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*Research article*

## **Dietary inclusion of enzyme- or probiotic-treated date palm leaves enhances the textural and sensory properties of cheese produced from sheep milk**

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**Abstract:** This study evaluated the effects of including date palm leaves (DPL) in the diets of lactating Farafra ewes on milk yield, fatty acid composition, and the quality of white soft cheese produced from their milk. The leaves were ensiled either untreated, with fibrolytic enzymes (ENZ), or with multi-species probiotics (MSP). Diets included DPL at 50% or 100% of the forage portion. Ensiling DPL with ENZ or MSP significantly increased milk yield (linear effect,  $p < 0.01$ ) and enhanced milk components, including total solids, fat, lactose, and energy content ( $p < 0.001$ ), with MSP diets producing higher solids-not-fat and lactose ( $p < 0.001$ ) than ENZ. Both treatments improved milk fatty acid profiles by increasing polyunsaturated and conjugated linoleic acids and elevating the unsaturated-to-saturated fatty acid ratio, while reducing the atherogenicity index ( $p < 0.001$ ). Cheese produced from milk of ewes fed treated DPL exhibited signs of accelerated ripening during 28 days of storage, as evidenced by increased titratable acidity, soluble nitrogen, and total volatile fatty acids, along with decreased pH. Moisture decreased significantly ( $p < 0.001$ ), whereas acidity increased markedly ( $p < 0.001$ ) during storage across all treatments, and total volatile fatty acids also increased ( $p < 0.001$ ), reaching the highest values in MSP100 cheeses at the end of storage. Total and soluble nitrogen contents also increased over storage ( $p < 0.001$ ), indicating enhanced proteolysis. Sensory scores for flavor, texture, and appearance improved significantly in ENZ and MSP groups ( $p \leq 0.011$ ), with higher overall acceptability observed in ENZ50 cheese, followed by MSP50 ( $p < 0.001$ ). Textural analysis showed marked increases in hardness, cohesiveness, springiness, gumminess, and chewiness during storage ( $p < 0.001$ ). Cheeses from the MSP50 group

exhibited superior texture attributes, including hardness, cohesiveness, gumminess, and chewiness values compared to other treatments ( $p < 0.01$ ). The interaction between treatment and storage time was significant for most parameters ( $p < 0.01$ ), confirming the dynamic influence of diet and ripening duration. In conclusion, feeding lactating ewes with DPL ensiled with fibrolytic enzymes or MSP improved milk yield, fatty acid composition, and the sensory and textural qualities of white soft cheese. The results highlight treated DPL as a promising feed option for sustainable dairy production.

**Keywords:** date palm leaves; white soft cheese; fibrolytic enzymes; multi-species probiotics; cheese texture; cheese ripening

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## 1. Introduction

Livestock production is a cornerstone of agricultural economies worldwide, particularly in arid and desert regions where crop production is constrained by harsh environmental conditions. In such environments, the availability of high-quality feed is a major constraint to sustainable animal production [1]. The use of agricultural by-products and crop residues offers a cost-effective and sustainable strategy to improve ruminant nutrition while reducing competition with human food resources [2].

Date palm leaves (DPL) are a widely available agricultural residue in many Middle Eastern and North African countries, including Egypt, where date palm cultivation is widespread. These leaves represent a potential roughage source for small ruminants, such as sheep and goats, which are well adapted to arid environments and contribute significantly to rural livelihoods [3]. However, the high lignocellulosic content in untreated DPL limits their digestibility and reduces their nutritional value, thereby restricting their inclusion in ruminant diets due to their adverse effects on intake and performance [4].

To overcome these limitations, several strategies have been employed to improve the nutritional quality of lignocellulosic feedstuffs. Ensiling, a fermentation-based preservation technique, can improve the palatability and digestibility of roughages by promoting the breakdown of structural carbohydrates and preserving nutrients [1,2]. Fibrolytic enzyme addition can further enhance fiber digestion and increase fermentable substrate availability for rumen microorganisms [5]. Similarly, multi-species probiotics (MSP) containing cellulolytic and lactic acid bacteria can improve silage fermentation quality, stabilize silage pH values, and enhance microbial efficiency in the rumen [6].

Improved utilization of treated DPL in the diets of lactating ewes could contribute to increased nutrient intake, better ruminal fermentation, and subsequently improved milk production and composition [1,2]. Milk quality is of paramount importance not only for direct consumption but also for the manufacture of high-value dairy products, such as white soft cheese, a common food in Mediterranean and Middle Eastern diets. Enhancing the fatty acid profile of sheep milk, particularly by increasing levels of polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA), and omega-3 fatty acids, can improve the nutritional and functional quality of dairy products, thereby enhancing both nutritional value and marketability [7].

The cheese ripening process and its final sensory, textural, and physicochemical characteristics are directly affected by the quality and composition of the raw milk. Dietary manipulation of

lactating animals to improve milk fat and protein fractions, antioxidant capacity, and fatty acid profile can positively affect cheese maturation and shelf life [8,9]. However, studies investigating the effects of feeding treated DPL on milk yield, milk fatty acids, and cheese quality in sheep are limited, especially under the specific environmental and production conditions of Egypt. Recently, Kholif and Kholif [10] investigated the effects of feeding lactating Farafra ewes diets containing DPL ensiled with either malic or lactic acid at inclusion levels of 50% and 100%. Their results showed that both diet and storage time significantly affected the chemical composition of white soft cheese. Cheeses produced from milk of ewes fed higher inclusion levels of treated DPL exhibited lower moisture and pH, alongside increased titratable acidity, volatile fatty acids (VFAs), salt content, total nitrogen (TN), and soluble nitrogen (SN). In addition, sensory attributes, including appearance, flavor, texture, and overall acceptability, improved significantly as storage progressed.

While previous studies have evaluated the effects of feeding treated DPL on animal performance and milk production, information regarding the impact of such treatments on cheese quality, ripening behavior, texture, and sensory properties remains limited. The present study was therefore designed to extend these findings by evaluating the downstream effects of feeding ensiled DPL treated with fibrolytic enzymes or MSP at two inclusion levels on milk composition and the physicochemical, ripening indices, sensory, and texture properties of white soft cheese produced from Farafra ewe milk. The findings provide new insights into how dietary strategies influence dairy product quality, supporting sustainable feed resource utilization and contributing to the development of cost-effective feeding strategies for dairy sheep production in arid regions.

## 2. Materials and methods

### 2.1. Experimental diets

The lactation trial was described in detail by Kholif et al. [1]. In summary, DPL were sun-dried for about 10 days and then ensiled anaerobically for 45 days using a urea-molasses mixture to reach a moisture content of 35–40%. The prepared materials were thoroughly mixed with the respective additives (enzyme or probiotic) and packed into polyethylene bag silos (approximately 40 × 70 cm). The material was manually compressed to expel air and ensure anaerobic conditions and then sealed tightly and stored at ambient temperature throughout the ensiling period. Three silage types were prepared: untreated DPL (control), DPL treated with fibrolytic enzymes (ENZ; Polyzyme, Zeus Biotech, India; applied at 4 g/kg DM; a multi-enzyme complex containing per kg:  $4 \times 10^6$  IU xylanase,  $4 \times 10^5$  IU cellulase,  $2.4 \times 10^5$  IU pectinase,  $2 \times 10^5$  IU  $\beta$ -glucanase,  $21.5 \times 10^6$  IU amylase,  $7.5 \times 10^5$  IU protease,  $4 \times 10^5$  IU galactosidase,  $2 \times 10^5$  IU mannanase,  $5 \times 10^4$  IU phytase, and  $4 \times 10^4$  IU lipase, with fermented rice bran as a carrier), and DPL treated with MSP (ProAct, India; applied at 4 g/kg DM; containing lactic acid bacteria and  $1.75 \times 10^{12}$  CFU/g each of *Bacillus subtilis* and *Bacillus licheniformis*, with dextrose monohydrate as a filler). Fifty lactating Farafra ewes ( $33.3 \pm 3.04$  kg BW;  $2 \pm 1.2$  parity) were randomly assigned into five groups (10 animals per treatment) for a 90-day feeding trial. The treatments included a control diet containing untreated DPL and four diets in which DPL was replaced by ENZ- or MSP-treated silage at 50% or 100% inclusion levels (ENZ50, ENZ100, MSP50, MSP100). Diets were formulated to meet NRC [11] nutrient requirements and were offered individually at 110% of the predicted intake. The chemical composition of the diets (g/kg DM) is presented in Table 1.

Milk samples were collected twice daily (09:00 and 21:00 h) during the final 10 days of each experimental period (days 20–30, 50–60, and 80–90). At each milking, 10% of the recorded milk yield was sampled, and subsamples were composited on a daily basis for each ewe prior to laboratory analysis. Samples were analyzed for fat, protein, lactose, and total solids using infrared spectrophotometry (Lactostar Dairy Analyzer, Funke Gerber, Berlin, Germany). Fatty acid composition was determined by gas chromatography (ISO 15884-IDF 182), and the atherogenicity index was calculated as described by Ulbricht and Southgate [12]. The gross energy content of milk was calculated according to Tyrrell and Reid [13].

