

Research article

IMPACT OF *NIGELLA SATIVA* FLOUR ON THE PHYSICOCHEMICAL, ANTIOXIDANT AND MICROBIOLOGICAL PROPERTIES OF TELEMEA CHEESE

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Abstract

Incorporating plant-derived bioactive ingredients into dairy products is a promising strategy for the development of functional foods with health-promoting properties. This study evaluated the effects of *Nigella sativa* (black cumin) flour (0.5% and 1%, w/w) on the physicochemical composition, total polyphenol content (TPC), antioxidant activity (DPPH and ABTS), microbiological stability and sensory properties of Romanian Telemea cheese. Fortification with *Nigella sativa* flour significantly increased total polyphenol content to 0.91 and 1.82 mg GAE/100 g for the 0.5% and 1% additions, respectively, while antioxidant capacity reached 1.53 and 3.05 mg TE/100 g (DPPH) and 40.00 and 80.00 mg TE/100 g (ABTS) ($p < 0.05$). Microbiological analyses revealed complete inhibition of *Enterobacteriaceae*, yeasts and molds in fortified cheeses. Sensory evaluation identified 0.5% as the optimal fortification level, balancing improved bioactivity and consumer acceptability.

The results demonstrate that moderate fortification (0.5%) effectively improves functional value while maintaining technological and sensory characteristics, supporting the development of clean-label functional brine cheeses.

1. Introduction

The growing demand for functional dairy products has stimulated research into plant-based bioactive enrichment strategies. Brined cheeses, such as Telemea cheese, represent suitable matrices for the incorporation of antioxidant-rich ingredients due to their high moisture and protein content. Due to its

advantageous nutritional profile and its well-established role in human diets across diverse cultural contexts, it is anticipated that global cheese production will continue to exhibit an upward trend in the forthcoming years (Moja et al., 2025). Cheese consumption in Romania is primarily centered on fresh cow's milk cheeses, produced through a process comparable to

tvorog cheese, though typically yielding a firmer texture (Pop, 2025). The second most popular variety is Telemea cheese, a white brined cheese closely related to Bulgarian and Albanian cheese and comparable to Greek feta, though with distinct ripening practices. Third in preference is cașcaval, a semi-matured pasta filata cheese similar in production to Italian mozzarella (Tamime, A. Y., 2011). At present, only a limited number of Romanian cheeses have achieved official recognition, notably Telemeaua de Ibănești (PDO) and Telemeaua de Sibiu (PGI) (Pop, 2025). Telemea is a traditional Romanian cheese variety, produced from cow, goat, or sheep milk, with its quality largely influenced by the type of raw milk utilized (Manolica et al., 2024). Telemea cheese is traditionally ripened in a 30% whey brine solution that is free of albumin and acidified with a starter culture of lactic bacteria (Mares & Dumitras, 2024). The utilization of brine as a ripening and preservation medium is particularly prevalent in regions characterized by warmer climates, such as the Mediterranean, the Balkans, the Middle East, and certain regions of Latin America. Cheeses that undergo maturation in brine develop a distinctive microbial community that differs from that of other cheese varieties (Hilma, 2022). This microflora plays a pivotal role in shaping the biochemical properties and volatile profile of the cheese. Spices are increasingly valued for their therapeutic potential, particularly due to their antioxidant and antimicrobial properties (El-Sayed & Youssef, 2019).

In the domain of cheesemaking, the enhancement of flavor is frequently accomplished through the incorporation of natural enhancers, such as smoke in both liquid and traditional forms, ashes, or nuts including almonds and walnuts. Spices and herbs are also widely used in this regard (c et al., 2024). Common examples include red and green peppers, black peppercorns, horseradish, thyme, cloves, cumin, black cumin, caraway, parsley, tarragon, nutmeg, basil, onion, garlic, and sundried tomatoes (Lutterodt et al., 2010). These ingredients are typically applied in minimal amounts, generally not exceeding 1%

of the curd, with the objective of complementing rather than overpowering the cheese's innate characteristics (Hassanien et al., 2014).

Among the various spices incorporated into cheese matrices, *Nigella sativa* is of particular interest due to the presence of bioactive compounds that contribute both sensory complexity and potential health-promoting effects (Lutterodt et al., 2010; Hassanien et al., 2014).

Nigella sativa (black cumin), a member of the Ranunculaceae family, is regarded as a medicinal food plant due to its bioactive constituents, the majority of which are concentrated in the seed's essential oil (Tanwar, 2021). The seeds typically exhibit a dark pigmentation, ranging from black to dark brown, and their morphology is characterized by angular or funnel-shaped structures. These seeds are notable for their strong aroma, accompanied by a slightly bitter, nutty, and peppery flavor profile (Voşgan et al., 2024). Ramadan (2007) indicates that the therapeutic potential of *Nigella sativa* is largely attributed to thymoquinone, the principal bioactive compound of its essential oil, in combination with other valuable constituents. These include alkaloids (e.g., nigellicine, nigellimine, and nigellidine), volatile oils (e.g., carvone, d-limonene, and cymene), fatty acids and terpenoids, phenolic acids, flavonoids, saponins, aliphatic alcohols, steroids, tannins, resins, as well as proteins, sugars, and vitamins (A1, A2, B1, B2) (Voşgan et al., 2024). A significant number of these metabolites have been shown to function by disrupting microbial cell membranes, impeding cell division, and hindering biofilm development (Bhatti et al., 2022). This underscores their promising potential for application in the context of controlling microbial contamination within the food industry (Friedlander et al., 2019). The antimicrobial activity of *Nigella sativa* has been the subject of extensive investigation in both in vivo and in vitro conditions (Forouzanfar et al., 2014). These studies demonstrated inhibitory effects against a broad spectrum of pathogens, including

Staphylococcus spp. (Voşgan et al., 2024), various fungi, and numerous bacteria such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella Enteritidis*, *Salmonella typhi*, *Shigella* spp., *Vibrio cholerae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, and *Proteus vulgaris* (Bakal et al., 2017).

The aim of this study is to investigate the impact of *Nigella sativa* flour fortification (0.5% and 1%, w/w) on the physicochemical characteristics, polyphenol content, antioxidant activity, microbiological stability, and sensory properties of Telemea cheese, with the objective of evaluating its potential for the

development of a clean-label functional brined dairy product.

2. Materials and methods

2.1. Experimental Design

The experimental design was structured to evaluate the dose-dependent effects of *Nigella sativa* flour incorporation on the physicochemical, functional, microbiological, and sensory properties of Telemea cheese. A completely randomized design was employed, consisting of three experimental variants produced under identical technological conditions. The samples codifications are presented in Table 1.

Table 1. Sample codification

Sample code	Description
PM	Control sample (Telemea cheese without <i>Nigella sativa</i> flour (0%))
P1	Telemea cheese fortified with 0.5% (w/w) <i>Nigella sativa</i> flour
P2	Telemea cheese fortified with 1% (w/w) <i>Nigella sativa</i> flour

The selected fortification levels were based on previous literature indicating that spice incorporation in cheese matrices typically does not exceed 1% of curd mass in order to preserve structural integrity and sensory balance.

