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# Mass balance of milk components and yield prediction in Caciocavallo Palermitano pasta filata cheese

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## ABSTRACT

This study evaluated, through closed mass balances, the partitioning of milk components during the cheesemaking process for the production of traditional pasta filata cheese. In addition, a statistical model was developed to predict cheese yield 24 h after stretching as a function of milk characteristics. The 1-year study was conducted in 3 cheesemaking plants using the traditional system for the production of Caciocavallo Palermitano cheese, carrying out measurements and sampling during bimonthly cheesemaking trials. The proximate composition of milk and of the fractions obtained after rennet coagulation was determined to assess the distribution of water, proteins, fat, ash, and lactose. Mass balance calculations showed closed mass balances greater than 98.7% for all the components analyzed. The average recovery in the pasta filata cheese was 72.0% for proteins and 76.9% for fat, whereas lactose remained predominantly in the whey (86.6%). Integrated ricotta production significantly contributed to the overall recovery of nutrients at the system level. Concurrently, in the 3 cheesemaking plants, bulk milk samples were collected and cheese yields were recorded 24 h after stretching on a weekly basis to build a database for developing a statistical model to estimate yield. Yield at 24 h after stretching was modeled using multiple linear regression. The final model, based on milk protein, fat, and urea contents, explained 82.2% of the observed variability ($R^2$ = 0.822; root mean square error = 0.1769). Leave-one-dairy-out cross-validation confirmed the stability and generalization ability of the model (r = 0.898). The mean absolute error was 0.147, and the mean bias error was negligible. Bland–Altman analysis showed limits of agreement of ~3% of the mean yield.

**Key words:** milk composition, cheese yield, milk yield index, ricotta cheese

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## INTRODUCTION

Cheese yield represents a key indicator of technological and economic efficiency in dairy production systems, particularly in traditional raw milk processes, where biological variability and artisanal management interact in complex ways. The main milk components, especially protein and fat, are recognized as the primary determinants of cheese yield, whereas other chemical and technological parameters exert more variable and process-dependent effects (Emmons et al., 1990; Verdier-Metz et al., 2001; Cipolat-Gotet et al., 2013; Stocco et al., 2022).

In pasta filata cheeses, curd transformation through syneresis, acidification, and stretching alters the partitioning of solids between the final product and by-products, making the system more complex than pressed cheeses (Walstra et al., 2006; Fox et al., 2017).

Mass balance analysis is a well-established approach for quantifying the recovery and distribution of components in dairy processes (Walstra et al., 2006; Fox et al., 2017). However, studies on traditional small-scale systems often focus only on final cheese yield, without experimentally verified mass balances across all process matrices (Cipolat-Gotet et al., 2013; Stocco et al., 2022). Consequently, comprehensive evaluations of the partitioning of proteins, fat, lactose, and minerals among curd, whey, and by-products using closed and reconciled mass balances remain limited. This gap hinders the identification of critical loss points and the development of reliable yield prediction models (Aldalur et al., 2019; Moatsou and Moschopoulou, 2021; Pires et al., 2021). A detailed assessment of component distribution across all cheesemaking stages is essential in artisanal pasta filata systems, where process-induced transformations may not be captured by final-yield measurements alone.

In this context, Caciocavallo Palermitano, a traditional pasta filata cheese produced mainly from whole raw milk of Cinisara cattle raised under extensive and semi-extensive systems (Giosuè et al., 2024; Maniaci et al., 2024), represents an ideal model for investigation as it integrates raw milk processing, traditional pasta filata technology, and significant biological and technological variability. The Cinisara, an indigenous breed from western Sicily, is characterized by relatively low milk yields but high technological quality, with fat and protein contents typically ranging from 2.9% to 3.2% and 3.2% to 3.5%, respectively, and favorable coagulation properties (Alabiso et al., 2020, 2021; Lanza et al., 2025). It is known that milk composition is strongly influenced by seasonal variations and pasture-based feeding systems, which are typical of extensive dairy production systems, thereby contributing to fluctuations in technological properties and variability in cheesemaking performance (Maniaci et al., 2021).

Therefore, the objectives of this study were as follows:

1. To quantify the partitioning of the main milk components throughout all stages of Caciocavallo Palermitano cheese using closed and closed mass balances;
2. To evaluate the variability among different artisanal cheesemaking plants;
3. To develop a statistical model to estimate cheese yield 24 h after stretching, based on compositional, coagulation, and seasonal variables.