**Table 1.** Proximate composition of experimental diets (g/kg DM).

|               | Diet <sup>1</sup> |       |        |       |        |
|---------------|-------------------|-------|--------|-------|--------|
|               | Control           | ENZ50 | ENZ100 | MSP50 | MSP100 |
| DM            | 838.3             | 840.9 | 843.5  | 842.5 | 846.7  |
| OM            | 916.5             | 916.7 | 916.9  | 917.3 | 918.1  |
| CP            | 117.8             | 118.4 | 119.0  | 118.4 | 119.0  |
| EE            | 36.9              | 36.7  | 36.5   | 36.9  | 36.9   |
| NSC           | 358.8             | 372.8 | 386.8  | 359.2 | 369.6  |
| NDF           | 403.1             | 388.9 | 374.7  | 402.9 | 402.7  |
| ADF           | 231.5             | 227.7 | 223.9  | 232.1 | 232.7  |
| Lignin        | 68.6              | 69.2  | 69.8   | 68.4  | 68.2   |
| Cellulose     | 162.8             | 158.4 | 154.0  | 163.6 | 164.4  |
| Hemicellulose | 171.6             | 161.2 | 150.8  | 170.8 | 170.0  |

ADF: acid detergent fiber; CP: crude protein; DM: dry matter; EE: ether extract; NDF: neutral detergent fiber; NSC: non-structural carbohydrates; OM: organic matter. <sup>1</sup>Control diet contained 600 g of concentrate feed mixture and 400 g of ensiled DPL without additives. In the other treatments, the ensiled DPL was partially or fully replaced with DPL treated with fibrolytic enzymes (ENZ50 and ENZ100) or multi-species probiotics (MSP50 and MSP100) at 50% or 100% replacement levels, respectively.

## 2.2. Materials and cheese manufacture

Ewes' milk obtained from the experimental animals assigned to each dietary treatment was collected individually during the final sampling period and composited per ewe across days and then pooled within each treatment to obtain a representative bulk milk sample for cheese manufacture. The pooled milk for each treatment was used to produce cheese under standardized conditions. Milk was characterized by a pH of  $6.63 \pm 0.1$  and titratable acidity of  $0.17 \pm 0.01\%$ . The New Valley University experimental farm in Al Kharga, New Valley, Egypt, was used for cheese manufacture. White soft cheese was prepared using microbial rennet (Chy-Max, Chr. Hansen Holding A/S, Hørsholm, Denmark) at 1 g per 100 L of milk. For each dietary treatment, three independent cheese-making batches (replicates) were prepared from separate pooled milk collections, and each batch was considered an experimental unit.

Cheese was manufactured from milk obtained from ewes fed five dietary treatments: Control, ENZ50, ENZ100, MSP50, and MSP100. The milk was heated to 72 °C for 15 seconds and then cooled to 37 °C. Salt was added at a concentration of 3%. Five types of soft cheese were produced, corresponding to the five dietary treatments (Control, ENZ50, ENZ100, MSP50, and MSP100). Each

type was inoculated with 1% active starter culture, containing equal parts of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*. Each treatment was manufactured in three independent replicates.

Each treatment received 0.02% calcium chloride, and the milk was kept at 37 °C for 1 hour. Microbial rennet was added to allow coagulation within 40 minutes. The milk was then incubated at 37 °C until a firm curd formed. The soft cheese was divided into plastic containers for chemical, rheological, and sensory testing. Containers were sealed and stored at  $5 \pm 2$  °C for 28 days. Samples were analyzed when fresh (day 0) and after 7, 14, and 28 days to monitor changes in composition and quality.

### 2.3. Physicochemical properties analysis

The physicochemical traits of milk and cheese were measured using standard methods. Moisture content was determined according to AOAC [14]. Ash content was measured by incinerating samples at 550 °C for 5 hours in a muffle furnace. Total nitrogen and SN were analyzed using semi-micro Kjeldahl distillation following AOAC guidelines [14]. Titratable acidity (TA) was assessed in milk and cheese and expressed as a percentage of lactic acid [14]. Fat content was measured by the Gerber method for both milk and cheese [14]. Total VFAs in cheese were measured by steam distillation and expressed as milliliters of 0.1 N NaOH per 100 g of cheese, following Kosikowski [15].

### 2.4. Sensory evaluation

Sensory evaluation of white soft cheese followed the organoleptic scoring method of Clark et al. [16]. Each treatment was evaluated using cheese samples obtained from three independent production batches (replicates). A trained panel of ten assessors (five men and five women, aged 25–45) from the Dairy Science Department, National Research Centre (Giza, Egypt) conducted the tests. Prior to the formal evaluation, panelists underwent a short training session to familiarize them with the scoring criteria and sensory attributes of white soft cheese. Reference samples representing a range of quality attributes were used to calibrate panelists and improve scoring consistency.

Each treatment was evaluated in three independent replicates. Panelists scored the samples for flavor (50 points), body and texture (40 points), and appearance (10 points), for a total of 100 points. Panel scores from assessors were averaged within each batch, and batch means were used as the experimental units for statistical analysis.

Cheese (~20 g) was cut into uniform cubes, labeled with random three-digit codes, and served in odor-free cups at  $15 \pm 1$  °C. Evaluations took place in controlled conditions (individual booths, white lighting,  $22 \pm 2$  °C). Water was provided between samples to avoid flavor carryover. For each dietary treatment and ripening period, three samples (one per batch) were assessed. The procedure was repeated three times across storage periods to ensure reproducibility. Mean panel scores were used for statistical analysis.

### 2.5. Textural profile analysis

Textural profile analysis was performed on white soft cheese samples using a double

compression test, following Glibowski et al. [17]. A TA-XT2i texture analyzer (Stable Micro Systems, UK) recorded force (N) versus time (s). A 25-mm Perspex conical probe was used to test samples in their storage containers, with five replicates per sample. Each sample was compressed to 30% of its original height at 2 cm/min during the pre-test and compression phases. Textural profile analysis parameters measured included:

- 1) Hardness: maximum force during the first compression
- 2) Cohesiveness: ability to withstand a second deformation relative to the first
- 3) Springiness: recovery of original height after compression
- 4) Gumminess: energy needed to break a semi-solid to a swallowable state (hardness  $\times$  cohesiveness)
- 5) Chewiness: energy required to chew a solid food until ready to swallow (gumminess  $\times$  springiness)

Textural measurements were performed on samples obtained from each independent cheese batch (replicate), with multiple readings averaged per batch prior to statistical analysis.

## 2.6. Statistical analysis

A completely randomized design with repeated measures over storage time was employed. Data from storage days 0, 7, 14, 21, and 28 were analyzed using the PROC MIXED procedure in SAS (SAS Institute Inc., Cary, NC, USA). Treatment (five dietary treatments) and storage period were included as fixed effects, and their interaction (treatment  $\times$  storage period) was also tested. Cheese-making batch (replicate) was considered the experimental unit and included as a random effect. Storage time was treated as a repeated measure within each batch. The model was:  $Y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + R_k + e_{ijk}$  where  $Y_{ijk}$  is the observation of the  $i$ th treatment in the  $j$ th storage period,  $T_i$  is the fixed effect of the  $i$ th treatment,  $P_j$  is the fixed effect of the  $j$ th storage period,  $(T \times P)_{ij}$  is the interaction between the treatments and storage period,  $R_k$  is the random effect of the  $k$ th cheese-making batch (replicate), and  $e_{ijk}$  is the experimental error. An appropriate covariance structure (e.g., compound symmetry or autoregressive) was selected based on the lowest Akaike information criterion. Linear and quadratic polynomial contrasts were applied to assess responses to increasing DPL levels. Results were considered significant at  $p < 0.05$ .

## 3. Results

### 3.1. Milk production and fatty acids

No significant differences were found between ENZ and MSP treatments in milk yield or milk component production ( $p = 0.645$ ; Table 2, ENZ vs. MSP comparison). However, ensiling DPL with either enzyme or MSP caused a linear increase ( $p < 0.01$ ) in milk production. Milk protein and ash contents were not affected. Both treatments nevertheless produced a linear rise ( $p < 0.001$ ) and a quadratic effect ( $p < 0.01$ ) in total solids, solids-not-fat, fat, lactose, and milk energy. MSP treatments produced significantly higher levels of solids-not-fat and lactose compared to enzyme treatments ( $p < 0.001$ ).

Both ENZ and MSP diets led to increases in PUFA, total CLA, and the ratio of total UFA to total SFA. At the same time, treatments caused a decrease (linear and quadratic effects,  $p < 0.001$ ) in the atherogenicity index (Table 2).

