered through Whatman 0.45 μm PTFE filters. The absorbance was reading 5 min after the addition of 3 mL of diluted $ABTS^{\cdot+}$ to 100 μL of sample. The decrease in absorbance caused by antioxidants, recorded at 734 nm against methanol, reflected the $ABTS^{\cdot+}$ free radical scavenging capacity. A calibration curve using Trolox in a concentration range of 1 μM–75 μM, was constructed. The obtained values were reported as Trolox equivalent antioxidant activity (TEAC) and expressed as mmol Trolox equivalent (TEAC) per 100 g of dried sample (d.m.). These analyses were performed in triplicate.

2.7.4. UHPLC-ESI-MS determination of phenolic profile

Phenolic compounds were analyzed by the UHPLC-ESI-MS method, according to procedures previously described (Bonacci et al., 2023; Di Stefano et al., 2022). Two grams of each freeze-dried sample were extracted with 40 mL 80% methanol/water solution for 45 min using an ultrasonic bath. After centrifugation at 5000×*g*, the supernatant was collected and evaporated with a rotary evaporator at 45 °C and the residue was redissolved in 2 mL of methanol. The solution, containing phenolics, was filtered through 0.45 μm PTFE syringe filter into glass vials prior UHPLC-ESI-MS analysis. For quantitative determination of phenolic compounds, seven calibration curves (gallic acid, quercetin, caffeic acid, ferulic acid, protocatechuic acid, p-coumaric acid, kaempferol) in a range of concentration (0.1–10 ppm) were developed. The identification and quantification of polyphenols were performed by UHPLC-ESI-MS analysis using Q-Exactive LCq/Orbitrap MS (Thermo Fisher Scientific), interfaced with UHPLC Ultimate 3000 RS (Dionex) and equipped with HESI (Heated Electrospray Ionization) source. The chromatographic separation was achieved using a Luna C18(2) (150 mm × 2.0 mm, 5 μm stationary phase), equipped with pre-column. The column was maintained at 30 °C and the flow rate was 400 μL/min. The mobile phases solutions were H_2O with 0.1% formic acid (A) and methanol with 0.1% formic acid (B). The binary gradient separation was as follows: 0–2 min 95% A; 2–18 min 95%–5% A; 18–20 min 5% A; 20–40 min 5%–95% A. The initial conditions were maintained for 3 min to equilibrate the column. One μL of each sample was injected with a run time of 40 min. The ESI source conditions were set as follows: Spray Voltage (±): 3000.00; Capillary Temperature (±): 300.00; Sheath Gas (±): 30.00; Aux Gas (±): 15.00; S-Lens RF Level: 50.00; Ion Source: ESI. Data were collected in full-scan negative ionization modes. The scan range was set between 80 and 1000 m/z.

2.7.5. Determination of fatty acids by GC-MS analysis

The fatty acid analysis was performed according to Di Stefano et al.

(2021). In detail, 2 g of each dried sample was treated with 10 mL $CHCl_3$/MeOH (2:1) and sonicated for 50 min at 60 °C. Then, the sample was cooled to room temperature (approximatively 20–22 °C), centrifuged at 4000 g for 20 min and the pellet was subjected to three extractions. To remove water-soluble compounds a separation with a separating funnel using a H_2O/NaCl solution (0.88%) and obtained solution was evaporated. The oily residue was resuspended in 1 mL of hexane and then 100 μL of KOH/MeOH (2 M) was added and the mixture was vortexed for 3 min. Subsequently, 100 μL of the hexanic phase, containing FAMEs, 100 μL of internal standard solution (ethyl myristate 150 ppm) and 800 μL of hexane were mixed prior Gas chromatography-mass spectrometry (GC/MS) analysis to evaluate fatty acid methyl esters (FAMEs). ISQTM 9000 Quadrupole GC-MS System (Thermo Fisher Scientific) gas chromatography-mass spectrometry (GC/MS) equipped with ZB-WAX® column (30 m × 0.25 mm x 0.25 μm, film thickness; Phenomenex, Italy) was used for the determination of fatty acid methyl esters. FAMEs were identified by a mass spectra database search (NIST 2011 Mass Spectral Library) and by comparing their retention times and MS spectra with the external standard mix solution (Supelco 37 Component FAME Mix C4–C24, Sigma-Aldrich). Peak integration was carried out using XcaliburTM software (Thermo Fisher Scientific). The amount of individual fatty acids methyl esters was expressed as g/100 g of sample. Analyses were performed in triplicate. Operating conditions were as follows: ultra-high purity helium was used as carrier gas at a flow rate of 1 mL; the injector temperature was set at 210 °C. The injected volume was 5 μL with a split ratio of 10:1. The initial oven temperature was maintained at 80 °C for 3 min and increased to 300 °C at a rate of 1.5 °C/min, and then increased at a rate of 10 °C/min to 350 °C and held isothermal for 6 min. The ionization potential of the mass-selective detector was 70 eV and the scan range was 33–550 m/z. These analyses were performed in triplicate.

2.8. Analysis of volatile organic compounds emitted from cheeses

The volatile organic compounds (VOCs) of cheese samples were determined using headspace solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS). Five grams of each sample were exposed to an SPME fiber (DVB/CAR/PDMS 50 mm, Supelco) for 15 min at 60 °C under continuous stirring. The SPME fiber was thermally desorbed through a splitless GC injector at 250 °C for 1 min. A DB-624 capillary column (60 m, 0.25 mm, 1.40 μm, Agilent Technologies, Folsom, CA, USA) was used for chromatographic separation of VOC compounds. The carrier gas (helium) was set at 1 mL/min, and the oven temperature program started with a 5 min isotherm at 40 °C, followed by a 5 °C/min linear increase up to 200 °C, held at 200 °C for 2 min. SCAN mode was used for mass acquisition in the range from 40 to 400, with the interface temperature set at 230 °C. VOC identification was performed by comparing MS spectra with a commercial library (NIST05). Identified compounds were reported by normalizing GC-MS peak areas with the total area of selected peaks. These analyses were performed in triplicate.

2.9. Sensory evaluation of cheeses

A trained panel of 15 judges (comprising nine men and six women, aged between 26 and 60), assessed the sensory characteristics of both control and experimental cheeses. The evaluation followed EN ISO 22935–2:2023 guidelines. The tasting session occurred in the Laboratory of Sensory Evaluation of the Department of Agricultural, Food and Forest Sciences (University of Palermo, Italy). The laboratory is equipped with well-lit individual chambers and controlled temperature conditions. The cheeses were acclimated at room temperature (approximatively 20–22 °C) for 1 h, cut into 2 cm × 2 cm × 2 cm cubes, and randomly served on white plastic plates labeled with unique digit codes. The judges evaluated 16 sensory attributes related to appearance, aroma, taste, and texture. Each descriptor received a score on a 9-point hedonic scale using an iPad connected to the Smart Sensory Box software (Smart Sensory Solutions S.r.l., Sassari, Italy).

2.10. Statistical analyses

The data of microbial dynamics, physicochemical parameters, and sensory evaluation were analyzed using the MIXED model procedure in SAS 9.2 software (SAS Institute Inc., Campus Drive Cary, NC, USA), in which the effect of cheese trials (CTR, 1-MOLP, 2-MOLP) was the fixed factor and cheese making (2 levels as replicates) was the error term. The number of VOCs and their respective relative peak area percentage values were grouped based on their aroma profile using Agglomerative Hierarchical Clustering (AHC). Pairwise comparisons were performed with Tukey's test at a significance level of $p \leq 0.05$.

3. Results and discussion

3.1. Microbial evolution during cheese productions

Table 1 shows plate count results along the cheese production chain, from raw materials (including MOLP) to CTR and MOLP-enriched cheeses after 2 months of ripening. Notably, *L. monocytogenes* and *Salmonella* spp., responsible for food-borne infections (Authority, Prevention, & Control, 2022), were not detected in any analyzed sample and, for this reason, not included in Table 1. Despite the potential risk associated with dried plant-derived products, aromatic herbs, and spices microorganisms (FAO/WHO, 2022; Sagoo et al., 2009), the commercial MOLP used in this study showed no detectable levels of any of the microbial groups object of investigation including mesophilic coccus LAB, spoilage and pathogenic populations. This underscores the hygienic and safety suitability of MOLP for cheese production. Raw ewes' milk initially contained approximately 10^5 CFU/mL of TMM and mesophilic coccus LAB. These levels are frequently detected in raw ewes' milk used for traditional Sicilian cheeses (Gaglio et al., 2019; Todaro et al., 2024). Following NMSC addition, mesophilic coccus LAB increased to 7.04 Log CFU/mL, data consistent with previous observations by Settanni et al. (2013) in PDO Pecorino Siciliano cheese production. Undesirable bacteria, in particular *Pseudomonas* spp., total coliforms, CPS, and *E. coli*, were present at 10^2–10^3 CFU/mL in raw milk and remained relatively after NMSC inoculation. A similar behavior was previously reported by Guarcello et al. (2016). Coagulation led to a tenfold increase in microbial groups in both control and experimental curds due to whey drainage (Cardamone et al., 2018). Immediately after production, all cheeses exhibited levels of TMM and mesophilic coccus LAB at approximately 8 Log CFU/g. Over the 2-month ripening period, these microbial levels remained relatively stable in both CTR and MOLP-enriched cheeses, consistent with the typical trend observed in pressed ovine cheeses (De Pasquale et al., 2016; Randazzo et al., 2006; Todaro et al., 2024). Notably, the addition of 1% and 2% (w/w) MOLP to the curd did not adversely affect the growth and survival of the two *Lc. lactis* strains used as starter cultures. In terms of safety, by the end of the ripening period, concentrations of CPS and *E. coli* had decreased to undetectable levels, aligning with the requirements outlined in Commission Regulation 2073/2005 regarding "microbiological criteria for foodstuff".