## MATERIALS AND METHODS

### *Experimental Design and Cheesemaking*

The study was conducted over the course of 1 year, from September 2024 to August 2025, and involved 3 artisanal cheesemaking plants (**Cp**) located in the typical production area of Caciocavallo Palermitano cheese, operating according to the traditional method described by Bonanno et al. (2013). The 3 artisanal Cp were selected to represent different production contexts within the traditional system. The selection was based on their location in distinct altitudinal zones (plain, hill, and mountain), which are associated with different environmental conditions. This approach allows an adequate representation of the variability of the production system and is consistent with previous studies conducted in Sicilian dairy systems, in which variability related to production conditions has been analyzed through comparison of different farm contexts (Marino et al., 2014; Mangione et al., 2025).

For a detailed analysis of component distribution throughout the cheesemaking process, 6 experimental trials were carried out at each plant on a bimonthly basis, for a total of 18 trials.

Bulk milk was sourced from local farms with Cinisara cows and their crossbreeds, primarily pasture-fed with supplemental hay and concentrates according to grass availability. Milk was heated to 37°C and coagulated for 40 min using lamb rennet paste (Caglio in pasta Provo 5, Caglificio Clerici S.p.A.; 1:15,000 international milk clotting units [IMCU] per gram; 50 g/100 kg of milk) in a wooden vat, resulting in curd–whey separation, calculating the percentage distribution of mass based on the initial milk weight (phase 1). The whey was then subjected to thermo-acidic coagulation at 90°C for ricotta cheese production, with the addition of "agra" (5 kg/100 L of whey), a natural whey starter obtained from the spontaneous acidification of whey derived from previous production batches, to promote protein coagulation and separation of ricotta cheese from the residual whey (ricotta whey). To ensure a closed technological system, no milk was added in the production of ricotta. The mass yield of each fraction was quantified (phase 2). The curd was then processed through successive technological steps. Cooking (phase 3) was carried out in wooden vats by covering the curd with ricotta whey at 80°C for 3 h, obtaining cooked curd and cooked curd whey. Cooking was followed by acidification for 20 h at room temperature, obtaining acid curd, pH 5.42 ± 0.05 (phase 4). Finally, stretching (phase 5) was performed by immersing the sliced acid curd in cooked curd whey (80°C) and manually stretching the curd mass until a fibrous structure was obtained (stretched curd), and stretching whey as waste. At each stage, percentage weight changes relative to the initial milk were recorded. To ensure a closed technological system, ricotta whey was reused during curd cooking, whereas whey obtained from the cooking phase (cooked curd whey) was subsequently used for stretching. At the end of the process, both stretched curd and stretched whey were collected and quantified.

All operations were carried out using traditional artisanal equipment, including wooden vats and manual handling tools, consistent with the historical manufacturing method of Caciocavallo Palermitano cheese (Bonanno et al., 2013).

During each cheesemaking trial, the masses of all input and output fractions (milk, curd, whey, agra, ricotta cheese, cooked curd whey, and stretched whey) were recorded for each stage of the process, ensuring complete traceability of material flows. Simultaneously, representative samples of each input and output matrix were collected at every stage of cheesemaking. The quantitative data obtained were used to develop mass balances: for each phase, mass balance closure was verified by evaluating the correspondence between total inputs and the sum of measured outputs. Mass balance closure was considered acceptable when the imbalance between total inputs and outputs was ≤1%. This stringent tolerance was adopted to account for analytical uncertainty, sampling variability, and minor technological losses, thereby ensuring the accuracy and reliability of the experimental data (Walstra et al., 2006). When deviations exceeded this threshold, raw data were rechecked and measurement procedures were verified. Two trials that failed to meet the ≤1% closure requirement after secondary verification were excluded and subsequently repeated.

The samples were immediately refrigerated and transported to the laboratory to be frozen at −20 °C and subsequently freeze-dried for analysis (SCANVAC Coolsafe 55-9, Labogene Aps, Lynge, Denmark).

Furthermore, systematic production monitoring was conducted at each plant through weekly assessments over a full year. For each sampling event, the volume of processed bulk milk was recorded, milk samples were collected, and the 24-h post-stretching cheese yield was measured. A total of 52 measurements were performed per plant, resulting in 156 observations. All samples were immediately refrigerated and transported to the laboratory for analysis.