**Table 2.** Milk yield, composition, and fatty acid profile of ewes fed treated or untreated DPL<sup>1,2</sup>.

|                                   | ENZ     |       |        | MSP   |        | SEM   | P-ENZ  |           | P-MSP  |           | ENZ vs. MSP |
|-----------------------------------|---------|-------|--------|-------|--------|-------|--------|-----------|--------|-----------|-------------|
|                                   | Control | ENZ50 | ENZ100 | MSP50 | MSP100 |       | Linear | Quadratic | Linear | Quadratic |             |
| Milk production, g/d              | 603     | 657   | 667    | 652   | 660    | 13.3  | 0.001  | 0.182     | 0.003  | 0.209     | 0.645       |
| Composition, g/kg                 |         |       |        |       |        |       |        |           |        |           |             |
| Total solids                      | 133     | 139   | 140    | 140   | 141    | 0.4   | <0.001 | <0.001    | <0.001 | <0.001    | 0.808       |
| Solids non-fat                    | 91.5    | 93.6  | 93.3   | 94.8  | 95.4   | 0.3   | <0.001 | 0.001     | <0.001 | 0.001     | <0.001      |
| Fat                               | 41.6    | 45.7  | 46.2   | 45.4  | 45.4   | 0.3   | <0.001 | <0.001    | <0.001 | <0.001    | 0.055       |
| Protein                           | 41.5    | 41.7  | 41.6   | 41.7  | 42.2   | 0.23  | 0.818  | 0.69      | 0.051  | 0.692     | 0.178       |
| Lactose                           | 42.3    | 44.1  | 43.9   | 45.3  | 45.4   | 0.17  | <0.001 | <0.001    | <0.001 | <0.001    | <0.001      |
| Ash                               | 7.65    | 7.87  | 7.83   | 7.8   | 7.77   | 0.12  | 0.248  | 0.341     | 0.423  | 0.498     | 0.584       |
| Milk energy content, MJ/kg        | 3.25    | 3.45  | 3.47   | 3.46  | 3.48   | 0.012 | <0.001 | <0.001    | <0.001 | <0.001    | 0.347       |
| Fatty acid profile, %             |         |       |        |       |        |       |        |           |        |           |             |
| SFA                               | 70.3    | 68.5  | 68.6   | 68.5  | 68.7   | 0.94  | 0.661  | 0.601     | 0.089  | 0.088     | 0.516       |
| UFA                               | 30      | 31.8  | 31.9   | 31.9  | 31.6   | 0.94  | 0.568  | 0.083     | 0.081  | 0.024     | 0.482       |
| MUFA                              | 28.7    | 30.4  | 30.4   | 30.4  | 30.2   | 0.94  | 0.056  | 0.082     | 0.111  | 0.146     | 0.376       |
| PUFA                              | 1.32    | 1.46  | 1.46   | 1.5   | 1.47   | 0.014 | <0.001 | 0.001     | <0.001 | <0.001    | 0.074       |
| Total CLA                         | 0.45    | 0.5   | 0.49   | 0.52  | 0.5    | 0.012 | 0.011  | 0.11      | 0.003  | 0.004     | 0.224       |
| n-6: n-3 FA                       | 2.2     | 2.22  | 2.22   | 2.22  | 2.26   | 0.045 | 0.662  | 0.867     | 0.3    | 0.949     | 0.620       |
| UFA: SFA ratio                    | 0.43    | 0.46  | 0.46   | 0.47  | 0.46   | 0.003 | <0.001 | <0.001    | <0.001 | <0.001    | 0.700       |
| Atherogenicity index <sup>3</sup> | 2.2     | 2.01  | 2      | 2.01  | 2.04   | 0.015 | <0.001 | <0.001    | <0.001 | <0.001    | 0.287       |

SEM: standard error of the mean; CLA: conjugated linoleic acid (C18:2 trans-10, cis-12 and C18:2 cis-9, trans-11); MUFA: mono-unsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; UFA: unsaturated fatty acids. <sup>1</sup>Control diet included 600 g of concentrate feed and 400 g of date palm leaves (DPL) ensiled without additives. In other treatments, the additive-free DPL was partially or fully replaced with DPL ensiled with fibrolytic enzymes (ENZ50, ENZ100) or multi-species probiotics (MSP50, MSP100) at 50% or 100% replacement levels, respectively. <sup>2</sup>Previously reported by Kholif et al. [1]. <sup>3</sup>Calculated according to Ulbricht and Southgate [12] equation.

### 3.2. Chemical analysis of soft cheese

Table 3 shows the chemical analysis of cheese produced from the milk of ewes fed diets containing DPL ensiled with or without additives over a 28-day storage period. Across all treatments and storage intervals, multiple cheese quality indicators exhibited significant trends. Moisture content decreased linearly ( $p < 0.01$ ) over time in all treatments. The highest moisture levels were recorded on day 0, with the lowest at day 28. This decline was more pronounced in cheeses derived from the ENZ100, MSP50, and MSP100 groups. In contrast, ash content showed only minor variations among treatments, displaying slight linear or quadratic trends without significant treatment effects ( $p = 0.238$ ). However, ash showed a significant linear decline over the storage period ( $p < 0.001$ ).

**Table 3.** Chemical composition and ripening changes of the white soft cheese produced from milk of lactating Farafra ewes fed diet containing treated or untreated DPL during storage<sup>1</sup>.

|         | Storage day | Moisture<br>(%) | Ash<br>(%) | Acidity<br>(%) | pH<br>value | Salt<br>(%) | TN<br>(%) | SN<br>(%) | VFAs<br>(mL 0.1 N NaOH/100 g) |
|---------|-------------|-----------------|------------|----------------|-------------|-------------|-----------|-----------|-------------------------------|
| Control | 0           | 64.2            | 3.69       | 0.22           | 6.26        | 2.53        | 2.15      | 0.19      | 5.57                          |
|         | 7           | 63.3            | 3.67       | 1.01           | 6.06        | 2.53        | 2.31      | 0.26      | 7.79                          |
|         | 14          | 62.5            | 3.26       | 1.19           | 5.81        | 2.66        | 2.46      | 0.32      | 12.6                          |
|         | 21          | 62.3            | 3.32       | 1.48           | 5.5         | 2.62        | 2.62      | 0.52      | 15.28                         |
|         | 28          | 60.8            | 3.18       | 1.73           | 5.26        | 2.62        | 2.83      | 0.61      | 18.19                         |
|         | SEM         | 0.29            | 0.117      | 0.03           | 0.043       | 0.028       | 0.043     | 0.033     | 0.726                         |
|         | Linear      | 0.003           | 0.027      | <0.001         | <0.001      | 0.009       | 0.001     | 0.014     | <0.001                        |
|         | Quadratic   | 0.946           | 0.198      | <0.001         | 0.601       | 0.077       | 0.975     | 0.903     | 0.177                         |
| ENZ50   | 0           | 64.6            | 3.77       | 0.32           | 6.08        | 2.57        | 2.18      | 0.19      | 4.43                          |
|         | 7           | 63.6            | 3.6        | 1.27           | 5.5         | 2.7         | 2.46      | 0.38      | 9.33                          |
|         | 14          | 62.2            | 3.55       | 1.68           | 4.96        | 2.79        | 2.4       | 0.51      | 12.33                         |
|         | 21          | 61.7            | 3.45       | 1.83           | 4.41        | 2.84        | 2.85      | 0.59      | 15.55                         |
|         | 28          | 60.3            | 3.3        | 2.35           | 4.18        | 2.94        | 2.88      | 0.61      | 22.69                         |
|         | SEM         | 0.403           | 0.071      | 0.07           | 0.067       | 0.026       | 0.126     | 0.035     | 1.626                         |
|         | Linear      | 0.002           | 0.053      | <0.001         | <0.001      | 0.001       | 0.244     | <0.001    | 0.006                         |
|         | Quadratic   | 0.745           | 0.506      | 0.011          | 0.797       | 0.571       | 0.295     | 0.546     | 0.643                         |
| ENZ100  | 0           | 63.3            | 3.75       | 0.33           | 6.37        | 2.62        | 2.19      | 0.2       | 6.2                           |
|         | 7           | 62.6            | 3.65       | 1.29           | 5.66        | 2.72        | 2.4       | 0.35      | 9.25                          |
|         | 14          | 60.5            | 3.52       | 1.51           | 4.94        | 2.91        | 2.47      | 0.47      | 13.13                         |
|         | 21          | 59.4            | 3.39       | 1.77           | 4.49        | 3.01        | 2.73      | 0.57      | 16.42                         |
|         | 28          | 57.3            | 3.37       | 1.98           | 4.46        | 3.31        | 2.84      | 0.58      | 20.49                         |
|         | SEM         | 0.925           | 0.109      | 0.061          | 0.118       | 0.04        | 0.028     | 0.024     | 0.479                         |
|         | Linear      | 0.059           | 0.171      | <0.001         | <0.001      | 0.001       | <0.001    | <0.001    | <0.001                        |
|         | Quadratic   | 0.57            | 0.903      | 0.001          | 0.955       | 0.359       | 0.096     | 0.665     | 0.489                         |
| MSP50   | 0           | 64              | 3.77       | 0.32           | 6.03        | 2.61        | 2.13      | 0.18      | 6.01                          |
|         | 7           | 62.6            | 3.72       | 1.56           | 5.69        | 2.66        | 2.34      | 0.33      | 7.4                           |
|         | 14          | 61.2            | 3.56       | 1.71           | 5.02        | 2.76        | 2.5       | 0.51      | 12.52                         |
|         | 21          | 57.4            | 3.31       | 1.9            | 5.01        | 2.85        | 2.63      | 0.57      | 17.31                         |
|         | 28          | 56.1            | 3.4        | 2.32           | 4.27        | 3.18        | 2.75      | 0.65      | 22.06                         |
|         | SEM         | 0.432           | 0.109      | 0.059          | 0.166       | 0.044       | 0.042     | 0.028     | 0.577                         |
|         | Linear      | 0.001           | 0.21       | <0.001         | 0.002       | 0.029       | 0.001     | <0.001    | <0.001                        |
|         | Quadratic   | 0.998           | 0.663      | <0.001         | 0.432       | 0.607       | 0.662     | 0.567     | 0.025                         |
| MSP100  | 0           | 64              | 3.8        | 0.27           | 6.26        | 2.57        | 1.96      | 0.16      | 6.96                          |
|         | 7           | 62.6            | 3.61       | 1.23           | 5.88        | 2.64        | 2.23      | 0.26      | 10.36                         |
|         | 14          | 61.4            | 3.37       | 1.43           | 5.63        | 2.76        | 2.54      | 0.35      | 17.5                          |
|         | 21          | 60.2            | 3.35       | 1.83           | 4.78        | 2.82        | 2.65      | 0.5       | 18.52                         |
|         | 28          | 59.6            | 3.17       | 2.14           | 4.42        | 2.87        | 2.77      | 0.58      | 24.83                         |
|         | SEM         | 0.526           | 0.108      | 0.038          | 0.087       | 0.021       | 0.061     | 0.029     | 1.534                         |
|         | Linear      | 0.006           | 0.019      | <0.001         | 0.001       | <0.001      | <0.001    | 0.001     | 0.001                         |