3.2. Characterization of cheese microbiota by MiSeq illumina

A thorough investigation of the total bacterial composition in both MOLP and ripened cheese samples was conducted using DNA-based Illumina technology. This widely used culture-independent approach provides valuable insights into the microbiota associated with raw materials and processed foods (Jagadeesan et al., 2019). In our analysis, operational taxonomy units (OTUs) with a relative abundance (RA) > 0.1%, were considered abundant bacterial communities (Logares et al., 2014). These results are reported in Fig. 3. Eighteen taxonomic groups were detected, primarily at the genus level. The order Actinobacteria,

Table 1
Microbial loads of samples collected during experimental cheese productions.

Samples	Growth media					
	SMA	M17	PAB	VRBA	BP	HEA
Raw materials						
MOLP	<2 c	<1 c	<2 b	<1 b	<2 b	<2 b
RM	5.05 b	5.09 b	3.07 a	2.85 a	2.15 a	1.85 a
IM	7.13 a	7.04 a	3.08 a	2.97 a	2.21 a	1.79 a
SEM	0.749	0.742	0.364	0.345	0.260	0.218
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Curd						
CTR	7.94	7.99	4.13	3.21	3.10	2.39
1-MOLP	7.96	7.93	4.39	3.47	2.87	2.18
2-MOLP	7.99	7.91	4.22	3.38	2.95	2.24
SEM	0.050	0.044	0.045	0.055	0.043	0.058
p value	0.978	0.928	0.380	0.573	0.483	0.734
Cheese after production						
CTR	8.12	8.11	4.31	3.11	3.33	2.94
1-MOLP	8.28	8.14	4.01	3.24	3.14	2.66
2-MOLP	8.19	8.15	4.14	3.08	3.27	2.75
SEM	0.033	0.047	0.048	0.050	0.043	0.062
p value	0.540	0.985	0.325	0.734	0.592	0.603
Ripened cheese						
CTR	7.94	7.88	<2	<1	<2	<2
1-MOLP	7.83	7.95	<2	<1	<2	<2
2-MOLP	7.91	7.90	<2	<1	<2	<2
SEM	0.043	0.041	n.e.	n.e.	n.e.	n.e.
p value	0.844	0.931	n.e.	n.e.	n.e.	n.e.

Loads are reported as Log CFU/mL for milk samples, and Log CFU/g for curd. Results indicate the mean of six plate counts (carried out in duplicate for three independent productions). Abbreviations: SMA, Skim Milk Agar for total mesophilic microorganisms; M17, medium 17 agar for mesophilic coccus LAB; PAB, *Pseudomonas* agar base for pseudomonads; VRBA, violet red bile agar for total coliforms; BP, baird-parker agar for coagulase-positive staphylococci; HEA, hektoen enteric agar for *E. coli*; MOLP, *Moringa oleifera* leaves in powder form; RM, raw ewes' milk; IM, inoculated milk; CTR, control production prepared from raw ewes' milk inoculated with the NMSC without MOLP addition; 1-MOLP, experimental production prepared from raw ewes' milk inoculated with the NMSC and enriched with 1% (w/w of curd) of MOLP; 2-MOLP experimental production prepared from raw ewes' milk inoculated with the NMSC and enriched with 2% (w/w of curd) of MOLP; SEM, standard error of the mean; n.e., not evaluated. On the column: a, b, c = $p < 0.05$.

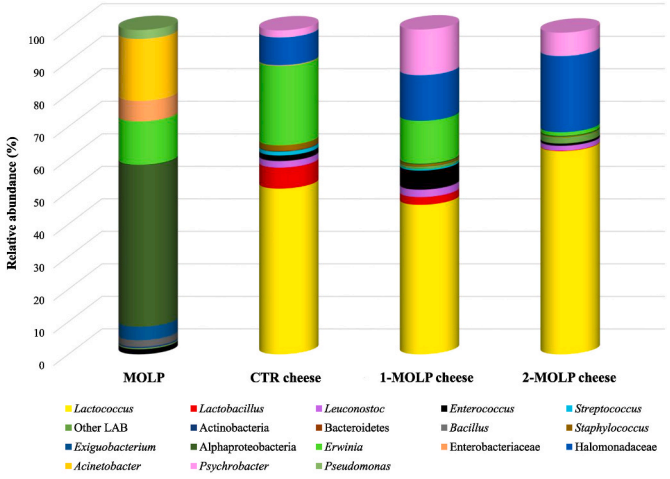

Fig. 3. Relative abundances (%) of bacteria identified by MiSeq Illumina. Abbreviations: MOLP, *Moringa oleifera* leaves in powder form; CTR, control cheese prepared without MOLP; 1-MOLP, experimental cheese enriched with 1% (w/w) of MOLP; 2-MOLP, experimental cheese enriched with 2% (w/w) of MOLP.

the class Alphaproteobacteria, the family of Enterobacteriaceae, and genera such as *Acinetobacter*, *Bacillus*, *Exiguobacterium*, *Pseudomonas*, and *Staphylococcus* were present in MOLP, but not in ripened cheeses. While the occurrence of these bacteria in MOLP is not surprising as they are commonly associated with wilted and unwilted *M. oleifera* leaf silage (Wang et al., 2018, 2019), enterococci were the sole LAB found in MOLP, with a low RA (1.56%). Their presence remained relatively stable in all cheeses, with RAs ranging from 0.71% to 5.90%. Enterococci are ubiquitous bacteria commonly found on plants and food items, including cheese (Foka et al., 2024). In all analyzed samples, the bacterial genus *Erwinia* was detected. This genus is commonly associated with the microbiota of pasture herbs (Schiavon et al., 2021). Specifically, in the MOLP sample, *Erwinia* was found at a RA of 13.35%. However, during CTR cheese production, its presence increased significantly (24.41% of RA). Interestingly, as the percentage of MOLP added to the cheese increased, the RA of *Erwinia* consistently decreased. This reduction was particularly pronounced in 2-MOLP cheese, where *Erwinia* accounted for only 1.08% of the bacterial RA. This decline is likely linked to the anti-*Erwinia* activity of *M. oleifera* leaves (Fontana et al., 2022).

The dominant bacterial group across all cheeses, regardless of MOLP addition, was *Lactococcus*. Its abundance ranged from 45.98% (in 1-MOLP cheese) to 62.48% (in 2-MOLP cheese) of the bacterial RA. Traditionally, *Lactococcus* is a key member of LAB starter cultures used in dairy production (Grujović et al., 2022). Notably, the high RA observed in ripened cheeses may be attributed to the presence of DNA from the two *Lc. lactis* strains added as fermenting agents. Similar findings were reported in PDO Silter cheese after 2 months of ripening with the addition of *Lc. lactis* starter culture (Silvetti et al., 2017). Among the non-dominant bacterial groups, enterococci, lactobacilli, leuconostocs, and streptococci were also detected in the cheese samples. Their presence is not surprising, as these bacteria are commonly found in raw ewes' milk cheeses (Yeluri Jonnala et al., 2018). The family Halomonadaceae and the genus *Psycrobacter*, known as halophilic/halotolerant bacteria (Almeida et al., 2014), were found in all cheeses. Although there is limited research on evaluating the primary dairy characteristics of these bacteria in cheese production, some authors have suggested that they may have a positive impact during ripening (Kothe et al., 2021; Wolfe et al., 2014). Notably, pathogenic bacteria such as CPS, *E. coli*, *L. monocytogenes*, and *Salmonella* spp., were never found in any cheese at the end of ripening, confirming results obtained through culture-dependent methods.

3.3. Physicochemical characterization of cheeses

Regarding the physicochemical traits of the final cheeses sampled after 2 months of ripening (Table 2), the addition of MOLP exclusively affected the dry matter and protein content. Additionally, the enrichment with MOLP influenced the TBARS value, albeit only at a trend level ($p = 0.0731$).

Interestingly, the lower dry matter value observed in 2-MOLP cheese appears to be associated with its lighter hardness. In fact, 2-MOLP cheese was more tender than the other two different cheeses. This phenomenon could be attributed to MOLP's ability to absorb water. In experimental cheeses where MOLP was used at a higher concentration, the increased moisture content likely contributed to the lower level of hardness.