### Analytical Procedures for Component Partitioning

Samples collected from the 18 cheesemaking trials (milk, curd, whey, ricotta cheese, cooked curd whey, and stretched whey) were analyzed for DM, ether extract (EE), CP (N × 6.38), and ash according to International Dairy Federation standards (IDF, 1964a,b, 1982, 1986). Total carbohydrate content was calculated by difference, assuming that carbohydrates were mainly represented by lactose, as commonly applied in proximate composition analyses (Juthi et al., 2025):

$$\text{Carbohydrates (\%)} = 100 - (\text{moisture \%} + \text{ash \%} + \text{CP \%} + \text{EE \%}).$$

Quantitative data, initially expressed in grams, were reconciled according to the mass balances described in the section "Mass Balance Across Cheesemaking Stages." Changes in proximate composition were calculated for each phase, accounting for relative deviations from the initial mass. This approach allowed for the quantification of syneresis, concentration, and selective losses without imposing forced closure constraints. Proximate composition data were then converted to a DM basis to enable consistent comparisons across processing stages and different trials.

Finally, overall component recovery throughout the cheesemaking process was quantified to evaluate the partitioning of nutrients between final products and by-products.

For each cheesemaking trial, the mass (kg) of a given component in each matrix was calculated as the product of the matrix weight (kg), the DM content (expressed as a fraction), and the component concentration on a DM basis ($C_{DM}$, expressed as a fraction).

The recovery (%) of each component in a specific matrix was calculated as follows:

$$\text{Recovery (\%)} = \frac{W_{\text{matrix}} \times DM_{\text{matrix}} \times C_{\text{DM matrix}}}{W_{\text{milk}} \times DM_{\text{milk}} \times C_{\text{DM milk}}} \times 100,$$

where W is the matrix weight (kg), DM is the dry matter fraction, and $C_{DM}$ is the component concentration on a DM basis. Total recovery was determined as the sum of recoveries across all output matrices and was used to verify mass balance closure at the system level.

### Analytical Methods for Yield Evaluation

The 156 bulk milk samples were analyzed for fat, protein, casein, lactose, and SCC using a CombiFoss 6000 (Foss Electric A/S, Hillerød, Denmark); total bacterial count using a BactoScan (Foss Electric A/S, Hillerød, Denmark); urea using a CL-10 Plus (Eurochem S.r.l., Rome, Italy); pH with a digital pH meter (Thermo Orion 710 A+). Milk coagulation properties, including rennet coagulation time (r, min), curd firming time ($k_{20}$, min), and curd consistency ($a_{30}$, mm), were measured using a Formagraph (Foss Electric A/S, Hillerød, Denmark) according to the method described by Annibaldi et al. (1977).

### Statistical Analysis

Statistical analyses were performed using SAS 9.2 (SAS Institute Inc., 2010). Proximate composition data of milk, curd, whey, ricotta cheese, cooked curd whey, and stretched whey were analyzed using a linear mixed model (PROC MIXED). The model included the cheesemaking phase (5 levels), plant (3 levels), and their interaction as fixed effects. The cheesemaking trial was included as a random effect nested within plant to account for interbatch variability. Results are presented as LSM, and differences between means were compared using Tukey's honestly significant difference test. Significance was declared at $P < 0.05$.

The relationship between the 24-h post-stretching cheese yield and milk characteristics was initially evaluated using multiple linear regression (**MLR**), including all compositional variables, coagulation parameters, and seasonal effects in a full model. A backward stepwise elimination procedure was then applied to develop the final model. Specifically, nonsignificant predictors ($P >$ 0.05) were sequentially removed, and model performance ($R^2$ and adjusted $R^2$) and multicollinearity (variance inflation factor, **VIF**) were continuously monitored. At each step, the model was re-estimated until all remaining predictors were significant and VIF values indicated the absence of relevant multicollinearity. The final model included protein, fat, and urea as significant milk compositional predictors, whereas coagulation traits and seasonal effects were excluded due to lack of significance.

Model assumptions of normality and homoscedasticity of residuals were evaluated using diagnostic plots, and influential observations were assessed through Cook's distance (Cook, 1977), difference in fits, and difference in betas. Significance was set at $P < 0.05$.

To assess model stability and predictive performance across the 3 cheesemaking plants, a leave-one-dairy-out (**LODO**) cross-validation was performed. In this approach, each plant was sequentially excluded from the training set and used as an independent test set. Predictive ability was further evaluated using mean absolute error (**MAE**), mean bias error (**MBE**), and root mean square error (**RMSE**).