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| Storage day           | Moisture (%) | Ash (%) | Acidity (%) | pH value | Salt (%) | TN (%) | SN (%) | VFAs (mL 0.1 N NaOH/100 g) |
|-----------------------|--------------|---------|-------------|----------|----------|--------|--------|----------------------------|
| Quadratic             | 0.896        | 0.835   | <0.001      | 0.556    | 0.35     | 0.777  | 0.817  | 0.342                      |
| SEM for treatment     | 0.25         | 0.047   | 0.024       | 0.047    | 0.015    | 0.031  | 0.013  | 0.494                      |
| P value for treatment | <0.001       | 0.238   | <0.001      | <0.001   | <0.001   | 0.055  | <0.001 | <0.001                     |
| SEM for storage       | 0.25         | 0.047   | 0.024       | 0.047    | 0.015    | 0.031  | 0.0134 | 0.494                      |
| P value for storage   |              |         |             |          |          |        |        |                            |
| Linear                | <0.001       | <0.001  | <0.001      | <0.001   | <0.001   | <0.001 | <0.001 | <0.001                     |
| Quadratic             | 0.642        | 0.398   | <0.001      | 0.688    | 0.103    | 0.216  | 0.685  | 0.143                      |
| ENZ vs. MSP           | <0.001       | <0.001  | <0.001      | <0.001   | <0.001   | <0.001 | <0.001 | <0.001                     |
| Treatment × Storage   | <0.001       | <0.001  | <0.001      | <0.001   | <0.001   | <0.001 | <0.001 | <0.001                     |

<sup>1</sup>Control cheese was manufactured from milk of ewes fed a diet containing 600 g of concentrate feed mixture and 400 g of DPL ensiled without additives. Cheeses from the other treatments were manufactured from milk of ewes fed DPL ensiled with fibrolytic enzymes (ENZ50 and ENZ100) or MSP (MSP50 and MSP100) at 50 or 100% level, respectively. TN = total nitrogen, SN = soluble nitrogen, VFAs = total volatile fatty acids.

Acidity levels increased significantly during storage, both linearly and quadratically ( $p < 0.001$ ), in all treatment groups. The largest increase occurred in the ENZ50 (2.35%) and MSP50 (2.32%), followed by MSP100 (2.14%) on day 28. Correspondingly, pH values declined significantly over time ( $p < 0.001$ ) across all treatments. The ENZ and MSP groups exhibited a more rapid acidification compared to the control, with pH values reaching as low as 4.18 in treated groups, whereas the control remained at 5.26 on day 28. The lowest pH was recorded in the ENZ50 and MSP50 groups, reaching 4.18 and 4.27, respectively.

Salt concentration increased gradually throughout the storage period ( $p < 0.001$ ), particularly in the ENZ100 and MSP50 cheeses. In terms of protein content, TN was numerically higher ( $p = 0.055$ ) in cheeses from ewes fed ENZ- or MSP-treated DPL relative to the control, with a significant linear increase observed over time ( $p < 0.001$ ), particularly in the ENZ100 and MSP100 groups. Similarly, SN, which is a key indicator of proteolysis and cheese ripening, also increased significantly over the storage period ( $p < 0.001$ ). The highest SN content was detected in MSP50 cheese at day 28 (0.65%), followed by ENZ50 (0.61%) and MSP100 (0.58%).

Total VFAs, which reflect lipolysis and contribute to cheese flavor development, rose markedly during storage in all groups ( $p < 0.001$ ). The highest concentration of VFAs was recorded in the MSP100 cheese at day 28 (24.83 mL 0.1 N NaOH/100 g), followed by ENZ50 (22.69 mL 0.1 N NaOH/100 g) and MSP50 (22.06 mL 0.1 N NaOH/100 g).

### 3.3. Sensory evaluation

The sensory characteristics of the cheese produced from milk of ewes fed diets containing DPL ensiled with or without ENZ or MSP over a 28-day storage period are shown in Table 4. Appearance scores improved significantly ( $p = 0.003$ ) over storage, particularly in cheeses from ENZ- and MSP-fed animals. The highest score (8.50) was observed in MSP50 at day 28. Flavor scores, a critical determinant of cheese acceptability, increased significantly over time ( $p < 0.001$ ), especially in cheeses from the ENZ and MSP groups. ENZ50 followed by ENZ100 treatments showed notably higher flavor scores than the control by day 28. There were significant effects of treatment ( $p < 0.001$ ) and a strong interaction between treatment and storage period ( $p < 0.001$ ).

Texture scores also improved across all treatments ( $p = 0.008$ ), with MSP50 yielding cheese with the highest texture ratings at the later stages of storage. Total sensory scores increased significantly over time ( $p < 0.001$ ). Cheeses from ewes fed ENZ50, MSP50, and ENZ100 had the highest scores by day 28 (90.0, 88.1, and 87.5, respectively), compared to 85.0 in the control. The results showed a clear effect of treatment ( $p < 0.001$ ), time ( $p < 0.001$ ) and their interaction ( $p < 0.001$ ).

**Table 4.** Sensory evaluation of the white soft cheese produced from milk of lactating Farafra ewes fed diet containing treated or untreated DPL during storage<sup>1</sup>.

|         | Storage day | Appearance (10) | Flavor (50) | Texture (40) | Total score (100) |
|---------|-------------|-----------------|-------------|--------------|-------------------|
| Control | 0           | 6.83            | 39.8        | 31.3         | 78.0              |
|         | 7           | 6.67            | 39.6        | 35.5         | 81.8              |
|         | 14          | 7.90            | 42.7        | 32.9         | 83.5              |
|         | 21          | 6.59            | 43.3        | 34.7         | 84.6              |
|         | 28          | 7.40            | 42.9        | 34.8         | 85.0              |
|         | SEM         | 0.478           | 1.07        | 0.81         | 1.71              |
|         | Linear      | 0.147           | 0.087       | 0.197        | 0.045             |
|         | Quadratic   | 0.260           | 0.241       | 0.007        | 0.626             |
| ENZ50   | 0           | 6.50            | 41.6        | 33.0         | 81.1              |
|         | 7           | 7.17            | 42.3        | 33.1         | 82.6              |
|         | 14          | 7.58            | 45.7        | 32.1         | 85.4              |
|         | 21          | 7.83            | 46.5        | 34.7         | 89.1              |
|         | 28          | 7.78            | 47.2        | 35.0         | 90.0              |
|         | SEM         | 0.221           | 0.54        | 1.04         | 1.02              |
|         | Linear      | 0.006           | 0.001       | 0.573        | 0.013             |
|         | Quadratic   | 0.654           | 0.073       | 0.709        | 0.583             |
| ENZ100  | 0           | 6.83            | 41.0        | 31.1         | 78.9              |
|         | 7           | 7.17            | 41.8        | 32.5         | 81.4              |
|         | 14          | 7.17            | 42.9        | 34.3         | 84.4              |
|         | 21          | 7.67            | 44.0        | 35.0         | 86.6              |
|         | 28          | 8.33            | 44.3        | 34.8         | 87.5              |
|         | SEM         | 0.459           | 0.32        | 0.47         | 0.57              |
|         | Linear      | 0.619           | 0.002       | 0.001        | <0.001            |
|         | Quadratic   | 0.773           | 0.628       | 0.671        | 0.694             |
| MSP50   | 0           | 6.83            | 41.3        | 34.3         | 82.5              |
|         | 7           | 7.50            | 42.3        | 34.3         | 84.1              |
|         | 14          | 7.67            | 43.5        | 35.6         | 86.8              |
|         | 21          | 8.00            | 43.0        | 37.7         | 88.7              |
|         | 28          | 8.50            | 43.7        | 36.0         | 88.1              |
|         | SEM         | 0.211           | 0.64        | 0.72         | 0.65              |
|         | Linear      | 0.019           | 0.036       | 0.246        | 0.001             |
|         | Quadratic   | 0.356           | 0.903       | 0.455        | 0.522             |
| MSP100  | 0           | 6.33            | 40.3        | 31.1         | 77.7              |
|         | 7           | 6.67            | 40.7        | 31.5         | 78.8              |