The addition of MOLP to cheese resulted in an increasing trend in protein content, likely due to the high protein content of moringa. This observation aligns with similar findings reported in previous studies (El-Siddig et al., 2018; Mahami et al., 2012; Miwada et al., 2023). Regarding lipid oxidation, only the secondary oxidation index of cheese fat exhibited a decreasing trend, albeit at a tendency level, from CTR cheeses to 1-MOLP and 2-MOLP cheeses. This reduction may be attributed to the antioxidant protection provided by moringa (Barzan et al., 2024).

Statistically significant differences were observed in all color indexes. Experimental cheeses with both MOLP levels (1-MOLP and 2-MOLP) displayed lower values of L* (lightness) and a* (redness) values, along with higher b* (yellowness) values, indicating their acquisition of greenish-yellow tones. In contrast, the color indexes in CTR cheeses were comparable to those typically observed for this type of cheese (Bonanno et al., 2019; Gaglio, Barbaccia, et al., 2021).

3.4. Chemical characterization

3.4.1. Total phenolic content and anti-radical activity of raw materials and cheeses

The study investigated the total polyphenol content (TPC) and antiradical activity in both raw materials [raw ewes' milk (RM), and MOLP] and cheeses (CTR, 1-MOLP, and 2-MOLP). As shown in Table 3, the MOLP sample exhibited the highest TPC value (40.189 mg GAE/g) compared to RM (1.391 mg GAE/g d.m.). MOLP exhibited also the highest anti-radical activity, with values of 211.414 mmol TEAC/100 g d.m. (DPPH test) and 266.157 mmol TEAC/100 g d.m. (ABTS test). In contrast, RM had lower values (0.044 and 0.387 mmol TEAC/100 g d.m. for DPPH and ABTS tests, respectively). Adding MOLP to cheeses increased TPC; specifically, in 2-MOLP production, the TPC reached 3.639 mg GAE/g. The 2-MOLP cheeses showed the highest antiradical values (1.202–1.351 mmol TEAC/100 g d.m. for DPPH and ABTS, respectively). These values exceeded those of CTR cheese, which had lower antiradical potential (0.061–0.109 mmol TEAC/100 g d.m. for DPPH and ABTS, respectively). Enriching cheeses with MOLP improved their ability to counteract free radicals compared to the control cheese.

3.5. Phenolic profile of cheeses

The qualitative and quantitative analysis of the polyphenolic profile of MOLP showed numerous phenolic compounds. Notably, it contained abundant levels of chlorogenic acid (950.13 mg/100g), 4-caffeoylquinic acid (2622.61 mg/100g), 4-coumaroylquinic acid (471.51 mg/100g), quinic acid (1208.34 mg/100g), feruloylquinic acid (325.62 mg/100g), astragalin (840.69 mg/100g), kaempferol 3-O-rutinoside (991.83 mg/100g), and kaempferol malonyl glycoside (919.98 mg/100g) (Table 4). The presence of phenolic compounds in milk and cheese originates from plant and can impact their antioxidant activity (Rocchetti et al., 2023). Additionally, milk and cheeses may contain bioactive phenolic compounds, resulting from biochemical transformations by intestinal microbiota (Rocchetti et al., 2022). In the studied milk and cheese samples, phenolic compounds were relatively scarce, primarily belonging to the class of phenolic acids. However, in cheese samples enriched with MOLP, there was a proportional increase in chlorogenic acid, protocatechuic acid, caffeic acid, and ferulic acid. In particular, chlorogenic acid, initially minimal in CTR cheese (0.505 mg/100g), was significantly higher in the MOLP-enriched cheese, especially in 2-MOLP (24.309 mg/100g). Similarly, ferulic acid content increased (14.681 mg/100g) compared to CTR cheese (0.284 mg/100g). Quercetin and quercitrin, undetected in RM and CTR, were highlighted in the enriched cheeses 1-MOLP (0.035–33.405 mg/100 g d.m.) and 2-MOLP (0.155–34.794 mg/100 g d.m.) (Table 4). These findings underscore

Table 2
Physicochemical analysis of cheeses.

	Samples			SEM	*p value*
	CTR	1-MOLP	2-MOLP		
Cheese weight, kg	3.89	3.60	3.80	0.417	0.4936
Cheese yiel, g/100 g	17.39	15.91	16.88	0.617	0.3999
Cheese yield, g/100 g dry matter	10.39	9.49	9.41	0.547	0.4883
Dry matter (DM), %	60.15 a	59.04 a	55.98 b	1.718	<0.0001
Ash, % DM	7.23	7.17	7.80	0.917	0.1529
Protein, % DM	40.07 b	45.93 a	46.60 a	0.832	<0.0001
Fat, % DM	43.70	43.71	42.41	0.462	0.1027
pH	5.52	5.67	5.61	0.166	0.7353
Water activity, a_w	0.932	0.953	0.948	0.012	0.3367
POV, mEq O_2/kg fat	2.14	2.21	2.00	0.720	0.9319
TBARs, mg MDA/kg DM	0.111	0.091	0.071	0.012	0.0731
Hardness, N/mm^2	0.546 a	0.560 a	0.387 b	0.208	0.0008
Color					
Lightness L*	78.45 a	72.07 b	68.38 c	0.924	<0.0001
Redness a*	−3.814 a	−5.312 b	−5.861 b	0.219	<0.0001
Yellowness b*	12.99 b	18.62 a	20.26 a	0.844	<0.0001

Results indicate mean values of six determinations (carried out in triplicate for two independent productions). Abbreviations: CTR, control production prepared from raw ewes' milk inoculated with the NMSC without MOLP addition; 1-MOLP, experimental production prepared from raw ewes' milk inoculated with the NMSC and enriched with 1% (w/w of curd) of MOLP; 2-MOLP experimental production prepared from raw ewes' milk inoculated with the NMSC and enriched with 2% (w/w of curd) of MOLP; SEM, standard error of the mean; POV, peroxide value; TBARs, thiobarbituric acid–reactive substances; MDA, malonylaldehyde. On the column: a, b, c = $p < 0.05$.

Table 3
Antioxidant and antiradical activity of raw materials and cheese samples.

Samples	TPC (mg GAE/g DM)	DPPH (mmol TEAC/100 g DM)	ABTS (mmol TEAC/100 g DM)
Raw materials			
MOLP	40.189 b	211.414 b	266.157 b
RM	1.391 a	0.044 a	0.387 a
SEM	8.713	33.432	55.453
p value	0.0003	<0.0001	<0.0001
Ripened cheese			
CTR	1.276 a	0.061 a	0.109 a
1-MOLP	2.697 b	0.992 b	0.813 b
2-MOLP	3.639 c	1.202 c	1.351 c
SEM	0.349	0.124	0.180
p value	<0.0001	<0.0001	<0.0001

Results indicate mean values of six determinations (carried out in triplicate for two independent productions). Abbreviations: TPC, total phenolic content; MOLP, *Moringa oleifera* leaves in powder form; RM, raw ewes' milk; CTR, control production prepared from raw ewes' milk inoculated with the NMSC without MOLP addition; 1-MOLP, experimental production prepared from raw ewes' milk inoculated with the NMSC and enriched with 1% (w/w of curd) of MOLP; 2-MOLP experimental production prepared from raw ewes' milk inoculated with the NMSC and enriched with 2% (w/w of curd) of MOLP; SEM, standard error of the mean. On the column: a, b, c = $p < 0.05$.

Table 4
Polyphenolic profile of raw materials and cheese samples.

Items	Raw materials		*p* value	SEM	Ripened cheese			*p* value	SEM
	MOLP	RM			CTR	1-MOLP	2-MOLP		
Chlorogenic acid	942.977 b	1.048 a	<0.0001	210.635	0.505 a	22.468 b	24.309 b	<0.0001	3.826
Protocatechuic acid	7.917 b	2.645 a	<0.0001	1.185	0.405 a	0.480 b	0.734 c	<0.0001	0.050
Caffeic acid	13.700 b	1.146 a	<0.0001	2.808	1.186 a	2.269 b	3.142 c	<0.0001	0.284
Ferulic acid	18.780 b	3.360 a	<0.0001	3.450	0.284 a	11.246 b	14.681 c	<0.0001	2.172
Quercetin	2.917	n.d.	n.e.	n.e.	n.d.	0.038 a	0.145 b	<0.0001	0.024
Quercitrin	17.110	n.d.	n.e.	n.e.	n.d.	0.806 a	1.676 b	<0.0001	0.196
4-Caffeoylquinic acid	2622.61	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
4-Coumaroylquinic acid	471.512	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
Feruloylquinic acid	325.623	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
Gallic acid	2.432	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
Quinic acid	1208.341	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
p-Coumaric acid	3.962	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
Quercetin malonyl glucoside	489.243	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
Vitexin	312.936	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
Quercetin 3-O-glucoside	4.591	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
Rutin	421.112	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
Astragalin	840.691	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
Kaempferol	19.622	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
Kaempferol 3-O-rutinoside	991.834	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
kaempferol acetyl glycoside	528.031	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.
Kaempferol malonyl glycoside	919.985	n.d.	n.e.	n.e.	n.d.	n.d.	n.d.	n.e.	n.e.