Each experimental variant was manufactured in triplicate on separate production days to ensure reproducibility and to account for technological variability. For

each batch, cheese samples were collected after 48 h of brining, when the final pH (4.4–4.5) was reached, and subsequently subjected to analytical determinations.

As illustrated in Figure 1, the experimental design enabled the systematic evaluation of fortification level effects, functional responses, microbiological stability, and sensory acceptability.

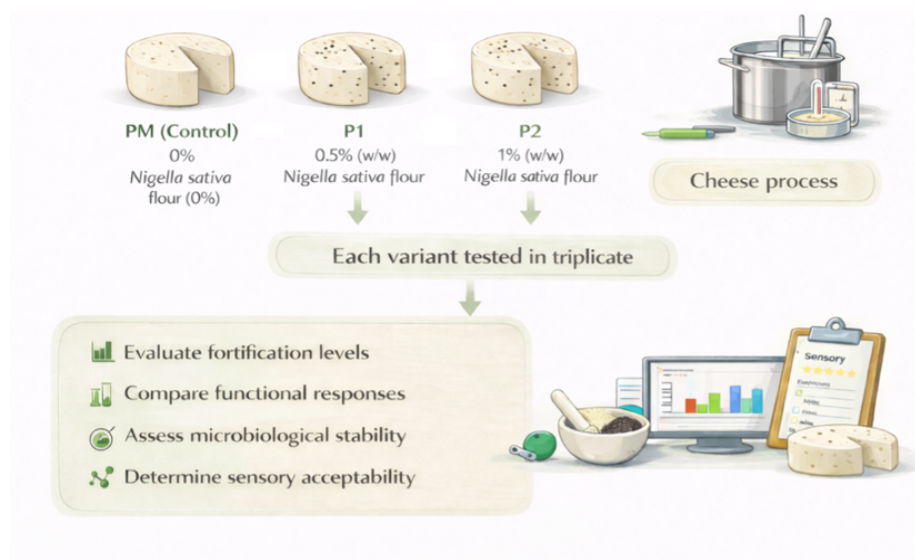


Figure 1. The experimental design

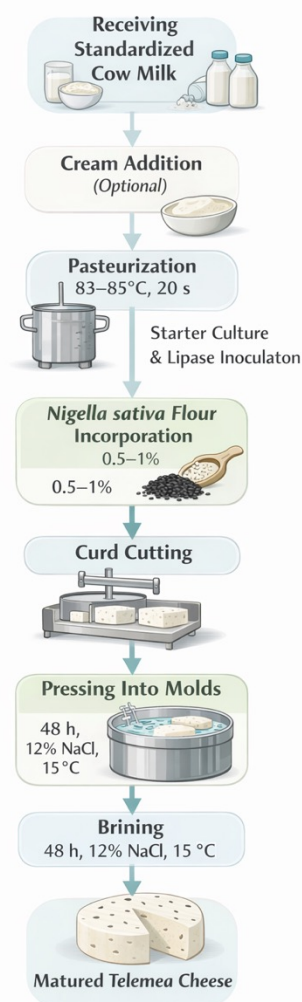
All analytical measurements were performed in triplicate for each batch, and results were expressed as mean $\pm$ standard deviation. Statistical comparisons between experimental groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, with significance established at $p < 0.05$.

2.2. Cheese Manufacture

Telemea cheese was produced using standardized cow's milk (9% fat, 7–8% protein), supplemented with whey protein powder. The milk was subjected to pasteurization at temperatures ranging from 83 to 85°C for a duration of 15 to 20 seconds within a plate heat exchanger (Alfa Laval AB, Sweden). Thereafter, the temperature was reduced to 34°C. Subsequently, starter cultures were inoculated, comprising a mesophilic blend (*Streptococcus lactis* and *Streptococcus cremoris*, 3%) and a thermophilic strain (*Streptococcus thermophilus*, 0.15%). To facilitate coagulation, a 40% calcium chloride solution (Merck KGaA, Darmstadt, Germany) and microbial rennet (Naturen® Extra, Chr. Hansen, Denmark) (diluted 1:1000) were added.

The cheesemaking process was carried out in an industrial coagulator (ALPMA GmbH, Germany), which was equipped with automatic stirring, cutting knives, and temperature control. Following inoculation, the milk was permitted to coagulate for approximately 10 minutes. The coagulum was then meticulously divided into uniform cubes, allowed to repose, and stirred gradually to encourage whey expulsion. The curd was subsequently transferred into molds under whey drainage conditions. At this stage, black cumin (*Nigella sativa*) seed flour (Die Ölfreunde GmbH, Germany) was incorporated at two levels: 0.5% (15 g/3 kg curd) and 1% (30 g/3 kg curd) as is shown in Table 1.

The molds were arranged in a stack and subjected to self-pressing, with three successive turnings at decreasing temperatures (29 °C, 27 °C, 25 °C, and 23 °C). After the pressing process, cheese blocks weighing approximately 2 kilograms each were extracted from the molds and immersed in brine at a temperature of 15°C. The cheeses were subjected to a ripening process until they attained a final pH of 4.4–4.5. Control samples were produced under identical conditions, but without the incorporation of black cumin flour. The technological process for obtaining Telemea cheese is illustrated in Figure 2.

**Figure 2.** Technological Telemea cheese process

2.3. Raw material analysis

For the milk samples, a comprehensive set of physicochemical and microbiological analyses were conducted to determine their compositional quality and hygienic condition. The determinations encompassed titratable acidity, aflatoxin M1 concentration, cryoscopic point, density, somatic cell count, fat, protein, total solids, pH, and total bacterial count.

2.3.1. Determination of titratable acidity

The determination of titratable acidity in milk was performed according to the Romanian standard SR 2418:2008 (ASRO, 2008). The method relies on the neutralization of organic acids and acid salts present in a defined milk volume by titration with sodium hydroxide solution (0.1 N, Merck, Darmstadt, Germany), using phenolphthalein (1% alcoholic solution, Sigma-Aldrich, St. Louis, MO, USA) as the pH indicator. For the analysis, 10 mL of milk were pipetted into a 100 mL Erlenmeyer flask (Duran, Wertheim, Germany), mixed with 20 mL of distilled water (Millipore Milli-Q system, Burlington, MA, USA), and supplemented with 3–4 drops of the indicator solution. The mixture was titrated using a 25 mL graduated burette (Brand, Wertheim, Germany) until a faint pink coloration appeared, persisting for at least one minute (Olariu et al., 2017). The titratable acidity was calculated according to the following equation.

$$\text{Acidity } [^{\circ}\text{T}] = \frac{V_{\text{NaOH}}}{V_{\text{sample}}} \times 100$$

where V_{NaOH} is the volume of sodium hydroxide solution used for titration (mL), and V_{sample} is the volume of milk analyzed (mL).