*Continued on the next page*

| Storage day            | Appearance (10) | Flavor (50) | Texture (40) | Total score (100) |
|------------------------|-----------------|-------------|--------------|-------------------|
| 14                     | 7.17            | 41.3        | 32.8         | 81.3              |
| 21                     | 7.33            | 42.2        | 34.0         | 83.5              |
| 28                     | 7.50            | 42.3        | 35.0         | 84.8              |
| SEM                    | 0.149           | 0.60        | 0.82         | 1.07              |
| Linear                 | 0.003           | 0.267       | 0.164        | 0.039             |
| Quadratic              | 0.658           | 0.912       | 0.641        | 0.631             |
| SEM treatment          | 0.149           | 0.30        | 0.35         | 0.48              |
| P value treatment      | 0.011           | <0.001      | <0.001       | <0.001            |
| SEM storage period     | 0.149           | 0.30        | 0.35         | 0.48              |
| P value storage period |                 |             |              |                   |
| Linear                 | 0.003           | <0.001      | 0.008        | <0.001            |
| Quadratic              | 0.794           | 0.079       | 0.263        | 0.707             |
| ENZ vs MSP             | 0.006           | <0.001      | <0.001       | <0.001            |
| Treatment × storage    | <0.001          | <0.001      | <0.001       | <0.001            |

<sup>1</sup>Control cheese was manufactured from milk of ewes fed a diet containing 600 g of concentrate feed mixture and 400 g of DPL ensiled without additives. Cheeses from the other treatments were manufactured from milk of ewes fed DPL ensiled with fibrolytic enzymes (ENZ50 and ENZ100) or MSP (MSP50 and MSP100) at 50 or 100% level, respectively.

### 3.4. Rheological properties of soft cheese

The texture profile of white soft cheese was significantly affected by both ewe diet and storage time (Table 5). Hardness increased significantly ( $p < 0.001$ ) in all treatments from day 0 to day 28. On day 28, the highest hardness was observed in MSP50 (8.28), followed by ENZ50 (7.55), ENZ100 (6.95), MSP100 (6.69), and Control (5.97). Cohesiveness was influenced by both diet ( $p = 0.008$ ) and storage duration ( $p < 0.001$ ). By day 28, MSP50 (0.77) and MSP100 (0.75) showed the highest cohesiveness, indicating that cheeses from ewes fed probiotic-treated DPL retained a more stable structure. No significant interaction was observed between treatment and storage time for cohesiveness ( $p = 0.114$ ).

Springiness, reflecting the cheese's ability to recover its shape after deformation, also improved significantly over the storage period ( $p < 0.001$ ). Cheeses produced from the MSP50 treatment (0.87) and ENZ50 (0.77) exhibited the greatest elasticity by day 28. The presence of a significant treatment × time interaction ( $p = 0.002$ ) indicates that the development of springiness was influenced by the specific ensiling treatment over time.

Gumminess and chewiness were both affected by dietary treatment ( $p = 0.002$  and  $p < 0.001$ , respectively), storage time ( $p < 0.001$ ), and their interaction ( $p = 0.003$  and  $p < 0.001$ , respectively). The highest gumminess (6.37) and chewiness (5.51) values were observed in the MSP50 treatment on day 28.

**Table 5.** Texture profile analysis of white soft cheese produced from milk of lactating Farafra ewes fed diet containing treated or untreated DPL during storage <sup>1</sup>.

| Treatment <sup>1</sup> | Storage day | Hardness<br>(N) | Cohesiveness<br>(A2/A1, area) | Springiness<br>(mm) | Gumminess<br>(N) | Chewiness<br>(N/mL) |
|------------------------|-------------|-----------------|-------------------------------|---------------------|------------------|---------------------|
| Control                | 0           | 4.83            | 0.46                          | 0.68                | 2.21             | 1.51                |
|                        | 28          | 5.97            | 0.62                          | 0.72                | 3.69             | 2.66                |
|                        | SEM         | 0.074           | 0.020                         | 0.008               | 0.112            | 0.089               |
|                        | P value     | 0.001           | 0.005                         | 0.025               | 0.001            | 0.001               |
| ENZ50                  | 0           | 5.03            | 0.52                          | 0.64                | 2.63             | 1.62                |
|                        | 28          | 7.55            | 0.68                          | 0.77                | 5.11             | 3.90                |
|                        | SEM         | 0.248           | 0.017                         | 0.027               | 0.263            | 0.101               |
|                        | P value     | 0.002           | 0.003                         | 0.026               | 0.003            | <0.001              |
| ENZ100                 | 0           | 5.07            | 0.55                          | 0.59                | 3.11             | 1.87                |
|                        | 28          | 6.95            | 0.68                          | 0.77                | 4.71             | 3.60                |
|                        | SEM         | 0.328           | 0.022                         | 0.032               | 0.436            | 0.316               |
|                        | P value     | 0.016           | 0.013                         | 0.017               | 0.060            | 0.018               |
| MSP50                  | 0           | 5.10            | 0.53                          | 0.62                | 2.70             | 1.69                |
|                        | 28          | 8.28            | 0.77                          | 0.87                | 6.37             | 5.51                |
|                        | SEM         | 0.157           | 0.028                         | 0.029               | 0.232            | 0.144               |
|                        | P value     | 0.001           | 0.004                         | 0.004               | 0.001            | <0.001              |
| MSP100                 | 0           | 5.17            | 0.52                          | 0.70                | 2.68             | 1.87                |
|                        | 28          | 6.69            | 0.75                          | 0.71                | 5.00             | 3.55                |
|                        | SEM         | 0.125           | 0.023                         | 0.028               | 0.082            | 0.116               |
|                        | P value     | 0.001           | 0.002                         | 0.700               | <0.001           | 0.001               |
| SEM treatment          |             | 0.146           | 0.016                         | 0.019               | 0.182            | 0.123               |
| P value treatment      |             | <0.001          | 0.008                         | 0.2307              | 0.002            | <0.001              |
| SEM day                |             | 0.093           | 0.010                         | 0.012               | 0.115            | 0.078               |
| P value day            |             | <0.001          | <0.001                        | <0.001              | <0.001           | <0.001              |
| Treatment × storage    |             | 0.006           | 0.114                         | 0.002               | 0.003            | <0.001              |

<sup>1</sup>Control cheese was manufactured from milk of ewes fed a diet containing 600 g of concentrate feed mixture and 400 g of DPL ensiled without additives. Cheeses from the other treatments were manufactured from milk of ewes fed DPL ensiled with fibrolytic enzymes (ENZ50 and ENZ100) or MSP (MSP50 and MSP100) at 50% or 100% level, respectively. N = Newton, N/mL= newton/mL. A2/A1 = The area under the second compression divided by the area under the first compression.

## 4. Discussion

### 4.1. Milk production and composition

Fibrolytic enzyme and MSP treatments increased daily milk yield by 9%, 10.7%, 8.1%, and 9.5% for ENZ50, ENZ100, MSP50, and MSP100, respectively. A previous study by Anee et al. [18] has reported positive effects of MSP supplementation and enzymatic treatments on milk production. Probiotics are known to improve microbial ecology, feed conversion ratios, and nutrient intake, thereby promoting better overall animal performance [19]. MSP supplementation specifically alters

ruminal bacteria populations, enriching bacteria such as those from the *Lactobacillales* family, which play a key role in stabilizing ruminal pH [19].

The combined effects of improved nutrient digestibility and ruminal fermentation characteristics, especially higher propionate concentrations, are likely the primary drivers of the increased daily milk yield [1]. As previously shown by Kholif et al. [1], propionate serves as a precursor for glucose and lactose synthesis and enhances energy availability, which positively influences milk production [20]. Elevated blood glucose levels, indicating better energy status, may also contribute to the increased milk yield observed in ewes fed DPL [20].

Milk from ewes treated with MSP showed higher lactose concentrations than those treated with enzymes, although the reason for this difference remains unclear. Lactose levels depend largely on nutrient digestibility (particularly OM and nonfibrous carbohydrates) and ruminal propionate concentration, all of which showed similar trends under both fibrolytic enzyme and MSP treatments [1]. Nutritional factors explain roughly 50% of variations in milk composition and yield [21].

Both ENZ and MSP treatments improved milk fat and energy content, likely due to elevated ruminal acetate concentrations resulting from enhanced fiber digestion of the DPL diets [1]. Acetate acts as a major precursor for the synthesis of fatty acids in the mammary gland [21]. While treatments had little effect on individual fatty acids, they did increase concentrations of total PUFA, CLA, and the UFA:SFA ratio, while reducing the atherogenicity index. Since over half of milk fatty acids come from plasma uptake, with the rest synthesized in the mammary gland [21], enhanced fiber digestion and shifts in ruminal acetate-to-propionate ratios likely drove the observed changes in milk fatty acid profiles.