Results as expressed in mg/100g of DM and indicate mean values of six determinations (carried out in triplicate for two independent productions). Abbreviations: MOLP, *Moringa oleifera* leaves in powder form; RM, raw ewes' milk; CTR, control production prepared from raw ewes' milk inoculated with the NMSC without MOLP addition; 1-MOLP, experimental production prepared from raw ewes' milk inoculated with the NMSC and enriched with 1% (w/w of curd) of MOLP; 2-MOLP experimental production prepared from raw ewes' milk inoculated with the NMSC and enriched with 2% (w/w of curd) of MOLP; SEM, standard error of the mean; n.d., not detected; n.e., not evaluated. On the row: a, b, c = $p < 0.05$.

the contribution of MOLP to the phenolic composition of cheeses.

Research on the phenolic profile of vegetable matrix-enriched cheeses is limited. When compared to the plant matrix (MOLP), fortified cheese samples experience a significant loss of polyphenols. This loss is attributed to phenolic compounds interacting with proteins, especially proline-rich proteins like caseins (Li et al., 2021; Spencer et al., 1988). The flexible secondary structure and increased hydrogen bonding of these proteins enhance their interaction with phenolic compounds. Cheese processing may alter phenolic compound solubility, resulting in some losses. Phenolic acids found in enriched cheeses render various biological activities, including antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, antihypertensive, antiaging effects, and regulation of glucose and lipids metabolism. The proposed functional product, ovine cheese enriched with MOLP rich in phenolic compounds, aims to prevent lifestyle disorders related to lipid metabolism and enhance antiradical activity (Kiokias & Oreopoulou, 2021; Kumar & Goel, 2019; Meng et al., 2013).

3.6. Fatty acid composition of cheeses

The composition of fatty acids in raw materials (RM and MOLP) and cheese samples (CTR, 1-MOLP, 2-MOLP) was analyzed and expressed as

Table 5
Fatty acids composition of raw materials and cheese samples.

Items		Raw materials		*p* value	SEM				*p* value	SEM
		MOLP	RM			CTR	1-MOLP	2-MOLP		
Hexanoic acid	C6:0	6.70 b	1.92 a	<0.0001	1.08	2.00 b	1.42 a	1.30 a	0.017	0.13
Octanoic acid	C8:0	n.d.	2.27	n.e.	n.e.	2.30 b	1.48 a	1.23 a	0.005	0.18
Decanoic acid	C10:0	n.d.	5.37	n.e.	n.e.	8.63 b	4.32 a	3.51 a	0.0001	0.82
Lauric acid	C12:0	n.d.	4.73	n.e.	n.e.	5.10 a	3.89 a	4.62 a	0.146	0.26
Miristic acid	C14:0	n.d.	11.89	n.e.	n.e.	13.47 b	10.06 a	10.28 a	0.003	0.60
Pentadecanoic acid	C15:1	34.44 b	5.34 a	<0.0001	6.51	5.40 a	7.54 b	8.57 b	0.0004	0.48
Palmitoleic acid	C16:1	n.d.	1.71	n.e.	n.e.	1.25 a	1.90 b	1.93 b	0.001	0.11
Palmitic acid	C16:0	18.64 a	20.71 b	0.045	0.56	21.61 a	22.79 a	24.67 b	0.002	0.48
Heptadecanoic acid	C17:0	n.d.	0.87	n.e.	n.e.	1.41 b	0.75 a	0.82 a	0.0001	0.11
γ-Linolenic acid	C18:3	6.70 b	1.28 a	0.0004	1.23	1.29 a	2.23 a	2.24 a	0.118	0.22
Oleic acid	C18:1	19.21 a	22.26 b	0.002	0.71	19.02 a	20.80 ab	24.22 b	0.016	0.88
Stearic acid	C18:0	13.36 b	10.07 a	0.001	0.75	14.02 a	16.17 b	16.03 b	0.011	0.39
Arachidic acid	C20:0	n.d.	0.45	n.e.	n.e.	0.52 a	0.49 a	0.51 a	0.769	0.01
Eicosanoic acid	C20:1	n.d.	0.33	n.e.	n.e.	0.25 a	0.29 ab	0.30 b	0.034	0.01
Eicosadienoic acid	C20:2	5.94 b	0.64 a	<0.0001	1.19	0.33 a	0.34 a	0.39 a	0.181	0.01
Eicosatetranoic acid	C20:4	8.88 b	0.77 a	<0.0001	1.81	0.24 a	0.37 b	0.39 b	0.019	0.03
Erucic acid	C22:1	n.d.	0.63	n.e.	n.e.	0.71 b	0.37 a	0.44 a	0.001	0.05
Behenic acid	C22:0	n.d.	0.36	n.e.	n.e.	0.75 b	0.38 a	0.48 a	0.001	0.06
Nervonic acid		2.80	n.d	n.e.	n.e.	n.d	0.89 a	0.91 a	0.542	0.13

Results as expressed in mg/g of DM and indicate mean values of six determinations (carried out in triplicate for two independent productions). Abbreviations: MOLP, *Moringa oleifera* leaves in powder form; RM, raw ewes' milk; CTR, control production prepared from raw ewes' milk inoculated with the NMSC without MOLP addition; 1-MOLP, experimental production prepared from raw ewes' milk inoculated with the NMSC and enriched with 1% (w/w of curd) of MOLP; 2-MOLP experimental production prepared from raw ewes' milk inoculated with the NMSC and enriched with 2% (w/w of curd) of MOLP; SEM, standard error of the mean; n.d., not detected. On the row: a, b = $p < 0.05$.

relative percentages (%) (Table 5). Raw ewes' milk samples contained numerous fatty acids, especially saturated ones, which were present in higher concentrations in all cheese samples. In MOLP-enriched cheeses, short-chain fatty acids (C6:0, C8:0, C10:0 and C12:0) decreased compared to CTR cheese. Palmitic and stearic fatty acids significantly increased in enriched cheeses. Notably affected by higher enrichment (2-MOLP) were oleic (24.22%), pentadecanoic (8.57%), and palmitoleic acids (1.93%), relative to CTR (19.02%, 5.40%, 1.25%). Oleic acid, known for its anti-cancer and anti-atherogenic properties, is beneficial in daily diets (Hanuš et al., 2018). Additionally, MOLP contained nine fatty acids, including three polyunsaturated ones (γ-linolenic, eicosadienoic, and eicosatetranoic acids) at discrete concentrations (6.70%, 5.94% and 8.88%, respectively). Long-chain polyunsaturated fatty acids (PUFA) are typically not synthesized by ruminant tissues and depend on animal feeding practices (Chilliard et al., 2000; Nudda et al., 2014; Vargas-Bello-Pérez et al., 2013).

The fatty acid composition of RM and cheese (CTR) aligned with literature data (Park et al., 2007; Paszczyk & Łuczyńska, 2020). E.g., the values reported in the study of Goudjil et al. (2004) were similar to those in Table 5. Specifically, sheep's milk contained abundant myristic (10.4%), palmitic (25.9%), oleic (21.1%), and stearic (9.57%) acids, consistent with RM sample. Nervonic acid from MOLP was detected only in enriched cheeses (Kumar et al., 2022). The presence of moringa powder, as highlighted in Tables 5 and in 1-MOLP and 2-MOLP increased the relative percentage of unsaturated fatty acids compared to CTR. In the enriched cheese samples, there was a noticeable increase in γ-linolenic acid due to the contribution of MOLP, which was proportional to the percentage added eicosadienoic acid, eicosatetranoic acid and γ-linoleic acid, for example, play a significant role from a nutritional point of view, thanks to their hypocholesterolemic and anti-inflammatory action (in particular against the "bad" LDL cholesterol). In addition, ω-6 and ω-3 PUFAs compete in metabolic pathways that influence cellular responses to physiological stress and constitute the substrates for the synthesis of eicosanoids (prostaglandins, leukotrienes, thromboxanes and lipoxins) (Shinagawa et al., 2015). For this reason, the contribution of moringa powder to cheeses is very important, because it allows to increase the nutritional role of ovine's cheese. Also, in presence of other plant-derived materials, sheep's cheese enrichment increased the level of bioactive compounds present in the functional food. Enriching sheep's cheese with *Portulaca oleracea* seed oil also increased omega-6 fatty acid content proportionally to the enrichment level (Keyvani & Bolandi, 2015).

3.7. Volatile organic compounds profiles of cheeses

VOC profiles generated from cheese samples were determined using the SPME-GC–MS technique. Fig. 4 illustrates the VOCs emitted from cheese samples, both with and without MOLP additions. In the CTR production, a total of 20 compounds were detected, belonging to classes such as acids, ketones, aldehydes, alcohols, and esters. Notably, free fatty acids constituted the main class of VOCs in the cheese samples. Among these, hexanoic acid and butyric acid were the most abundant, followed by acetic acid. These compounds directly and indirectly contribute to cheese flavor formation. For instance, hexanoic acid provides a sour note, butyric acid imparts a cheesy flavor, and acetic acid contributes vinegar and acidic notes (McSweeney & Sousa, 2000). Additionally, they serve as precursors to odor-active compounds. Other compounds detected included alcohols (1-butanol-3-methyl), aldehydes (hexenal and heptanal), and esters (ethyl hexanoate).