Results were expressed in Thörner degrees ($^{\circ}\text{T}$), a unit commonly applied in dairy science to assess milk freshness and technological suitability.

This method is widely recognized as a reliable quality indicator for milk and is consistent with international analytical standards.

2.3.2. Determination of aflatoxin

The Symmetric M1 Lateral Flow Test Kit is a rapid immunoassay (LFIA) designed to detect aflatoxin M1 in milk. It uses antibodies that specifically bind to this contaminant. The method enables quantitative assessment of aflatoxin M1 levels in cow, sheep, and goat milk, within a sensitivity range of 8–150 ppt (Brănescu et al., 2025). The procedure necessitates the utilization of a commercial test kit, a single-channel 100 μL pipette with sterile tips, and representative milk samples. Prior to analysis, the milk is subjected to homogenization and equilibration to ambient temperature. A volume of 100 μL is dispensed into the designated well on the test strip, which is then subjected to an incubation period ranging from five to fifteen minutes, thereby allowing for antigen–antibody interactions to occur. The evaluation of the outcome can be conducted visually or through the utilization of a specialized reader. The device measures the intensity of both the control and test lines, subsequently converting the optical response into a numerical result expressed in parts per trillion (ppt). This guarantees precise and replicable quantification of aflatoxin M1 concentrations within the established range of the kit (Brănescu et al., 2025).

2.3.3. Determination of cryoscopic point

The freezing point of milk is a highly sensitive quality parameter that is frequently employed to detect adulteration practices, such as the addition of water (Ceniti et al., 2019). For this determination, raw cow and goat milk samples were collected under hygienic conditions and homogenized to ensure uniformity prior to testing (Otwinowska-Mindur et al., 2017). A 2-mL aliquot of milk was placed in a cryoscope, which gradually decreased the sample's temperature until ice nucleation occurred. This temperature was designated as the cryoscopic point. Pure milk generally undergoes a freezing point depression between $-0.512\text{ }^{\circ}\text{C}$ and $-0.550\text{ }^{\circ}\text{C}$, with cow and goat milk typically ranging from $-0.525\text{ }^{\circ}\text{C}$ to $-0.550\text{ }^{\circ}\text{C}$. The presence of values outside

this interval may be indicative of water dilution or other compositional alterations. The freezing point of milk is closely related to its soluble components, thereby providing indirect insights into its natural composition and authenticity (Brănescu et al., 2025).

2.3.4. Determination of density

The determination of density is a critical step in the analysis of materials. Milk density was evaluated using an automated infrared spectroscopic system, the MilkoScan FT2 (Foss, Denmark), which is widely applied in the dairy industry (Souhassou et al., 2018). Prior to analysis, the instrument was calibrated with distilled water as a reference. Subsequently, cow and goat milk samples were introduced into the device. The MilkoScan quantifies density by evaluating mass-to-volume relationships in conjunction with compositional parameters, including protein, fat, and lactose contents (Parmar et al., 2020). The instrument automatically displayed and stored the density results. For interpretation, the measured values were then compared against standard reference ranges. According to the findings of Park et al., (2007) the range of milk solids in bovine milk is from 1.028 to 1.035 g/mL, and in caprine milk, it is from 1.030 to 1.037 g/mL.

2.3.5. Determination of somatic cell count, fat, protein, total solids and pH

The somatic cell count was measured using a CombiFoss analyzer (FOSS Analytical, Hillerød, Denmark). Prior to measurement, the instrument underwent calibration in accordance with the manufacturer's protocol (FOSS Analytical, 2020). Milk samples were equilibrated to 20°C and subsequently aspirated automatically through the device's sampling pipette. The dedicated software generated results in an expeditious manner. In addition to somatic cell count, the system concurrently furnished data on fat, protein, lactose, total solids, and pH content, thereby providing a comprehensive evaluation of milk quality parameters (Fonseca et al., 2025).

2.3.6. Determination of total bacterial count

The total microbial load was determined using a BactoScan system (FOSS Analytical,

Hillerød, Denmark). Prior to utilization, the instrument underwent a rigorous calibration process. The method is predicated on the staining of bacteria with a fluorescent dye, followed by optical detection (Ramsahoi et al., 2011). The results were expressed as colony-forming units (CFU), enabling rapid quantification of the total bacterial count in raw milk samples.

2.3.7. Detection and enumeration of Enterobacteriaceae

The presence of *Enterobacteriaceae* was evaluated according to the horizontal method described in ISO 21528-2:2017 (International Organization for Standardization, 2017). These microorganisms form colonies on glucose-enriched agar and are characterized by glucose fermentation and oxidase-negative reactions. The culture medium, Violet Red Bile Glucose Agar (VRBG; Merck, Darmstadt, Germany) and Nutrient Agar (Biokar Diagnostics, Beauvais, France), was prepared by suspending the dehydrated medium in distilled water and homogenizing it on a magnetic stirrer (IKA, Staufen, Germany). The medium was subjected to boiling and subsequently cooled to an appropriate temperature. Milk samples (10 mL) were serially diluted to 10^{-2} using sterile distilled water. From the diluted samples, 1 mL was transferred with a sterile pipette (Eppendorf, Hamburg, Germany) into a Petri dish (Sarstedt, Nümbrecht, Germany), followed by the addition of approximately 15 mL of molten culture medium. The plates were then subjected to gentle agitation to ensure uniform distribution of the samples. Following this, the plates were allowed to solidify, and subsequently, they were placed in an incubator (Mettler, Schwabach, Germany) set to 30°C. The plates were left to incubate for 24 hours at this temperature. All inoculation procedures were carried out under a laminar flow cabinet (Telstar, Terrassa, Spain) to maintain aseptic conditions (Mokadem et al., 2020).

2.4. Telemea cheese analyses

A series of physicochemical, microbiological, and sensory determinations

were performed on the Telemea cheese samples to evaluate product quality, safety, and overall acceptability. The analyses encompassed a comprehensive array of parameters, including pH, moisture levels, the chemical composition of the sample (fat, protein, salt, total solids, and acidity), and the antioxidant activity. In addition, the *Enterobacteriaceae* count was determined, and a sensory evaluation was conducted to assess the overall quality of the sample.

2.4.1. Determination of pH

The pH of the milk and cheese samples was measured using a standard laboratory pH meter (WTW Multi 3510 IDS, Xylem Analytics, Germany). Prior to each measurement, the instrument was calibrated with buffer solutions of pH 4.0 and 7.0 to ensure accuracy. pH determination was carried out both during the production process and in the final product. Monitoring pH during cheese manufacture is essential, as it decreases during coagulation and curd formation and strongly influences texture, microbial stability, and overall cheese quality (Bansal & Veena, 2024).