The rise in PUFA levels and UFA:SFA ratio suggests that DPL treatments influenced the activity of the ruminal bacteria involved in the biohydrogenation of dietary PUFA [21]. Minor increases in several n-3 fatty acids in milk were also observed with probiotic or enzyme supplementation. PUFA levels in milk primarily depend on the extent to which dietary PUFA escape ruminal biohydrogenation and are absorbed in the small intestine [21]. Milk CLA is formed in the rumen through partial biohydrogenation of linoleic acid by rumen bacteria and is also synthesized in the mammary gland via desaturation of rumenic acid, accounting for approximately 70% of total milk CLA [22].

Date palm leaves contain phytochemicals with antimicrobial properties. Kholif and Kholif [10] demonstrated that feeding ewes malic- or lactic-acid-treated DPL, especially at higher inclusion levels, improved the sensory characteristics of white soft cheese throughout storage. Appearance, flavor, texture, and total sensory scores increased progressively over the 28-day ripening period, with the highest values consistently observed in cheeses from the 100% lactic-acid-treated DPL treatment, followed by the 50% malic- and lactic-acid-treated groups [10]. Ensiling DPL with MSP or enzymes may release additional phenolic and saponin compounds, which can suppress ruminal biohydrogenation of unsaturated fatty acids. This allows more fatty acids to be absorbed into the bloodstream and incorporated into milk, thereby elevating PUFA and CLA concentrations in milk, and consequently, in the derived cheese products.

#### 4.2. Chemical analysis of soft cheese

The composition of white soft cheese derived from the milk of ewes fed diets incorporating DPL ensiled with or without additives (ENZ or MSP) exhibited marked alterations throughout the

28-day storage period. These variations highlight the complex interplay between dietary input and storage duration on cheese maturation. A progressive decline in moisture content was observed across all treatment groups, a characteristic change during ripening primarily driven by evaporation and whey separation. This trend aligns with Moreira et al. [23], who reported that acidification and microbial activity during storage promote syneresis, leading to moisture loss in ripening cheeses. As the cheese pH approaches the isoelectric point of casein, reduced casein hydration and increased protein aggregation further accelerate whey expulsion. The notably greater moisture reduction observed in cheeses from the ENZ100, MSP50, and MSP100 groups suggests that both the presence and level of silage additives can influence the cheese's water retention capacity. Such differences may stem from diet-induced modifications in milk composition, particularly protein and fat concentrations, or from variations in enzymatic activity that affect curd structure and the rate of syneresis [23].

Ash content, which reflects the mineral composition of cheese, remained relatively constant across all treatments, suggesting that the inclusion of ensiled DPL, whether untreated or supplemented with ENZ or MSP, had minimal effect on mineral retention or salt uptake in the final product. This constancy may indicate efficient nutrient transfer from the ewe's diet to the milk and suggests that mineral equilibrium is preserved despite dietary modifications. Maintaining consistent ash levels is particularly relevant given the role of minerals in shaping cheese texture and flavor. According to Nájera et al. [24], such stability is advantageous because it ensures product uniformity and quality irrespective of the feeding strategy employed.

Acidity increased significantly over the storage period, with the highest level recorded in the ENZ50 cheese on day 28 (2.35%). This rise in acidity corresponded with a notable decline in pH values, indicating intensified lactic acid fermentation and proteolytic activity [25]. Elevated acidity during cheese maturation also plays a protective role by inhibiting the growth of spoilage organisms such as butyric acid bacteria [26]. The rapid acidification observed in treated groups is likely driven by increased microbial activity, either through the direct action of dietary probiotics or indirectly via enhanced ruminal fermentation and improved milk composition, which in turn stimulates starter culture performance during ripening [27]. Lower pH not only shapes microbial dynamics but also influences enzymatic functions and protein interactions, contributing to the development of cheese flavor and texture [28].

Salt concentration increased steadily throughout the storage period, with the most pronounced rise observed in ENZ100 and MSP50 cheeses. This trend is consistent with typical ripening behavior, where moisture loss concentrates the salt content and facilitates its diffusion throughout the cheese matrix [29]. Adequate salt levels play a vital role in modulating microbial populations, enzymatic reactions, and overall flavor development, aligning with the expected maturation dynamics of soft cheeses [29]. Moreover, salt influences casein hydration and structural arrangement, which are essential factors in shaping the biochemical and sensory properties of ripened cheese [29].

Protein degradation, assessed through TN and SN content, showed a significant increase over the storage period in cheeses from ewes fed ENZ- or MSP-treated DPL, reflecting intensified proteolysis in these groups [30]. Proteolysis is a critical process in cheese maturation, contributing to the release of peptides and amino acids that enhance both flavor complexity and texture. The elevated SN levels observed in the ENZ50 and MSP50 treatments indicate a more extensive breakdown of caseins, leading to a softer texture and richer umami profile [31]. These findings highlight the influence of dietary interventions on milk composition and, consequently, on the

ripening behavior and sensory quality of cheese [31].

The increase in total VFAs during storage, particularly in MSP100 cheese, highlights enhanced lipolytic activity and the formation of flavor compounds [32]. Volatile fatty acids contribute to the characteristic aromas and tastes of cheese, and the elevated levels in MSP and ENZ groups suggest that feeding ensiled DPL with additives promotes microbial activity or enhances milk substrate availability for flavor development [33]. These results align with the higher sensory flavor scores reported later, showing that biochemical changes in cheese composition translated into noticeable improvements in overall quality. Taken together, the observed changes in moisture, protein fractions, ash, salt, acidity, and VFA levels reflect the integrated biochemical processes governing soft cheese ripening. Notably, the results support the concept that dietary strategies can indirectly modulate cheese composition, not only through their effects on the initial milk matrix but also by shaping microbial metabolism throughout storage [10].

Although fat concentration was not analyzed in the present study, priority was given to VFA quantification because it serves as a more sensitive indicator of ripening progression and flavor development. In contrast, cheese fat concentration typically exhibits only minor fluctuations during storage. Future research should integrate both fat and VFAs determinations to yield a more comprehensive understanding of cheese maturation processes.

Overall, the accelerated ripening patterns, manifested by faster acidification, enhanced proteolysis (TN and SN), and elevated VFAs, were observed in cheeses produced from milk of ewes fed ENZ- and MSP-treated DPL. These findings suggest that ENZ and MSP in DPL silage improve not only animal performance but also the biochemical processes during cheese production, ultimately enhancing cheese quality and sensory attributes. The significant treatment  $\times$  storage time interactions underscore the importance of both feeding strategy and ripening duration in shaping cheese characteristics. These findings are consistent with the broader body of evidence demonstrating that dietary manipulation of lactating animals can modify milk substrate composition in ways that accelerate or modulate the biochemical cascades of ripening, including changes in the availability of proteolytic substrates and lipolytic precursors [8,9].

Kholif and Kholif [10] reported that replacing conventional diets with malic- or lactic-acid-treated DPL, particularly at 100% inclusion, significantly altered cheese composition and ripening dynamics. During storage, moisture content declined and CP, TN, and SN increased, indicating enhanced protein concentration and proteolysis, while ash content decreased. In contrast, salt content, titratable acidity, and total VFAs increased markedly over time, accompanied by a progressive decline in pH, reflecting intensified fermentation and flavor development.

#### *4.3. Sensory evaluation*

Sensory assessment of the cheese demonstrated marked enhancements in appearance, flavor, texture, and overall acceptability throughout the 28-day storage period. These positive changes underscore the combined effect of dietary treatments and ripening duration on the organoleptic properties of cheese. The progressive improvement with time suggests a synergistic effect between diet-induced alterations in milk composition and the biochemical transformations occurring during cheese maturation [34].

Appearance scores improved significantly during storage, with cheeses from ewes fed ENZ- and MSP-treated DPL achieving the highest visual appeal by day 28. Notably, the MSP50 group

achieved the top score (8.50), suggesting that moderate inclusion of probiotics enhanced key visual attributes, such as uniform color, smooth surface texture, and moisture-associated glossiness [25]. The presence of bioactive compounds in DPL, including  $\beta$ -glucans [3,35], may have contributed to these improvements by subtly influencing the cheese's color profile and visual characteristics [35]. The significant effects of both treatment and the treatment  $\times$  storage interaction indicate that the type and inclusion level of ensiling additives can shape the visual development of cheese over time, likely through alterations in milk composition and the curd matrix structure formed during coagulation and ripening.

Flavor, a key determinant of consumer preference, improved significantly over the course of ripening, with ENZ50 cheese achieving notably higher flavor scores than the control by day 28. These enhancements are likely linked to increased proteolysis and lipolysis in the treated groups, as reflected by increased production of SN and total VFAs. These compounds contribute to the complex and desirable sensory profile characteristic of well-ripened cheeses [36]. The intensified flavor development in cheeses from ewes fed functional diets aligns with previous studies showing that bioactive feed components influence milk composition and fermentation [30,32]. Moreover, the phytochemicals present in DPL may have indirectly influenced the milk's microbial ecosystem [3], thereby enhancing enzymatic activity during cheese maturation. The significant treatment and treatment  $\times$  storage interactions further support the notion that dietary inclusion of fibrolytic enzymes or probiotics enhances flavor formation during ripening, possibly through modulation of milk substrates or by promoting beneficial microbial dynamics in the cheese matrix.