The volatile composition observed in the control cheese aligns with the profile typically found in cheeses produced from sheep's milk, as reported in various studies (Busetta et al., 2023; Gaglio, Barbaccia, et al., 2021, 2024; Kırmacı et al., 2015). However, when MOLP was added to the cheese, 24 additional VOCs were emitted. These included

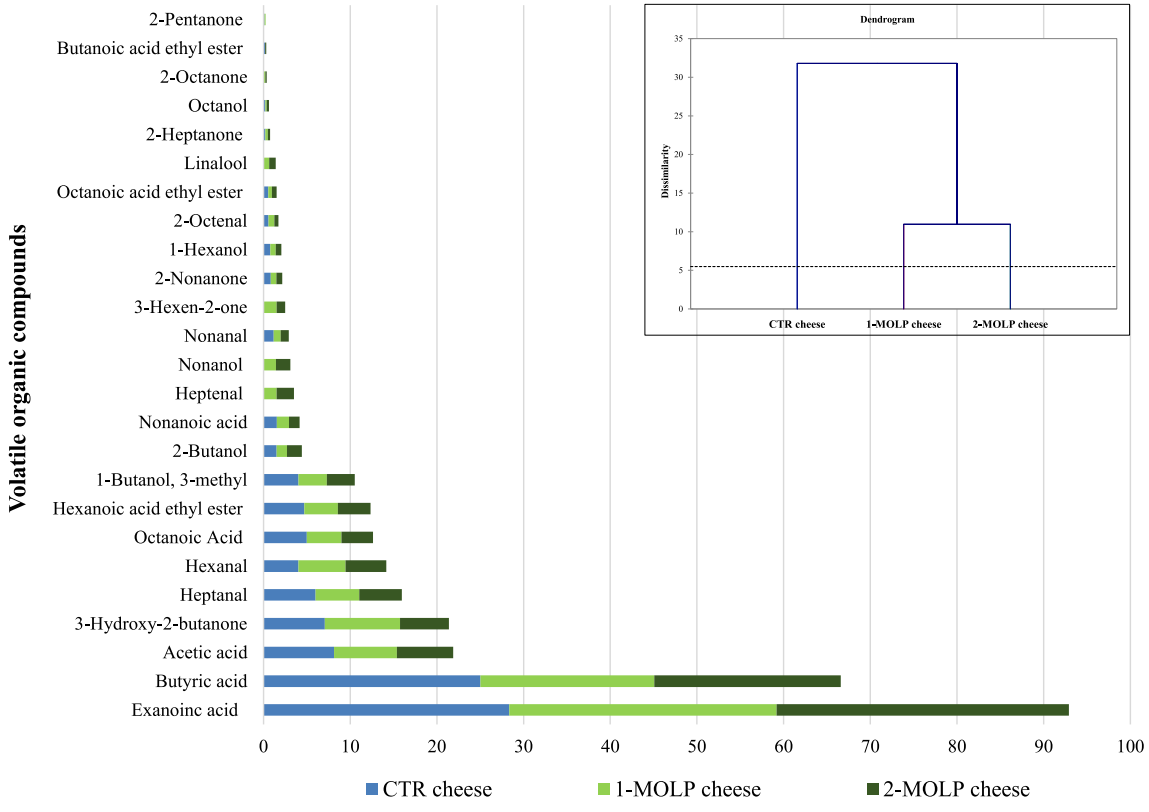

Fig. 4. Distribution and clustering of volatile organic compounds in cheeses. Abbreviations: CTR, control cheese prepared without *Moringa oleifera* leaves in powder form (MOLP); 1-MOLP, experimental cheese enriched with 1% (w/w) of MOLP; 2-MOLP, experimental cheese enriched with 2% (w/w) of MOLP.

compounds such as 2-octanone, 3-hexen-2-one, heptane, nonanol, and linalool. Interestingly, these compounds have also been identified in *M. oleifera*-derived products (Ismael et al., 2016; Li et al., 2023; Mukunzi et al., 2011). It is however, only a few studies on the volatile composition of moringa leaves are available, and the data vary significantly depending on geographical origin (Mukunz et al., 2011). It's worth noting that the exclusive compounds detected in the MOLP-added cheeses constituted less than 6% of the total aromatic profile. The slight variations in VOC profiles may be attributed to the low percentage of MOLP added (1%–2%). Other studies reported similar trends even with higher addition rates (Ismael et al., 2016). However, the impact of MOLP on VOCs emitted from cheese needs to be further investigated.

3.8. Sensory evaluation of cheeses

The sensory evaluation of MOLP-enriched cheeses was analyzed using a radar chart. The chart depicted various sensory attributes, comparing CTR cheese with experimental cheese productions (Fig. 5). While it is well-known that incorporating fruit and vegetable by-products affects dairy product sensory characteristics (Costa et al., 2018), the comparison between CTR and MOLP-enriched cheeses did not reveal statistically significant differences ($p > 0.05$) for most evaluated attributes. The addition of different MOLP percentages (1% and 2% w/w) significantly affected specific attributes, including color, intensity of odor, milk odor, butter odor, and bitterness. Notably, these differences increased with higher MOLP levels, confirming the trend reported by Moneeb et al. (2024) for cow soft cheeses enriched with 0.5%, 0.75%, and 1.5% of MOLP. This effect is mainly due to the bitter taste and herbal flavor of moringa (Zhang et al., 2019). The most significant difference was observed in color. The green tonality increased from a score of 5.01 for CTR cheese to 7.16 for 2-MOLP cheese. Bourekoua et al. (2018) attributed this enhanced green color to the high chlorophyll content in MOLP. In terms of overall evaluation, both CTR and 1-MOLP cheeses received similar scores and were highly appreciated by panelists. However, the sensory acceptance of 2-MOLP cheese was negatively affected, confirming previous results published by Bermudez-Beltrán et al. (2020).

4. Conclusion

Incorporating MOLP into Pecorino cheese at 1% and 2% (w/w) concentrations had no impact on the *Lc. lactis* fermenting agents. Both MOLP and final cheeses were free from spoilage and pathogenic microorganisms. MOLP-enriched cheeses exhibited unique properties: increased protein and phenolic content, enhanced anti-radical activity, higher oleic acid levels, and reduced secondary lipid oxidation. Surprisingly, sensory evaluation found little difference between MOLP-enriched and control cheeses. These findings suggest that these dairy products may mitigate lifestyle disorders and offer opportunities in the functional foods market.