2.4.2. Determination of moisture

The moisture content was determined using a moisture analyzer (KERN, Germany) that operates on the thermogravimetric principle. Prior to analysis, the instrument was activated and the drying parameters (time and temperature) were set according to the sample characteristics. The cheese sample was evenly distributed on a weighing pan and dried automatically, with moisture content determined gravimetrically from mass loss and displayed digitally at the end of the cycle (Vera Zambrano et al., 2019).

2.4.3. Physico-chemical determinations using FoodScan

The chemical composition of cheese samples was assessed with a FoodScan analyzer (FOSS Analytical, Denmark), which operates on the principle of near-infrared transmission (NIRT) spectroscopy (FOSS Analytical, 2020). The instrument was calibrated according to the manufacturer's instructions. Homogenized samples were evenly spread on a Petri dish cover and placed

in the measurement chamber. A cheese-specific program was selected, providing rapid, non-destructive determination of protein, fat, moisture, salt, total solids, and acidity (Anjos, 2020).

2.4.4. Determination of Total Polyphenol content

2.4.4.1. Extraction of Phenolic Compounds

Phenolic compounds were extracted from cheese samples using a hydroalcoholic solvent system adapted for dairy matrices with minor modification after Abd El-Fat et al., (2018). Briefly, 5 g of finely homogenized cheese were mixed with 25 mL of methanol–water solution (80:20, v/v). The mixture was subjected to ultrasonic-assisted extraction in an ultrasonic bath for 30 min at room temperature to enhance the release of phenolic compounds from the protein–lipid matrix. Following sonication, the samples were centrifuged at 5000 rpm for 10 min, and the supernatant was carefully collected and used for subsequent analyses. All extractions were performed in triplicate to ensure reproducibility.

2.4.4.2 Total Polyphenol Content (TPC)

The total polyphenol content (TPC) of the cheese extracts was determined using the Folin–Ciocalteu colorimetric method, adapted from previously published procedures applied to dairy products (Weragama et al., 2021). An aliquot of 0.5 mL of the extract was mixed with 2.5 mL of Folin–Ciocalteu reagent previously diluted (1:10, v/v) with distilled water. After 5 min, 2 mL of sodium carbonate solution (7.5%, w/v) were added to the reaction mixture. The samples were incubated for 30 min in the dark at room temperature to allow color development. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer (SPECORD 200 PLUS, Analytik Jena, Germany). Gallic acid was used as the calibration standard, and results were expressed as milligrams of gallic acid equivalents (mg GAE) per 100 g of cheese.

2.4.5. Determination of Antioxidant Activity

The combined application of DPPH and ABTS assays provides a complementary evaluation of antioxidant capacity, as the two radicals differ in solubility and reaction

mechanisms. While DPPH is primarily suitable for assessing the scavenging activity of lipophilic antioxidants, the ABTS assay allows the detection of both hydrophilic and lipophilic compounds (Floegel et al., 2011). Therefore, the use of both methods offers a more comprehensive characterization of the antioxidant potential of the fortified cheese matrix.

2.4.5.1. DPPH

The antioxidant capacity of the cheese extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a widely applied method for assessing the free radical neutralization potential of food matrices (Gulcin & Alwasel, 2023).

A 0.1 mM DPPH solution was prepared in methanol prior to analysis. For each determination, 0.5 mL of cheese extract were mixed with 2.5 mL of the DPPH solution. The reaction mixture was incubated for 30 min in the dark at room temperature to allow sufficient interaction between antioxidant compounds and the DPPH radical. The decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer (SPECORD 200 PLUS, Analytik Jena, Germany). Trolox was used as the reference antioxidant for calibration, and results were expressed as micromoles of Trolox equivalents ($\mu\text{mol TE}$) per 100 g of cheese. The radical scavenging activity was additionally calculated as percentage inhibition according to the following equation:

$$\% \text{ inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

where A_0 represents the absorbance of the control solution (DPPH without extract) and A_s represents the absorbance of the sample.

2.4.5.2. ABTS

The antioxidant activity was further assessed using the ABTS radical cation decolorization assay (Kut et al., 2022). The $\text{ABTS}^{\bullet+}$ radical was generated by reacting 7 mM ABTS solution with 2.45 mM potassium

persulfate. The mixture was stored in the dark at room temperature for 12–16 h to allow complete radical formation before use. For the assay, 0.2 mL of cheese extract were mixed with 2 mL of the ABTS radical solution. After appropriate equilibration, absorbance was measured at 734 nm. Trolox was used to construct the calibration curve, and results were expressed as micromoles of Trolox equivalents ($\mu\text{mol TE}$) per 100 g of cheese. All determinations were carried out in triplicate to ensure analytical reliability.

2.4.6. Microbiological quality assesment

The enumeration of *Enterobacteriaceae* was performed in accordance with ISO 21528-2:2017 (International Organization for Standardization, 2017), a standardized colony-count method that is widely employed in the field of food microbiology to evaluate hygiene and contamination indicators. A total of 10 grams of the sample were meticulously collected from various regions and transferred into a sterile stomacher bag equipped with a filter. The automated diluter (DILUMAT, AES Chemunex, France) was utilized to prepare a 1:10 dilution, with 90 milliliters of sterile distilled water being added to the initial volume. The suspension was subsequently homogenized in a Stomacher 400 (Seward, UK) for a duration of 2–4 minutes.

From the homogenate, 1 mL was inoculated onto Violet Red Bile Glucose (VRBG) agar (Oxoid™, Thermo Fisher Scientific, UK), overlaid with the same medium, and incubated at 20–30°C for 72–120 hours. For yeasts and molds, Oxytetracycline Glucose Agar (OGA) (Oxoid™, Thermo Fisher Scientific, UK) was used, and the incubation was conducted at 30–44°C for 24 hours. Following the incubation period, the colonies were enumerated as colony-forming units (CFU) and expressed per gram of product (Manolica et al., 2024).

2.4.7. Sensory evaluation

The sensory evaluation of Telemea cheese was conducted in accordance with established methods using a 0–5 scoring scale (Civille & Carr, 2015). The attributes evaluated encompassed external characteristics such as

shape, surface color, and the presence of cracks. Internal attributes included homogeneity and the presence of fermentation holes. Textural attributes such as elasticity of the curd were also assessed, along with odor and taste. The evaluations were carried out by a trained panel of assessors ($n = 50$) under standardized conditions.

2.4.8. Statistical analysis

All experiments were conducted in triplicate, with results expressed as the mean $\pm$ standard deviation (SD). Statistical analysis was performed using analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant differences between samples, with a significance threshold of $p < 0.05$.

3. Results and discussions

3.1. Raw milk results and discussions

3.1.1. Titrable acidity results

Titrate acidity (TA) is a significant quality parameter of raw milk, reflecting its buffering capacity and the presence of lactic acid and related compounds. According to the standards outlined in SR 2418:2008, the pH level of fresh raw milk should ideally range from 6.6 to 6.8, with a temperature between 15 and 19°C. In the present study, raw milk samples exhibited a mean TA of 17°T, based on triplicate determinations, thus falling within the expected range, as is presented in Table 2. Yuan et al., (2022) reported comparable results, finding TA values of 16.5–17.2°T in bulk cow's milk under controlled refrigeration. Puga-Torres et al. (2024) observed similar values (16–18°T) in province of Pichincha – Ecuador raw milk, linking higher acidity to microbial activity during transport and storage.