Texture scores improved significantly across all treatments over the storage period, with MSP50 cheese receiving the highest ratings in the later stages of ripening. These enhancements likely reflect progressive structural changes in the cheese matrix, including proteolysis and moisture redistribution, which are influenced by diet-induced modifications in milk composition associated with DPL-based diets [34]. The enhanced protein solubilization in these groups likely promoted the formation of firmer, more cohesive curds, which is important for consumer acceptance. Additionally, as highlighted by Gulati et al. [37], feeding strategies that alter milk casein composition can affect curd microstructure and elasticity, supporting the observed improvements in cheese texture among treated groups. The significant interaction between treatment and storage time underscores the dynamic interplay between feed-induced changes in milk quality and ripening processes that shape cheese texture, influencing attributes, such as softness, cohesiveness, and mouthfeel.

Comparative analysis indicated that both MSP and ENZ treatments significantly enhanced all sensory attributes compared to the control, with ENZ50 achieving the highest overall sensory scores, while MSP50 recorded the highest appearance scores. This suggests that moderate levels of probiotic supplementation may be particularly effective in improving cheese sensory quality. The observed enhancements are likely due to the beneficial effects of probiotics on rumen microbial populations and subsequent alterations in milk composition [19], increased proteolysis (i.e., SN) and VFAs, which are known contributors to flavor development, as well as their potential role in shaping cheese microbial ecology during ripening. Notably, compared with organic acid-treated DPL [10], fibrolytic enzyme and MSP treatments appear to produce more consistent sensory improvements across inclusion levels, suggesting that biological modification of DPL fiber, rather than simple pH reduction through acid treatment, may more effectively alter the milk substrate in ways that favor progressive sensory development during ripening.

#### 4.4. Rheological properties of soft cheese

Cheese texture was significantly affected by both the diet of lactating ewes and the storage period, highlighting the complex interplay between dietary treatments, milk composition, and cheese maturation processes. These variations reflect the dynamic structural changes characteristic of soft cheese ripening, driven by biochemical processes, such as proteolysis, moisture redistribution, and gradual development of the casein network [38]. The evaluated texture parameters (i.e., hardness, cohesiveness, springiness, gumminess, and chewiness) varied markedly depending on the DPL ensiling method and the length of storage, underscoring the role of both feed interventions and ripening time in shaping cheese texture attributes.

Hardness, a key indicator of cheese firmness, gradually increased over the 28-day storage period in all treatments, reflecting typical ripening-related processes, such as moisture loss and matrix consolidation [39]. Among the treatments, the highest hardness at day 28 was observed in the MSP50 group (8.28), followed by ENZ50 (7.55), ENZ100 (6.95), MSP100 (6.69), and Control (5.97). The significant main effects of treatment and storage time, along with their interaction, underscore the synergistic influence of dietary interventions and ripening duration on cheese firmness. These findings suggest that the inclusion of ensiling additives, particularly MSP at moderate levels, enhanced curd firmness, potentially by promoting structural consolidation through improved protein matrix formation. The superior hardness in MSP50 cheese may be associated with differences in moisture loss and proteolysis patterns observed among treatments. These components may modulate proteolytic pathways or affect casein micelle stability in milk, leading to denser, more cohesive curd structures during cheese maturation [40].

Cohesiveness, representing the internal bonding and structural integrity of the cheese, was also significantly influenced by diet and storage time. The highest cohesiveness values were found in cheeses from MSP-treated DPL diets (MSP50 at 0.77 and MSP100 at 0.75) at day 28, suggesting that probiotic supplementation contributes to a more uniform and resilient matrix structure. The absence of a significant treatment  $\times$  storage interaction suggests that cohesiveness improved steadily with storage irrespective of treatment, though probiotic diets consistently outperformed control.

Springiness, which reflects the elasticity and the ability of cheese to recover its original shape after deformation, increased significantly as the storage period increased. Cheeses produced from MSP50 and ENZ50 diets exhibited the highest springiness values at day 28, indicating enhanced structural resilience. This improvement may be linked to alterations in casein micelle interactions and matrix flexibility induced by the dietary inclusion of probiotics or fibrolytic enzymes [37]. The significant interaction between treatment and storage duration further indicates that springiness development is modulated by both the nature of the DPL ensiling additive and the length of ripening. Although mineral composition of milk was not measured in this study, previous reports suggest that mineral balance can influence casein interactions and texture development [41]. These findings underscore the role of feed-induced mineral composition and milk matrix dynamics in influencing cheese textural properties during maturation.

Derived texture parameters, gumminess and chewiness, both closely associated with hardness and cohesiveness, were significantly influenced by diet, storage duration, and their interaction. The highest gumminess (6.37) and chewiness (5.51) values were observed in cheeses from the MSP50 treatment at day 28, underscoring the positive effect of probiotic-treated DPL diets on the formation of a firm yet elastic cheese matrix. These enhancements likely arise from increased proteolytic and

enzymatic activity during ripening, which contribute to structural refinement of the curd and improved mastication properties. These textural qualities are highly valued in semi-soft cheeses because they enhance mouthfeel and overall sensory appeal [42]. The gradual rises in gumminess and chewiness correspond with the increases in SN and total VFAs, both key indicators of cheese matrix breakdown and the integration of flavor and texture [8]. These results suggest that probiotic supplementation at moderate inclusion levels effectively promotes desirable rheological characteristics in ripened soft cheese.

For comparison, Kholif and Kholif [10] reported that organic acid-treated DPL, particularly at 100% inclusion, increased hardness, cohesiveness, gumminess, and chewiness during storage, which responded primarily to storage time rather than to dietary treatment. The present study extends these findings: ENZ and MSP treatments at moderate inclusion levels (50%) produced superior hardness, springiness, gumminess, and chewiness relative to the 100% inclusion levels, suggesting that partial replacement of the forage fraction preserves sufficient milk protein and fat fractions to support optimal curd network development. The consistent superiority of MSP50 across all texture parameters underscores the potential of probiotic-based silage treatments to improve not only animal performance but also the rheological quality of the resulting cheese, a finding with practical relevance for small-scale dairy operations in arid regions.

## 5. Conclusions

This study investigated the effects of feeding lactating Farafra ewes with date palm leaves ensiled either without additives or treated with fibrolytic enzymes or multi-species probiotics, applied at two dietary inclusion levels. The research focused on evaluating milk production performance, milk fatty acid composition, and the quality characteristics of white soft cheese produced from the milk. Results demonstrated that dietary treatments influenced milk yield, increased concentrations of beneficial fatty acids, including polyunsaturated fatty acids and conjugated linoleic acid; and improved the cheese's physicochemical, sensory, and textural properties. These results emphasize the potential of using treated date palm leaves as a sustainable, cost-effective feed resource that can improve dairy sheep productivity and product quality, particularly in arid regions. Further research is warranted to evaluate long-term effects, economic feasibility, and applicability under commercial production conditions.

### Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

### Conflict of interest

The authors declare no conflicts of interest.

### Author Contributions

Conceptualization: A.M.M.K. and A.E.K.; methodology: A.M.M.K. and A.E.K.; data analysis:

A.M.M.K. and A.E.K.; writing original draft preparation: A.M.M.K. and A.E.K.; writing—review and editing: A.E.K.; visualization: A.M.M.K. and A.E.K.; supervision: A.E.K. Both authors have read and agreed to the published version of the manuscript.

## References

1. Kholif AE, Hamdon HA, Gouda GA, et al. (2022) Feeding date-palm leaves ensiled with fibrolytic enzymes or multi-species probiotics to Farafra ewes: intake, digestibility, ruminal fermentation, blood chemistry, milk production and milk fatty acid profile. *Animals* 12: 1107. <https://doi.org/10.3390/ani12091107>
2. Gouda GA, Kholif AE, Hamdon HA, et al. (2022) Utilization of waste date palm leaves biomass ensiled with malic or lactic acids in diets of Farafra ewes under tropical conditions. *Animals* 12: 1432. <https://doi.org/10.3390/ani12111432>
3. Kholif AE, Olafadehan OA, Anele UY (2026) Utilization of date palm leaves for livestock feed, In: Maqsood S, Roobab U, Aadil RM (Eds.), *Sustainable Valorization of Date Palm By-products and Wastes*, Elsevier, 185–206. <https://doi.org/10.1016/B978-0-443-29023-7.00010-0>
4. Hamdon HA, Kassab AY, Vargas-Bello-Pérez E, et al. (2022) Using probiotics to improve the utilization of chopped dried date palm leaves as a feed in diets of growing Farafra lambs. *Front Vet Sci* 9: 1048409. <https://doi.org/10.3389/fvets.2022.1048409>
5. Kholif AE, Patra AK (2024) Dietary applications of exogenous enzymes to improve nutrient utilization and performance in ruminants, In: Mahesh MS, Yata VK (Eds.), *Feed Additives and Supplements for Ruminants*, Singapore, Springer Nature Singapore, 1–28. [https://doi.org/10.1007/978-981-97-0794-2\\_1](https://doi.org/10.1007/978-981-97-0794-2_1)
6. Saleem ASA, Abdelnour S, Bassiony SM, et al. (2025) Probiotic supplementation in sustainable sheep production: impacts on health, performance, and methane mitigation. *Trop Anim Health Prod* 57: 1–22. <https://doi.org/10.1007/s11250-025-04439-y>
7. Arda B, Akdeniz V, Kara HH, et al. (2025) Fatty acid profile, conjugated linoleic acid (CLA) content, microbiological and sensory properties of kefir produced from sheep milk using kefir grains and starter culture in comparison with the respective kefir from cow milk. *Int Dairy J* 162: 106157. <https://doi.org/10.1016/j.idairyj.2024.106157>
8. Di Paolo M, Vuoso V, Ambrosio RL, et al. (2023) Role of feeding and novel ripening system to enhance the quality and production sustainability of curd buffalo cheeses. *Foods* 12: 704. <https://doi.org/10.3390/foods12040704>
9. Ioannidou MD, Maggira M, Samouris G (2022) Physicochemical characteristics, fatty acids profile and lipid oxidation during ripening of graviera cheese produced with raw and pasteurized milk. *Foods* 11: 2138. <https://doi.org/10.3390/foods11142138>
10. Kholif AMM, Kholif AE (2025) Organic acid-treated ensiled date palm leaves improve texture and sensory quality of white soft cheese from Farafra sheep milk in arid environments. *Biocatal Agric Biotechnol* 69: 103798. <https://doi.org/10.1016/j.bcab.2025.103798>
11. NRC (2007) Nutrient requirements of small ruminants: Sheep, goats, cervids, and new world camelids, Washington DC, National Academies Press.
12. Ulbricht TLV, Southgate DAT (1991) Coronary heart disease: Seven dietary factors. *The Lancet* 338: 985–992. [https://doi.org/10.1016/0140-6736\(91\)91846-M](https://doi.org/10.1016/0140-6736(91)91846-M)