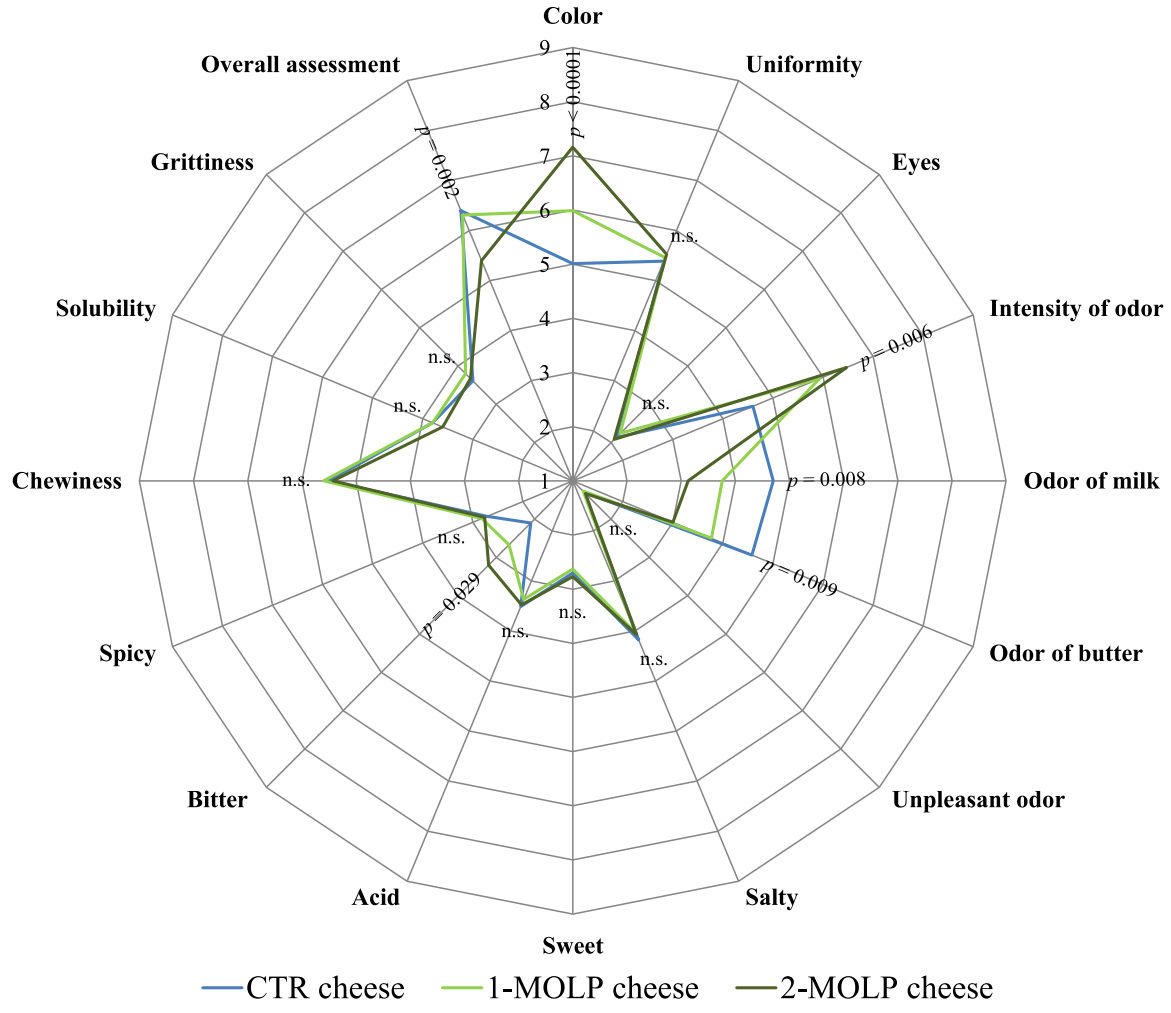

Fig. 5. Radar chart of the descriptive sensory assessment of cheeses. Abbreviations: CTR, control cheese prepared without *Moringa oleifera* leaves in powder form (MOLP); 1-MOLP, experimental cheese enriched with 1% (w/w) of MOLP; 2-MOLP, experimental cheese enriched with 2% (w/w) of MOLP; n.s., not significant.

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CRediT authorship contribution statement

Giuliana Garofalo: Software, Investigation, Formal analysis, Data curation. **Carla Buzzanca:** Software, Investigation, Formal analysis, Data curation. **Marialetizia Ponte:** Software, Investigation, Formal analysis, Data curation. **Marcella Barbera:** Writing – original draft, Formal analysis, Data curation. **Angela D'Amico:** Software, Investigation, Formal analysis, Data curation. **Carlo Greco:** Resources, Project administration, Funding acquisition. **Michele Massimo Mammano:** Resources, Project administration, Funding acquisition. **Elena Franciosi:** Software, Investigation, Formal analysis. **Daniela Piazzese:** Methodology. **Valeria Guarrasi:** Software. **Salvatore Ciulla:** Project administration. **Santo Orlando:** Software. **Antonino Di Grigoli:** Methodology, Conceptualization. **Adriana Bonanno:** Writing – original draft, Validation, Methodology. **Vita Di Stefano:** Writing – original draft, Validation, Methodology. **Luca Settanni:** Writing – review & editing. **Raimondo Gaglio:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

References

Alegbeleye, O. O. (2018). How functional is *Moringa oleifera*? A review of its nutritive, medicinal, and socioeconomic potential. *Food and Nutrition Bulletin, 39*, 149–170. https://doi.org/10.1177/0379572117749814

Almeida, M., Hébert, A., Abraham, A. L., Rasmussen, S., Monnet, C., Pons, N., Delbes, C., Loux, V., Batto, J. M., Leonard, P., Kennedy, S., Ehrlich, S. D., Pop, M., Montel, M. C., Irlinger, F., & Renault, P. (2014). Construction of a dairy microbial genome catalog opens new perspectives for the metagenomic analysis of dairy fermented products. *BMC Genomics, 15*, 1–16. http://www.biomedcentral.com/1471-2164/15/1101.

Authority, E. F. S., Prevention, E., & C.f. D., & Control. (2022). The European Union one health 2021 zoonoses report. *EFSA Journal, 20*, Article e07666.

Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S. F., & Nabavi, S. M. (2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological Research, 196*, 44–68. https://doi.org/10.1016/j.micres.2016.12.003

Barzan, G., Sacco, A., Giovannozzi, A. M., Portesi, C., Schiavone, C., Salafranca, J., Wrona, M., Nerín, C., & Rossi, A. M. (2024). Development of innovative antioxidant food packaging systems based on natural extracts from food industry waste and *Moringa oleifera* leaves. *Food Chemistry, 432*, 137088. https://doi.org/10.1016/j.foodchem.2023.137088

Bermudez-Beltrán, K. A., Marzal-Bolaño, J. K., Olivera-Martínez, A. B., & Espitia, P. J. (2020). Cape gooseberry Petit Suisse cheese incorporated with moringa leaf powder and gelatin. *LWT - Food Science and Technology, 123*, 109101. https://doi.org/10.1016/j.lwt.2020.109101

Bonacci, S., Di Stefano, V., Sciacca, F., Buzzanca, C., Virzì, N., Argento, S., & Melilli, M. G. (2023). Hemp flour particle size affects the quality and nutritional profile of the enriched functional pasta. *Foods, 12*(774). https://doi.org/10.3390/foods12040774

Bonanno, A., Di Grigoli, A., Vitale, F., Di Miceli, G., Todaro, M., Alabiso, M., Gargano, M. L., Venturella, G., Anike, F. N., & Isikhuemhen, O. S. (2019). Effects of feeding diets supplemented with medicinal mushrooms myceliated grains on some production, health and oxidation traits of dairy ewes. *International Journal of Medicinal Mushrooms, 21*, 89–103. https://doi.org/10.1615/IntJMedMushrooms.2018029327

Bourekoua, H., Różyło, R., Gawlik-Dziki, U., Benatallah, L., Zidoune, M. N., & Dziki, D. (2018). Evaluation of physical, sensorial, and antioxidant properties of gluten-free bread enriched with *Moringa oleifera* leaf powder. *European Food Research and Technology, 244*, 189–195. https://doi.org/10.1007/s00217-017-2942-y

Busetta, G., Garofalo, G., Barbera, M., Di Trana, A., Claps, S., Lovallo, C., Franciosi, E., Gaglio, R., & Settanni, L. (2023). Metagenomic, microbiological, chemical and sensory profiling of Caciocavallo Podolico Lucano cheese. *Food Research International, 169*, 112926. https://doi.org/10.1016/j.foodres.2023.112926

Cardamone, C., Di Noto, A. M., Mancuso, I., Sciortino, S., & Scatassa, M. L. (2018). Behavior of *Escherichia coli* O157 during the manufacture and ripening of a traditional sicilian raw ewes'milk cheese. *Carpathian Journal of Food Science & Technology, 10*.

Chilliard, Y., Ferlay, A., Mansbridge, R. M., & Doreau, M. (2000). Ruminant milk fat plasticity: Nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. *Annales de Zootechnie, 49*, 181–205. https://doi.org/10.1051/animres:2000117

CIE. (1986). *Colorimetry. Publication CIE 15.2* (2nd ed.). Wien: Austria: Commission International de l'Éclairage.

Costa, C., Lucera, A., Marinelli, V., Del Nobile, M. A., & Conte, A. (2018). Influence of different by-products addition on sensory and physicochemical aspects of Primosale cheese. *Journal of Food Science and Technology, 55*, 4174–4183. https://doi.org/10.1007/s13197-018-3347-z

Dalei, J., Rao, V. M., Sahoo, D., Rukmini, M., & Ray, R. (2016). Review on nutritional and pharmacological potencies of. *Moringa oleifera. European Journal of Medical Research, 3*, 150–155.

De Pasquale, I., Di Cagno, R., Buchin, S., De Angelis, M., & Gobbetti, M. (2016). Spatial distribution of the metabolically active microbiota within Italian PDO ewes' milk cheeses. *PLoS One, 11*. https://doi.org/10.1371/journal.pone.0153213

Di Stefano, V., Bongiorno, D., Buzzanca, C., Indelicato, S., Santini, A., Lucarini, M., & Durazzo, A. (2021). Fatty acids and triacylglycerols profiles from Sicilian (cold pressed vs. soxhlet) grape seed oils. *Sustainability, 13*, 13038. https://doi.org/10.3390/su132313038

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

Di Stefano, V., Buzzanca, C., Ruvutuso, F., Scuderi, D., Palazzolo, E., Gugliuzza, G., Tinebra, I., & Farina, V. (2023). Chemical composition and anti-radical properties of coffee cherry cultivated in Mediterranean climate. *Food Bioscience, 56*, 103349. https://doi.org/10.1016/j.fbio.2023.103349

El-Siddig, E. E., Abdelgadir, W. S., Kabeir, B. M., Koko, M. Y., & Ibrahim, R. A. (2018). Quality of white cheese made using *Moringa oleifera* leaf extract. *Journal of Academia and Industrial Research, 7*, 7.

Falowo, A. B., Mukumbo, F. E., Idamokoro, E. M., Lorenzo, J. M., Afolayan, A. J., & Muchenje, V. (2018). Multi-functional application of *Moringa oleifera* Lam. In nutrition and animal food products: A review. *Food Research International, 106*, 317–334. https://doi.org/10.1016/j.foodres.2017.12.079

FAO/WHO. (2022). Microbiological hazards in spices and dried aromatic herbs: Meeting report. In *Food & agriculture organization, 27*. Geneva, Switzerland: World Health Organization.

Foka, F. E. T., Manganyi, M. C., Kumar, A., & Mufhandu, H. T. (2024). Enterococci in the food industry: Friends or foes? In R. P. Singh, G. Manchanda, S. Sarsan, A. Kumar, & H. Panosyan (Eds.), *Microbial essentialism* (pp. 87–112). Massachusetts: Academic Press.