3.1.2. Aflatoxin M1 results

In the event of dairy animals consuming feed contaminated with aflatoxin B1 (AFB1), it is subject to metabolism, resulting in the production of aflatoxin M1 (AFM1), which subsequently seeps into milk within hours (Pipoyan et al., 2024). AFM1 demonstrates heat stability, withstanding pasteurization processes, yet it poses potential hazards,

including liver toxicity and carcinogenesis (Gemede, 2025). Given its status as a staple food, particularly for children, the contamination of milk constitutes a significant safety concern. According to existing regulatory standards, these substances are subject to strict control, with allowable levels limited to 50 ng/L in the European Union and 500 ng/L in the United States (Malissiova et al., 2024).

The analysis of raw milk samples for aflatoxins yielded negative results, indicating that no detectable levels of aflatoxins were present, as presented in Table 2.

3.1.3. Cryoscopic point results

The cryoscopic point, defined as the temperature at which milk undergoes solidification under controlled conditions, represents a critical benchmark in the study of dairy science and technology (Souhassou et al., 2018). The freezing point of milk is a critical indicator of its quality and authenticity. Pure milk exhibits an average freezing point of approximately -0.530°C (Brănescu et al., 2025). It has been established that this temperature is influenced by the presence of solutes, including water, sugars, and salts. A higher freezing point is an indication of water adulteration, as dilution increases the temperature closer to 0°C (Senevirathne et al., 2016). This method has gained significant traction within the dairy industry, where it is employed as a standard practice for the detection of adulteration (Brănescu et al., 2025). In the present study, the cryoscopic point of bovine raw milk was established at $-0.540 \pm 0.010^{\circ}\text{C}$, as presented in Table 2, which falls within the tolerance limits defined by SR ISO 5764:2010 -0.515 to -0.555°C (ASRO, 2010). These findings are in close agreement with recent reports; for instance, Brito et al., (2020) documented an average freezing point of approximately -0.522°C in raw cow's milk using precision cryoscopy and FTIR spectroscopy. The existence of such concordance serves to substantiate the robustness of cryoscopic point determination as a criterion for authenticity and quality assessment in the domain of dairy science.

3.1.4. Density

Density is a fundamental physicochemical indicator of milk composition, primarily influenced by its content of fat, protein, lactose, and minerals. Standardized measurements at 20°C are widely employed in the field of dairy science. These measurements are used for two primary purposes: first, to verify the authenticity of a product, and second, to detect potential adulteration, particularly dilution with water (Yakubchak et al., 2021).

In the present study, the density of raw milk at 20°C was established at 1.030 ± 0.001 g/cm³, as shown in Table 2, which exceeds the minimum requirement of 1.029 g/cm³ as stipulated by SR 2418:2008 (ASRO, 2008). These findings are consistent with the values reported in recent literature. For instance, Parmar et al., (2020) investigated 1,035 raw cow milk samples and reported a mean density of 1.0308 ± 0.002 g/cm³, confirming that the obtained result lies well within the expected range for authentic milk. In a similar vein, (Senevirathne et al., 2016) underscored that the typical range of bovine milk's density, contingent on compositional variations, is between 1.028 and 1.033 g/cm³.

3.1.5. Somatic cells count, fat, protein, total solids, pH

Milk composition parameters, including fat, protein, lactose, total solids, pH, and somatic cell count (SCC), are widely recognized as fundamental indicators of quality and technological suitability (Bardales et al., 2024). These characteristics are further influenced by physiological and environmental determinants, such as the lactation stage, the nutritional regimen, and the health of the mammary gland (Gecaj et al., 2021).

In the present analysis, the fat content of the milk samples was found to be $9.26 \pm 0.17\%$, the protein content was $7.68 \pm 0.11\%$, the total solids content was $21.65 \pm 0.22\%$, the lactose content was $4.24 \pm 0.07\%$, the pH was $6.73 \pm 0.05\%$, and the SCC was 380000 ± 20000 cells/mL, as presented in Table 2. These values exceeded the minimum standards set by SR 2418:2008 (ASRO, 2008), with SCC remaining below the regulatory threshold of

400000 cells/mL (European Parliament and Council, 2004). A comparative analysis of the results with those of other studies reveals noteworthy disparities. Bardales et al., (2024) reported average fat and protein levels of $\sim 3.9\%$ and $\sim 3.3\%$, respectively, in raw cow milk, while Gecaj et al. (2021) documented SCC values typically ranging between 200.000 and 600.000 cells/mL. A comparison of the present results with recent findings in bovine milk reveals notable discrepancies. The current study documented protein levels of approximately 3.0–3.5%, lactose values of approximately 4.7%, total solids of 12.5–14.5%, and pH ranging from 6.6 to 6.8 (Chernet et al. 2024; Saleem et al. 2025). In contrast, the present results indicate a markedly higher protein and solids content, while lactose and pH remain closer to expected values.

3.1.6. Total bacterial count

The total bacterial count (TBC) is a critical microbiological indicator of raw milk quality, reflecting hygiene during milking, handling, and storage (Naing et al., 2019). This parameter is widely used for two purposes: first, to assess compliance with safety regulations, and second, to monitor overall microbial load in dairy products.

In the present study, the TBC of raw milk was determined using the BactoScan system and established at 95000 ± 5000 CFU/mL, as presented in Table 2. This value is in accordance with the maximum permissible limit of ≤ 100000 CFU/mL stipulated by EU Regulation (EC) No. 853/2004 (European Parliament and Council, 2004).

These results are consistent with previously reported data. For instance, Yuan et al., (2022) examined bulk tank raw milk samples and found a geometric mean TBC of 5.25 log CFU/mL ($\sim 1.8 \times 10^5$ CFU/mL), which is slightly higher than the values obtained in the present study.

3.1.7. Enterobacteriaceae

Enterobacteriaceae are regarded as significant indicators of hygiene in raw milk, as their presence may indicate fecal contamination or inadequate sanitary practices during milking and storage (Sobeih et al., 2020). In the present

study, the group exhibited a complete absence (0.00 ± 0.00 CFU/mL), thereby satisfying the ISO 21528-2:2017 requirement for absence in raw milk. Comparable results have not always been reported, for example, Pyz-Łukasik et al.,

(2015) found 1.0×10^3 – 1.2×10^6 CFU/mL in raw cow's milk, emphasizing that the absence observed here reflects superior hygienic condition.