13. Tyrrell HF, Reid JT (1965) Prediction of the energy value of cow's milk. *J Dairy Sci* 48: 1215–1223. [https://doi.org/10.3168/jds.S0022-0302\(65\)88430-2](https://doi.org/10.3168/jds.S0022-0302(65)88430-2)
14. AOAC (2019) Official Methods of Analysis of AOAC International, Washington DC, Oxford University Press.
15. Kosikowski F V. (1982) Cheese and fermented milk foods, Brooktonale, New York, F. V. Kosikowski and Associates.
16. Clark S, Costello M, Drake MA, et al. (2009) The sensory evaluation of dairy products, New York, NY, Springer US. <https://doi.org/10.1007/978-0-387-77408-4>
17. Glibowski P, Zarzycki P, Krzepkowska M (2008) The rheological and instrumental textural properties of selected table fats. *Int J Food Prop* 11: 678–686. <https://doi.org/10.1080/10942910701622599>
18. Anee IJ, Alam S, Begum RA, et al. (2021) The role of probiotics on animal health and nutrition. *The Journal of Basic and Applied Zoology* 82: 52. <https://doi.org/10.1186/s41936-021-00250-x>
19. Kholif AE, Anele A, Anele UY (2024) Microbial feed additives in ruminant feeding. *AIMS Microbiol* 10: 542–571. <https://doi.org/10.3934/microbiol.2024026>
20. Rigout S, Hurtaud C, Lemoscjuet S, et al. (2003) Lactational effect of propionic acid and duodenal glucose in cows. *J Dairy Sci* 86: 243–253. [https://doi.org/10.3168/jds.S0022-0302\(03\)73603-0](https://doi.org/10.3168/jds.S0022-0302(03)73603-0)
21. Kholif AE, Olafadehan OA (2022) Dietary strategies to enrich milk with healthy fatty acids—A review. *Ann Anim Sci* 22: 523–536. <https://doi.org/10.2478/aoas-2021-0058>
22. Corl BA, Baumgard LH, Dwyer DA, et al. (2001) The role of  $\Delta 9$ -desaturase in the production of cis-9, trans-11 CLA. *J Nutr Biochem* 12: 622–630. [https://doi.org/10.1016/S0955-2863\(01\)00180-2](https://doi.org/10.1016/S0955-2863(01)00180-2)
23. Moreira RV, Costa MP, Frasao BS, et al. (2020) Effect of ripening time on bacteriological and physicochemical goat milk cheese characteristics. *Food Sci Biotechnol* 29: 459–467. <https://doi.org/10.1007/s10068-019-00682-w>
24. Nájera AI, Nieto S, Barron LJR, et al. (2021) A review of the preservation of hard and semi-hard cheeses: quality and safety. *Int J Environ Res Public Health* 18: 9789. <https://doi.org/10.3390/ijerph18189789>
25. Zheng X, Shi X, Wang B (2021) A review on the general cheese processing technology, flavor biochemical pathways and the influence of yeasts in cheese. *Front Microbiol* 12: 703284. <https://doi.org/10.3389/fmicb.2021.703284>
26. Rilla N, Martínez B, Delgado T, et al. (2003) Inhibition of *Clostridium tyrobutyricum* in Vidiago cheese by *Lactococcus lactis* ssp. *lactis* IPLA 729, a nisin Z producer. *Int J Food Microbiol* 85: 23–33. [https://doi.org/10.1016/S0168-1605\(02\)00478-6](https://doi.org/10.1016/S0168-1605(02)00478-6)
27. Coelho MC, Malcata FX, Silva CCG (2022) Lactic acid bacteria in raw-milk cheeses: From starter cultures to probiotic functions. *Foods* 11: 2276. <https://doi.org/10.3390/foods11152276>
28. Bansal V, Veena N (2022) Understanding the role of pH in cheese manufacturing: general aspects of cheese quality and safety. *J Food Sci Technol* 61: 16–26. <https://doi.org/10.1007/s13197-022-05631-w>
29. Estrada O, Ariño A, Juan T (2019) Salt distribution in raw sheep milk cheese during ripening and the effect on proteolysis and lipolysis. *Foods* 8: 100. <https://doi.org/10.3390/foods8030100>
30. Zhao X, Zheng Z, Zhang J, et al. (2019) Change of proteolysis and sensory profile during ripening of Cheddar-style cheese as influenced by a microbial rennet from rice wine. *Food Sci Nutr* 7: 1540. <https://doi.org/10.1002/fsn3.1003>

31. Tavarria FK, Franco I, Carballo FJ, et al. (2003) Amino acid and soluble nitrogen evolution throughout ripening of Serra da Estrela cheese. *Int Dairy J* 13: 537–545. [https://doi.org/10.1016/S0958-6946\(03\)00060-8](https://doi.org/10.1016/S0958-6946(03)00060-8)
32. You L-Q, Wang Y-R, Bai S, et al. (2024) Impact of ripening periods on the key volatile compounds of Cheddar cheese evaluated by sensory evaluation, instrumental analysis and chemometrics method. *Appl Food Res* 4: 100578. <https://doi.org/10.1016/j.afres.2024.100578>
33. Sant'Ana AMS, Bessa RJB, Alves SP, et al. (2019) Fatty acid, volatile and sensory profiles of milk and cheese from goats raised on native semiarid pasture or in confinement. *Int Dairy J* 91: 147–154. <https://doi.org/10.1016/j.idairyj.2018.09.008>
34. Falih MA, Altemimi AB, Hamed Alkaisy Q, et al. (2024) Enhancing safety and quality in the global cheese industry: A review of innovative preservation techniques. *Heliyon* 10: e40459. <https://doi.org/10.1016/j.heliyon.2024.e40459>
35. Maqsood S, Adiamo O, Ahmad M, et al. (2020) Bioactive compounds from date fruit and seed as potential nutraceutical and functional food ingredients. *Food Chem* 308: 125522. <https://doi.org/10.1016/j.foodchem.2019.125522>
36. Ianni A, Bennato F, Martino C, et al. (2020) Volatile flavor compounds in cheese as affected by ruminant diet. *Molecules* 25: 461. <https://doi.org/10.3390/molecules25030461>
37. Gulati A, Galvin N, Hennessy D, et al. (2018) Grazing of dairy cows on pasture versus indoor feeding on total mixed ration: Effects on low-moisture part-skim Mozzarella cheese yield and quality characteristics in mid and late lactation. *J Dairy Sci* 101: 8737–8756. <https://doi.org/10.3168/jds.2018-14566>
38. Lamichhane P, Kelly AL, Sheehan JJ (2018) Symposium review: Structure-function relationships in cheese. *J Dairy Sci* 101: 2692–2709. <https://doi.org/10.3168/jds.2017-13386>
39. D'Incecco P, Limbo S, Hogenboom J, et al. (2020) Impact of extending hard-cheese ripening: A multiparameter characterization of Parmigiano Reggiano cheese ripened up to 50 months. *Foods* 9: 268. <https://doi.org/10.3390/foods9030268>
40. Wang W, Jia R, Hui Y, et al. (2023) Utilization of two plant polysaccharides to improve fresh goat milk cheese: Texture, rheological properties, and microstructure characterization. *J Dairy Sci* 106: 3900–3917. <https://doi.org/10.3168/jds.2022-22195>
41. Tsioulpas A, Lewis MJ, Grandison AS (2007) Effect of minerals on casein micelle stability of cows' milk. *J Dairy Res* 74: 167–173. <https://doi.org/10.1017/S0022029906002330>
42. Moss R, Le Blanc J, Gorman M, et al. (2023) A prospective review of the sensory properties of plant-based dairy and meat alternatives with a focus on texture. *Foods* 12: 1709. <https://doi.org/10.3390/foods12081709>



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