Fontana, R., Macchi, G., Caproni, A., Sicurella, M., Buratto, M., Salvatori, F., Pappadà, M., Manfredini, S., Baldisserotto, A., & Marconi, P. (2022). Control of *Erwinia amylovora* growth by *Moringa oleifera* leaf extracts: In vitro and in planta effects. *Plants, 11*(957). https://doi.org/10.3390/plants11070957

Gaglio, R., Barbaccia, P., Barbera, M., Restivo, I., Attanzio, A., Maniaci, G., Di Grigoli, A., Francesca, N., Tesoriere, L., Bonanno, A., Moschetti, G., & Settanni, L. (2021). The use of winery by-products to enhance the functional aspects of the fresh ovine "Primosale" cheese. *Foods, 10*(461). https://doi.org/10.3390/foods10020461

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

Gaglio, R., Franciosi, E., Todaro, A., Guarcello, R., Alfeo, V., Randazzo, C. L., Settanni, L., & Todaro, M. (2020). Addition of selected starter/non-starter lactic acid bacterial inoculums to stabilise PDO pecorino siciliano cheese production. *Food Research International, 136*, 109335. https://doi.org/10.1016/j.foodres.2020.109335

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

Gaglio, R., La Rosa, L., Serio, G., Mannino, G., Alfonzo, A., Franciosi, E., Settanni, L., & Gentile, C. (2023). *Rubus idaeus* by-products: Sustainable improvement of the antioxidant value of sourdough bread by a new end-use of exhausted seeds still containing bioactive compounds. *Innovative Food Science & Emerging Technologies, 103517*. https://doi.org/10.1016/j.ifset.2023.103517

Gaglio, R., Restivo, I., Barbera, M., Barbaccia, P., Ponte, M., Tesoriere, L., Bonanno, A., Attanzio, A., Di Grigoli, A., Francesca, N., Moschetti, G., & Settanni, L. (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

Gaglio, R., Todaro, M., Scatassa, M. L., Franciosi, E., Corona, O., Mancuso, I., Di Gerlando, R., Cardamone, C., & Settanni, L. (2019). Transformation of raw ewes' milk applying "Grana" type pressed cheese technology: Development of extra-hard

"Gran Ovino" cheese. *International Journal of Food Microbiology, 307*, 108277. https://doi.org/10.1016/j.ijfoodmicro.2019.108277

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

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

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness, 5*, 49–56. https://doi.org/10.1016/j.fshw.2016.04.001

Goudjil, H., Fontecha, J., Luna, P., de La Fuente, M. A., Alonso, L., & Juárez, M. (2004). Quantitative characterization of unsaturated and trans fatty acids in Ewe's milk fat. *Le Lait, 84*, 473–482. https://doi.org/10.1051/lait:2004017

Grujović, M.Ž., Mladenović, K. G., Semedo-Lemsaddek, T., Laranjo, M., Stefanović, O. D., & Kocić-Tanackov, S. D. (2022). Advantages and disadvantages of non-starter lactic acid bacteria from traditional fermented foods: Potential use as starters or probiotics. *Comprehensive Reviews in Food Science and Food Safety, 21*, 1537–1567. https://doi.org/10.1111/1541-4337.12897

Guarcello, R., Carpino, S., Gaglio, R., Pino, A., Rapisarda, T., Caggia, C., Marino, G., Randazzo, C. L., Settanni, L., & Todaro, M. (2016). A large factory-scale application of selected autochthonous lactic acid bacteria for PDO Pecorino Siciliano cheese production. *Food Microbiology, 59*, 66–75. https://doi.org/10.1016/j.fm.2016.05.011

Hanuš, O., Samková, E., Křížová, L., Hasoňová, L., & Kala, R. (2018). Role of fatty acids in milk fat and the influence of selected factors on their variability—a review. *Molecules, 23*(1636). https://doi.org/10.3390/molecules23071636

IDF. (1964a). *Determination of the ash content of processed cheese products. FIL-IDF 27*. Brussels: Belgium: International Dairy Federation Standard.

IDF. (1964b). *Determination of the protein content of processed cheese products. FIL-IDF 25*. Brussels: Belgium: International Dairy Federation Standard.

IDF. (1982). *Cheese and processed cheese product. Determination of the total solids content. FIL-IDF 4A*. Brussels: Belgium: International Dairy Federation Standard.

IDF. (1986). *Cheese and processed cheese product. Determination of fat content-gravimetric method. FIL-IDF 5B*. Brussels: Belgium: International Dairy Federation Standard.

IDF. (1991). *Determination of the peroxide Value.74A*. Brussels: Belgium: International Dairy Federation Standars.

Ismael, S., Farahat, A., Ebrahim, Y., Gohari, S., & Ibrahim, G. (2016). Fortification effect of *Moringa oleifera* leaves powder on nutritional and volatile compounds of sweet whey beverage. *Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences, 3*, 1–11.

Jagadeesan, B., Gerner-Smidt, P., Allard, M. W., Leuillet, S., Winkler, A., Xiao, Y., Chaffron, S., Van Der Vossen, J., Tang, S., Katase, M., McClure, P., Kimura, B., Chai, C. L., Chapman, J., & Grant, K. (2019). The use of next generation sequencing for improving food safety: Translation into practice. *Food Microbiology, 79*, 96–115. https://doi.org/10.1016/j.fm.2018.11.005

Keyvani, M., & Bolandi, M. (2015). Physicochemical and organoleptic properties of Lighvan cheese fortified with *Protulaca oleracea* seed oil. *Journal of Chemical Health Risks, 5*, 21–27.

Kiokias, S., & Oreopoulou, V. (2021). A review of the health protective effects of phenolic acids against a range of severe pathologic conditions (including coronavirus-based infections). *Molecules, 26*(5405). https://doi.org/10.3390/molecules26175405

Kırmacı, H. A., Hayaloğlu, A. A., Özer, H. B., Atasoy, A. F., & Levent, O. K. A. N. (2015). Effects of wild-type starter culture (artisanal strains) on volatile profile of urfa cheese made from Ewe milk. *International Journal of Food Properties, 18*, 1915–1929. https://doi.org/10.1080/10942912.2014.942782

Kothe, C. I., Bolotin, A., Kraïem, B. F., Dridi, B., Team, F. M., & Renault, P. (2021). Unraveling the world of halophilic and halotolerant bacteria in cheese by combining cultural, genomic and metagenomic approaches. *International Journal of Food Microbiology, 358*, 109312. https://doi.org/10.1016/j.ijfoodmicro.2021.109312

Kumar, N., & Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports, 24*, Article e00370. https://doi.org/10.1016/j.btre.2019.e00370

Kumar, G. P., Kumar, P. P., Jain, B., & Jain, U. (2022). Extraction of *Moringa oleifera* oil by cold press method and its physiochemical analysis. *Journal of Academia and Industrial Research, 10*, 30–33.

Li, M., Ritzoulis, C., Du, Q., Liu, Y., Ding, Y., Liu, W., & Liu, J. (2021). Recent progress on protein-polyphenol complexes: Effect on stability and nutrients delivery of oil-in-water emulsion system. *Frontiers in Nutrition, 8*, 765589. https://doi.org/10.3389/fnut.2021.765589

Li, X., Shi, C., Wang, S., Wang, S., Wang, X., & Lü, X. (2023). Uncovering the effect of *Moringa oleifera* Lam. leaf addition to Fuzhuan Brick tea on sensory properties, volatile profiles and anti-obesity activity. *Food & Function, 14*, 2404–2415. https://doi.org/10.1039/d2fo03531f

Lin, M., Zhang, J., & Chen, X. (2018). Bioactive flavonoids in *Moringa oleifera* and their health-promoting properties. *Journal of Functional Foods, 47*, 469–479. https://doi.org/10.1016/j.jff.2018.06.011

Lisak Jakopović, K., Repajić, M., Rumora Samarin, I., Božanić, R., Blažić, M., & Barukčić Jurina, I. (2022). Fortification of cow milk with *Moringa oleifera* extract: Influence on physicochemical characteristics, antioxidant capacity and mineral content of yoghurt. *Fermentation, 8*(545). https://doi.org/10.3390/fermentation8100545

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

Mahami, T., Ocloo, F. C. K., Odonkor, S. T., Owulah, C., & Coffie, S. A. (2012). Preliminary study on the influence of moringa seed extracts supplementation on the yield and quality of cottage cheese. *International Journal of Recent Trends in Science and Technology, 2*, 4–8.