Table 2. Results of physicochemical and microbiological analyses of milk samples compared with standard limits

Analysis/ Parameter	Value obtained $\pm$ SD	Standard limit (STAS / ISO / EU)
Titrate acidity (°T)	17 ± 0.50^b	15–19 (SR 2418:2008) (ASRO, 2008)
Aflatoxin M1 (µg/L)	Not detected $\pm$ 0.00^c	≤ 0.05 (EU Reg. 1881/2006) (European Commission, 2006)
Density (20 °C) (g/cm ³)	1.030 ± 0.001^c	≥ 1.029 (SR 2418:2008) (ASRO, 2008)
Cryoscopic point (°C)	-0.540 ± 0.010^c	-0.515 to -0.555 (SR ISO 5764:2010) (ASRO, 2010)
Fat (%)	9.26 ± 0.17^a	≥ 3.2 (SR 2418:2008) ASRO, 2008)
Protein (%)	7.68 ± 0.11^a	≥ 3.2 (SR 2418:2008) (ASRO, 2008)
Total solids (%)	21.65 ± 0.22^a	≥ 12.5 (SR 2418:2008)(ASRO, 2008)
Lactose (%)	4.24 ± 0.07^b	4.4 – 4.7 (SR 2418:2008) (ASRO, 2008)
pH	6.73 ± 0.05^c	6.6 – 6.8 (SR 2418:2008) (ASRO, 2008)
Somatic cell count (cells/mL)	$380,000 \pm$ $20,000^d$	≤ 400000 (EU Reg. 853/2004) (European Parliament and Council, 2004)
Total bacterial count (CFU/mL)	$95,000 \pm 5,000^d$	≤ 100000 (EU Reg. 853/2004)(European Parliament and Council, 2004)
<i>Enterobacteriaceae</i> (CFU/mL)	Absent $\pm 0.00^d$	Absence (ISO 21528-2) (ISO, 2017)

Values are expressed as mean $\pm$ standard deviation ($n = 3$). Different superscript letters within the same column indicate statistically significant differences (one-way ANOVA followed by Tukey's test, $p < 0.05$).

3.2. Telemea cheese results and discussions

3.2.1. pH of Telemea cheese

The pH level is a pivotal technological parameter in the cheesemaking process, exerting a direct influence on coagulation kinetics, microbial growth, and the eventual textural and sensory profile of the cheese (Bansal & Veena, 2024).

The progression of the pH level during the manufacturing process of Telemea cheese is outlined in Table 3. The initial pH level of 6.50 ± 0.02 underwent a gradual decline during the processing stage, ultimately reaching 6.30 ± 0.01 within the molded curd. Acidification progressed steadily during successive turnings, with pH values of 5.90 ± 0.02 (PM), 5.53 ± 0.01 (P1), and 5.48 ± 0.01 (P2) after the first

turning, decreasing to 4.68 – 4.73 ± 0.02 after the final turning. Following a maturation period of 48 hours in brine, the final pH levels were recorded at 4.45 ± 0.01 (P1) and 4.40 ± 0.01 (P2).

The slightly higher final pH in the 0.5% black cumin sample suggests a mild buffering effect attributed to the protein and mineral content of *Nigella sativa* flour. The pH range obtained (4.4–4.8) is consistent with the typical values reported for matured Telemea-type cheeses and aligns with the findings documented by Hassanien et al., (2014), Voşgan et al., (2024) and Alqahtani et al., (2023). This observation validates the hypothesis that there is a similarity in the

acidification patterns observed in soft and fortified cheeses.

Table 3. Results of pH variation during Telemea cheese processing with different levels of *Nigella sativa* addition

Processing stage	pH PM	pH P1	pH P2
Milk (before coagulation)	6.50 ± 0.02 ^a	6.50 ± 0.02 ^a	6.50 ± 0.02 ^a
After coagulation	6.45 ± 0.01 ^a	6.45 ± 0.01 ^a	6.45 ± 0.01 ^a
After curd cutting	6.40 ± 0.01 ^a	6.40 ± 0.01 ^a	6.40 ± 0.01 ^a
In molds (initial)	6.30 ± 0.01 ^a	6.30 ± 0.01 ^a	6.30 ± 0.01 ^a
After 1 st turning	5.90 ± 0.02 ^a	5.53 ± 0.01 ^b	5.48 ± 0.01 ^b
After 2 nd turning	5.07 ± 0.02 ^a	5.01 ± 0.01 ^{ab}	4.95 ± 0.01 ^b
After 3 rd turning	4.85 ± 0.02 ^a	4.82 ± 0.01 ^{ab}	4.79 ± 0.01 ^b
After 4 th turning (removal from molds)	4.68 ± 0.02 ^{ab}	4.73 ± 0.02 ^a	4.69 ± 0.02 ^{ab}
After brine maturation (48 h)	—	4.45 ± 0.01 ^a	4.40 ± 0.01 ^b

Values are expressed as mean ± standard deviation ($n = 3$). Different superscript letters within the same column indicate statistically significant differences (one-way ANOVA followed by Tukey's test, $p < 0.05$).

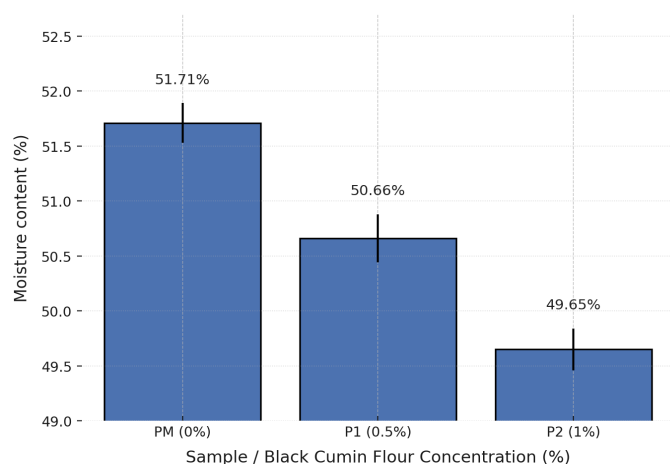


Figure 3. Variation of moisture content in Telemea cheese with different levels of black cumin flour addition

3.2.2. Moisture content

Moisture content is a key determinant of cheese texture, ripening rate, and microbial stability, directly influencing sensory perception and shelf life (Sarvary et al., 2025).

The moisture analysis revealed a gradual decrease with increasing black cumin flour concentration as is showed in Figure 3. The control sample (PM) exhibited a mean moisture

content of $51.71 \pm 0.18\%$, which decreased to $50.66 \pm 0.22\%$ in P1 (0.5%) and $49.65 \pm 0.19\%$ in P2 (1%). This reduction reflects the influence of *Nigella sativa* flour on the cheese's water retention capacity. The fibrous and polysaccharidic components of the flour likely absorbed part of the whey during coagulation and pressing, leading to a denser curd structure. Similar effects have been described in cheese enriched with dietary fibers or herbal flours,

which tend to lower moisture through increased binding of water molecules (Szafrńska et al., 2021). Despite the reduction, all samples remained within the optimal moisture range (48–52%) for matured Telemea cheese, indicating that fortification did not negatively affect product integrity.

3.2.3. Protein content

The protein content of cheese constitutes a pivotal nutritional and functional attribute, exerting a substantial influence on its texture, flavor development, and overall quality. The incorporation of *Nigella sativa* flour exerted a pronounced influence on the protein composition of the cheese. The protein content exhibited an increase from $17.68 \pm 0.12\%$ in the control to $18.53 \pm 0.10\%$ in P1 and $20.13 \pm 0.09\%$ in P2, attributable to the supplementary protein fraction contributed by *Nigella sativa*, as shown in Table 4. Cakir et al., (2016) have reported analogous trends, observing elevated protein levels in cheeses fortified with *Nigella sativa* and its essential oil. This finding suggests that the incorporation of herbs has a favorable effect on the nutritional quality of dairy products.

Conversely, the fat content decreased with higher levels of black cumin flour — from $23.97 \pm 0.20\%$ in PM to $20.60 \pm 0.15\%$ in P1 and $19.60 \pm 0.18\%$ in P2 — likely due to the lipid-modulating and emulsifying properties of black cumin bioactive compounds such as thymoquinone, as presented in Table 4. This agrees with the observations of Irfan et al.,

(2023), who reported reduced fat retention in Cheddar cheese supplemented with plant powders.

The salt content increased notably in fortified samples ($1.65 \pm 0.01\%$ in P1, $1.13 \pm 0.01\%$ in P2) compared with the control ($0.57 \pm 0.02\%$), as shown in Table 4, possibly due to the mineral composition of *Nigella sativa* and the enhanced ionic exchange during brining. Similar patterns were observed in functional cheeses containing herbs or spices (Ritota & Manzi, 2020).

The total solids exhibited a comparable upward trend, as the results from Table 4 showed, ($45.28 \pm 0.21\%$ in PM, $46.40 \pm 0.18\%$ in P1, and $49.34 \pm 0.20\%$ in P2), consistent with the observed reduction in moisture. This finding aligns with the observations reported by Ali et al., (2022), who noted that the incorporation of plant-based ingredients into soft cheese formulations led to an increase in dry matter and, consequently, total solids content.

Overall, the 0.5% fortification level (P1) provided the most balanced formulation, improving protein and mineral content while preserving an appropriate fat–moisture ratio and desirable texture. These results suggest that moderate *Nigella sativa* addition enhances the nutritional value of Telemea cheese without adversely affecting physicochemical stability, likely due to improved whey retention during coagulation and pressing, which led to a more compact curd structure.

Table 4. Chemical composition of Telemea cheese samples

Sample	Protein [%]	Fat [%]	Salt [%]	Total solids [%]
PM	17.68 ± 0.12^c	23.97 ± 0.20^a	0.57 ± 0.02^c	45.28 ± 0.21^c
P1	18.53 ± 0.10^b	20.60 ± 0.15^b	1.65 ± 0.01^a	46.40 ± 0.18^b
P2	20.13 ± 0.09^a	19.60 ± 0.18^c	1.13 ± 0.01^b	49.34 ± 0.20^a

Values are expressed as mean $\pm$ standard deviation ($n = 3$). Different superscript letters within the same column indicate statistically significant differences (one-way ANOVA followed by Tukey's test, $p < 0.05$).

3.2.4. Total Polyphenol Content (TPC)

The experimental results demonstrated a measurable increase in total polyphenol content following fortification with black cumin flour.

The control sample (PM) exhibited only trace levels of phenolic compounds, consistent with the inherently low polyphenol concentration of dairy matrices.

The incorporation of 0.5% *Nigella sativa* flour (P1) resulted in a TPC value of 0.91 mg GAE/100 g cheese, while the 1% addition (P2) led to a further increase to 1.82 mg GAE/100 g (Table 5). The near doubling of TPC between P1 and P2 confirms a concentration-dependent enrichment effect. Although the absolute values appear moderate, the increase is significant in the context of cheese, where phenolic compounds are not naturally abundant. Similar increases in phenolic content and antioxidant activity have been reported for fresh cheeses

fortified with plant-based ingredients. For instance, De Oliveira Mindelo et al., (2025) observed higher levels of bioactive compounds and antioxidant capacity in fresh cheese supplemented with guabiroba pulp compared with the control. In agreement with these findings, the present study shows that the addition of *Nigella sativa* flour leads to a clear, concentration-dependent increase in total polyphenol content and antioxidant activity in Telemea cheese, despite the naturally low phenolic levels of the dairy matrix

Table 5. Effect of *Nigella sativa* flour addition on total polyphenol content and antioxidant activity of Telemea cheese

Sample	TPC (mg GAE/100 g)	DPPH (mg TE/100 g)	ABTS (mg TE/100 g)
PM (Control)	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c
P1 (0.5%)	0.91 ± 0.03 ^b	1.53 ± 0.05 ^b	40.00 ± 1.20 ^b
P2 (1%)	1.82 ± 0.04 ^a	3.05 ± 0.07 ^a	80.00 ± 1.85 ^a

Values are expressed as mean ± standard deviation (n = 3). Different superscript letters within the same column indicate statistically significant differences (one-way ANOVA followed by Tukey's test, p < 0.05).

3.2.5. Determination of Antioxidant Activity

3.2.5.1. DPPH

The antioxidant capacity assessed by the DPPH assay followed a similar trend. The control sample (PM) exhibited negligible radical scavenging activity, whereas the fortified samples showed progressive increases proportional to the level of *Nigella sativa* flour added.

Sample P1 (0.5%) reached 1.53 mg TE/100 g, while P2 (1%) achieved 3.05 mg TE/100 g (Table 5). The approximately twofold increase between P1 and P2 indicates a direct relationship between fortification level and antioxidant efficiency.

An increase in DPPH antioxidant activity proportional to the amount of plant-derived powder added has been documented in fortified dairy matrices. For example, Salık & Çakmakçı, (2025) reported that Beyaz cheese enriched with 0.5% and 1% walnut leaf powder showed increases in DPPH• antioxidant activity compared to the control, with values increasing proportionally with the level of fortification. In the present study, the increase in DPPH activity from 1.53 to 3.05 mg TE/100 g with 0.5% and

1% *Nigella sativa* flour similarly confirms that the phenolic compounds introduced through fortification remain functionally active in the dairy matrix.

3.2.5.2 ABTS

The ABTS assay revealed higher antioxidant values compared to DPPH. The 0.5% sample (P1) exhibited 40 mg TE/100 g, whereas the 1% sample (P2) reached 80 mg TE/100 g, again showing a proportional increase (Table 5).

The higher ABTS values may be explained by the broader reactivity of the ABTS radical toward both hydrophilic and lipophilic antioxidant compounds. Given the complex protein–lipid structure of cheese, this assay may better reflect the overall antioxidant potential of the fortified matrix.