Matic, I., Guidi, A., Kenzo, M., Mattei, M., & Galgani, A. (2018). Investigation of medicinal plants traditionally used as dietary supplements: A review on *Moringa oleifera*. *Journal of Public Health in Africa, 9*, 191–199. https://doi.org/10.4081/jphia.2018.841

Mbikay, M. (2012). Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: A review. *Frontier in Pharmacology, 3*(24). https://doi.org/10.3389/fphar.2012.00024

McSweeney, P. L., & Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. *Le Lait, 80*, 293–324. https://doi.org/10.1051/lait:2000127

Meng, S., Cao, J., Feng, Q., Peng, J., & Hu, Y. (2013). Roles of chlorogenic acid on regulating glucose and lipids metabolism: A review. *Evidence-based Complementary and Alternative Medicine, 2013*, 801457. https://doi.org/10.1155/2013/801457

Miwada, I. N. S., Sutama, I. N. S., & Susilo, A. (2023). Antioxidant activity, texture attributes, and hedonic properties of fortified cheese with moringa leaf extract. *Advances in Animal and Veterinary Sciences, 11*, 1875–1880. https://doi.org/10.17582/journal.aavs/2023/11.11.1875.1880

Moneeb, A. H., Moawad, R., & Osman, D. M. (2024). Characteristics of soft cheese fortified by *Moringa oleifera* and *Mentha piperita* leaves. *Assiut Journal of Agricultural Sciences, 55*, 55–67. https://doi.org/10.21608/ajas.2024.280272.1351

Mukunzi, D., Nsor-Atindana, J., Xiaoming, Z., Gahungu, A., Karangwa, E., Mukamurezi, G., Al-Domi, H., Princewill-Ogbonna, I. L., Ogbonna, P. C., & Arief, N. J. (2011). Comparison of volatile profile of *Moringa oleifera* leaves from Rwanda and China using HS-SPME. *Pakistan Journal of Nutrition, 10*, 602–608.

Nudda, A., Battacone, G., Boaventura, O., Cannas, A., Francesconi, A. H. D., Atzori, A. S., & Pulina, G. (2014). Feeding strategies to design the fatty acid profile of sheep milk and cheese. *Revista Brasileira de Zootecnia, 43*, 445–456. https://doi.org/10.1590/S1516-35982014000800008

Oyeyinka, A. T., & Oyeyinka, S. A. (2018). *Moringa oleifera* as a food fortificant: Recent trends and prospects. *Journal of the Saudi Society of Agricultural Sciences, 17*, 127–136. https://doi.org/10.1016/j.jssas.2016.02.002

Park, Y. W., Juárez, M., Ramos, M., & Haenlein, G. F. W. (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research, 68*, 88–113. https://doi.org/10.1016/j.smallrumres.2006.09.013

Paszczyk, B., & Łuczyńska, J. (2020). The comparison of fatty acid composition and lipid quality indices in hard cow, sheep, and goat cheeses. *Foods, 9*(1667). https://doi.org/10.3390/foods9111667

Randazzo, C. L., Vaughan, E. E., & Caggia, C. (2006). Artisanal and experimental Pecorino Siciliano cheese: Microbial dynamics during manufacture assessed by culturing and PCR–DGGE analyses. International Journal of Dairy. *Technology, 109*, 1–8. https://doi.org/10.1016/j.ijfoodmicro.2005.11.002

Rocchetti, G., Becchi, P. P., Salis, L., Lucini, L., & Cabiddu, A. (2023). Impact of pasture-based diets on the untargeted metabolomics profile of sarda sheep milk. *Foods, 12*(143). https://doi.org/10.3390/foods12010143

Rocchetti, G., Ghilardelli, F., Mosconi, M., Masoero, F., & Gallo, A. (2022). Occurrence of polyphenols, isoflavonoids, and their metabolites in milk samples from different cow feeding regimens. *Dairy, 3*, 314–325. https://doi.org/10.3390/dairy3020024

Sagoo, S. K., Little, C. L., Greenwood, M., Mithani, V., Grant, K. A., McLauchlin, J., de Pinna, E., & Threlfall, E. J. (2009). Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom. *Food Microbiology, 26*, 39–43. https://doi.org/10.1016/j.fm.2008.07.005

Salehi, B., Azzini, E., Zucca, P., Maria Varoni, E., Anil Kumar, N. V., Dini, L., Panzarini, E., Rajkovic, J., Valere Tsouh Fokou, P., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., El Beyrouthy, M., Setzer, W. N., Polito, L., Iriti, M., Sureda, A., Magdalena Quetglas-Llabres, M., … Sharifi-Rad, J. (2020). Plant-derived bioactives and oxidative stress-related disorders: A key trend towards healthy aging and longevity promotion. *Applied Sciences, 10*(947). https://doi.org/10.3390/app10030947

Salem, A. S., Salama, W. M., Hassanein, A. M., & El Ghandour, H. M. (2013). Enhancement of nutritional and biological values of Labneh by adding dry leaves of *Moringa oleifera* as innovative dairy products. *World Applied Sciences Journal, 22*, 1594–1602. https://doi.org/10.5829/idosi.wasj.2013.22.11.13024

Schiavon, S., Paolini, M., Guzzon, R., Mancini, A., Larcher, R., Villegas, T. R., & Franciosi, E. (2021). Bacterial complexity of traditional mountain butter is affected by the malga-farm of production. *Microorganisms, 10*(17). https://doi.org/10.3390/microorganisms10010017

Sciacca, F., Virzì, N., Pecchioni, N., Melilli, M. G., Buzzanca, C., Bonacci, S., & Di Stefano, V. (2023). Functional end-use of hemp seed waste: Technological, qualitative, nutritional, and sensorial characterization of fortified bread. *Sustainability, 15*, 12899. https://doi.org/10.3390/su151712899

Settanni, L., Gaglio, R., Guarcello, R., Francesca, N., Carpino, S., Sannino, C., & Todaro, M. (2013). Selected lactic acid bacteria as a hurdle to the microbial spoilage of cheese: Application on a traditional raw ewes' milk cheese. *International Dairy Journal, 32*, 126–132. https://doi.org/10.1016/j.idairyj.2013.04.010

Silvetti, T., Capra, E., Morandi, S., Cremonesi, P., Decimo, M., Gavazzi, F., Giannico, R., Noni, I. D., & Brasca, M. (2017). Microbial population profile during ripening of protected designation of origin (PDO) Silter cheese, produced with and without

autochthonous starter culture. *LWT - Food Science and Technology, 84*, 821–831. https://doi.org/10.1016/j.lwt.2017.06.022

Sitzia, M., Bonanno, A., Todaro, M., Cannas, A., Atzori, A. S., Francesconi, A. H. D., & Trabalza-Marinucci, M. (2015). Feeding and management techniques to favour summer sheep milk and cheese production in the Mediterranean environment. *Small Ruminant Research, 126*, 43–58. https://doi.org/10.1016/j.smallrumres.2015.01.021

Spencer, C. M., Cai, Y., Martin, R., Gaffney, S. H., Goulding, P. N., Magnolato, D., Lilley, T. H., & Haslam, E. (1988). Polyphenol complexation—some thoughts and observations. *Phytochemistry, 27*, 2397–2409. https://doi.org/10.1016/0031-9422(88)87004-3

Todaro, M., Gannuscio, R., Mancuso, I., Ducato, B., & Scatassa, M. L. (2023). Relationships between chemical and physical parameters of bulk milk from Valle del Belice sheep. *Italian Journal of Animal Science, 22*, 953–958. https://doi.org/10.1080/1828051X.2023.2252846

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

Tóth, G. (2019). Circular economy and its comparison with 14 other business sustainability movements. *Resources, 8*(159). https://doi.org/10.3390/resources8040159

Vargas-Bello-Pérez, E., Vera, R. R., Aguilar, C., Lira, R., Peña, I., & Fernández, J. (2013). Feeding olive cake to ewes improves fatty acid profile of milk and cheese. *Animal Feed Science and Technology, 184*, 94–99. https://doi.org/10.1016/j.anifeedsci.2013.05.016

Viola, E., Buzzanca, C., Tinebra, I., Settanni, L., Farina, V., Gaglio, R., & Di Stefano, V. (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

Wang, Y., He, L., Xing, Y., Zheng, Y., Zhou, W., Pian, R., … Marco, M. L. (2019). Dynamics of bacterial community and fermentation quality during ensiling of wilted and unwilted *Moringa oleifera* leaf silage with or without lactic acid bacterial inoculants. *mSphere, 4*, Article e00341-19. https://doi.org/10.1128/mSphere.00341-19

Wang, Y., Wang, C., Zhou, W., Yang, F. Y., Chen, X. Y., & Zhang, Q. (2018). Effects of wilting and *Lactobacillus plantarum* addition on the fermentation quality and microbial community of *Moringa oleifera* leaf silage. *Frontiers in Microbiology, 9* (1817). https://doi.org/10.3389/fmicb.2018.01817

Wolfe, B. E., Button, J. E., Santarelli, M., & Dutton, R. J. (2014). Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity. *Cell, 158*, 422–433.

Yeluri Jonnala, B. R., McSweeney, P. L., Sheehan, J. J., & Cotter, P. D. (2018). Sequencing of the cheese microbiome and its relevance to industry. *Frontiers in Microbiology, 9*(1020). https://doi.org/10.3389/fmicb.2018.01020

Zhang, T., Jeong, C. H., Cheng, W. N., Bae, H., Seo, H. G., Petriello, M. C., & Han, S. G. (2019). Moringa extract enhances the fermentative, textural, and bioactive properties of yogurt. *LWT - Food Science and Technology, 101*, 276–284. https://doi.org/10.1016/j.lwt.2018.11.010