While the 1% addition provided the highest functional values, technological feasibility and sensory balance should be considered when determining the optimal inclusion level. From a functional perspective, even the 0.5% addition achieved a meaningful enhancement relative to the control.

Comparable ABTS antioxidant values have been reported for white brined cheeses fortified with plant-based ingredients. For example, Suna (2022) reported ABTS antioxidant activities ranging from approximately 30 to over 90 mg TE/100 g in white cheese enriched with increasing levels of microalgal powder. In comparison, the ABTS values obtained in the present study (40–80 mg TE/100 g) indicate a comparable enhancement of antioxidant capacity, confirming the effectiveness of *Nigella sativa* flour as a functional ingredient in Telemea cheese.

The laboratory results clearly demonstrate that *Nigella sativa* flour significantly enhances the functional profile of Telemea cheese in a dose-dependent manner. All measured parameters (TPC, DPPH, and ABTS) increased proportionally with the level of fortification.

3.2.6 Enterobacteriaceae

The assessment of the microbiological quality of the samples was conducted through analyses aimed at the detection of *Enterobacteriaceae*, yeasts, and molds (Manolica et al., 2024). The presence of yeast and mold was distinctly evident in the control sample, while the samples containing *Nigella sativa* exhibited no signs of microbial development. This finding confirms the antimicrobial potential of black cumin, which contributes to improved microbiological stability and safety of the cheese (Nazarparvar et al., 2020).

Comparable results were reported by Abdel-Latif et al., (2021) who demonstrated that supplementation of soft cheese with 0.5% *Nigella sativa* oil significantly reduced *Candida albicans* and *Aspergillus parasiticus* counts by approximately 1.4 log CFU/g and 2.3 log CFU/g. In a similar study, Ozcan et al., (2017) observed that increasing concentrations of black cumin in Tulum cheese led to a

statistically significant ($p < 0.05$) decrease in yeast and mold counts during ripening. These findings, which are consistent with the present study, highlight the effectiveness of *Nigella sativa* as a natural antimicrobial and preservative agent in dairy products.

3.2.7. Sensory evaluation of Telemea cheese

The control sample (PM) demonstrated the balanced sensory characteristics that are characteristic of traditional Telemea cheese. The 0.5% black cumin variant (P1) demonstrated the most favorable profile, exhibiting the highest overall sensory score (22.0 ± 1.8), while the 1% addition (P2) received slightly lower values (21.2 ± 2.1). The panelists described P1 as having a pleasant, harmonious flavor, with a subtle cumin aroma that complemented the natural lactic and slightly salty taste of Telemea cheese. In contrast, P2 was characterized by a darker surface and a more pronounced, slightly bitter flavor, which moderately reduced consumer acceptability. These findings suggest that a moderate inclusion level of *Nigella sativa* flour enhances the product's flavor complexity and aromatic profile without altering the characteristic texture and visual uniformity of Telemea cheese. Bakheit & Foda, (2012) also found that moderate cumin addition enhanced cheese acceptability, while higher levels led to a decline in consumer preference.

The bar chart from Figure 4 provides a clear illustration of the findings, demonstrating that moderate fortification (0.5%) enhances flavor and overall acceptability without compromising texture or visual attributes. These results are consistent with those reported by Hassanien et al., (2014) who demonstrated that low-level *Nigella sativa* enrichment improves sensory appeal, while excessive addition can negatively influence product balance.

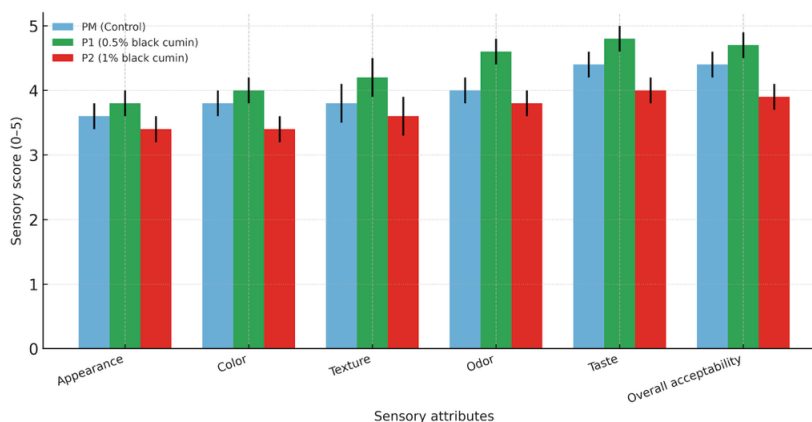


Figure 4. Sensory evaluation of Telemea cheese fortified with black cumin flour

4. Conclusions

The present study demonstrated that the incorporation of *Nigella sativa* flour into Telemea cheese significantly influenced its physicochemical, microbiological, and sensory characteristics in a concentration-dependent manner. The incorporation of 0.5% *Nigella sativa* flour (P1) resulted in an augmentation of the protein content ($18.53 \pm 0.10\%$) and total solids ($46.40 \pm 0.18\%$), while concurrently maintaining an optimal moisture level ($50.66 \pm 0.22\%$) and a desirable pH (4.45 ± 0.01) during the maturation process. In contrast, the 1% supplementation (P2) further increased protein ($20.13 \pm 0.09\%$) and total solids ($49.34 \pm 0.20\%$), but resulted in a denser curd and an overly intense flavor, slightly lowering sensory acceptance.

Fortification significantly increased total polyphenol content and antioxidant capacity (DPPH and ABTS) in a dose-dependent manner ($p < 0.05$), confirming the successful transfer and activity of plant-derived bioactive compounds within the cheese matrix.

A strong positive correlation between total polyphenol content and antioxidant activity further supports the role of phenolic compounds as primary contributors to the enhanced functional profile. The results indicate that even low inclusion levels (0.5%) can produce statistically significant improvements compared to the control, highlighting the efficiency of moderate fortification strategies. Although the 1% addition provided the highest functional values,

technological and sensory considerations suggest that the 0.5% level may represent a more balanced formulation, combining measurable bioactive enhancement with product stability and consumer acceptability.

Microbiological analyses revealed that the presence of *Nigella sativa* flour effectively inhibited the development of *Enterobacteriaceae*, yeasts, and molds, indicating a strong antimicrobial effect that may contribute to improved product safety and shelf stability. These findings support the potential use of *Nigella sativa* as a natural preservative in brined cheese systems. Sensory evaluation revealed that moderate enrichment (0.5%) achieved the highest overall score (22.0 ± 1.8), characterized by a pleasant, balanced flavor and typical Telemea texture.

Overall, the results suggest that a fortification level of 0.5% provides the most balanced formulation, combining enhanced functional value, microbiological stability, and favorable sensory characteristics. This study highlights the potential of *Nigella sativa* flour as a clean-label ingredient for the development of functional Telemea cheese and similar white brined cheeses. Further research should focus on the long-term stability of bioactive compounds during storage and on the scalability of the proposed formulation under industrial production conditions.

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