In addition, a Bland–Altman analysis was conducted to assess agreement between observed and predicted yield values, reporting limits of agreement as mean difference ± 1.96 × SD. Pearson correlation coefficients were calculated using PROC CORR to evaluate relationships among the 3 compositional variables, as well as the association between observed and predicted yields, further supporting model validity.

## RESULTS

### Mass Balance Across Cheesemaking Stages

Figure 1 illustrates the schematic of the Caciocavallo Palermitano cheese production process, detailing the proximate composition (mean of 3 cheesemaking plants ± SD) and mass partitioning (%) at each stage. Rennet coagulation resulted in a distinct mass partitioning between curd (23.0% ± 1.1%) and whey (77.0% ± 0.9%). This stage was characterized by a preferential concentration of proteins and fat in the solid fraction, whereas lactose was predominantly retained in the liquid fraction (2.38 g vs. 0.97 g for protein, 2.50 g vs. 0.62 g for fat, and 0.90 g vs. 3.84 g for lactose, respectively). Subsequent thermo-acid coagulation of the whey enabled the recovery of ~35% ± 3.8% and 76% ± 4.2% of the residual protein and fat fractions, respectively, based on mean mass balance calculations.

The technological treatments applied to the curd affected mass and chemical composition. Lactose was predominantly concentrated in the liquid matrices, whey (65.6% ± 4.9%), ricotta whey (76.8% ± 6.5%), cooked curd whey (76.8% ± 7.6%), and stretching whey (81.4% ± 4.3%), whereas it progressively decreased in the solid matrices, reaching low levels in the final stretched curd (9.2% ± 1.8%). Ash content, expressed on a DM basis, showed no significant differences among the analyzed matrices.

Results for the rebalanced DM of each cheesemaking plants are reported in Table 1. Notable differences were observed between Cp across various stages of the process. In the starting milk, Cp 3 exhibited higher DM values compared with Cp 1 and Cp 2 (12.33% vs. 11.67% and 11.72%, respectively; $P = 0.0114$), indicating a higher solids concentration in the raw milk. This trend was reflected in the curd, with higher values observed in Cp 2 and Cp 3 compared with Cp 1 (26.75% and 27.32% vs. 25.15%, respectively; $P = 0.0130$).

Conversely, whey from Cp 1 showed higher DM content than Cp 2 (7.89% vs. 7.14%; $P = 0.0337$). Significant differences were also noted in the cooked curd and acid curd, showing an increasing gradient from Cp 1 to Cp 3 (42.97% vs. 47.58% vs. 52.37% for cooked curd, $P = 0.0008$; 48.15% vs. 52.18% vs. 57.24% for acid curd, $P = 0.0091$). However, in the final stretched curd, differences between plants were not statistically significant ($P > 0.05$). Among the by-products, ricotta whey showed significant variability, with Cp 1 exhibiting a lower DM content compared with the higher values found in Cp 2 and Cp 3 (3.39% vs. 5.81% and 5.90%, respectively; $P = 0.0069$).

Table 2 reports the ash content of the masses across all cheesemaking stages; no significant differences were observed between the plants ($P > 0.05$). Results for CP on a DM basis are presented in Table 3. Significant differences were found in specific matrices: in ricotta cheese, CP was higher in Cp 1 compared with Cp 3 (36.14% vs. 29.36%; $P = 0.0498$), consistent with the findings for cooked curd (43.60% vs. 37.20%; $P = 0.0285$). In the stretched curd, no significant differences were detected ($P > 0.05$), although a decreasing trend in protein content from Cp 1 to Cp 3 was noted.

Table 4 details fat content on a DM basis. In the curd, Cp 3 exhibited higher values compared with Cp 1 and Cp 2 (44.96% vs. 39.39% and 39.07%, respectively; $P = 0.0233$). Similarly, in the cooked curd, fat content was higher in Cp 3 than in Cp 2 and Cp 1 (46.33% vs. 42.25% and 41.25%; $P = 0.0058$), whereas values remained relatively homogeneous in the stretched curd. Table 5 reports lactose content, which showed no significant differences between plants ($P > 0.05$).

Recovery values (mean ± SD) for milk components are summarized in Table 6. Protein recovery in the stretched curd averaged 72.04% ± 6.87%, with an additional 11.55% ± 1.48% recovered during ricotta cheese production; the remaining 16.04% ± 7.61% was partitioned into the stretching whey. Fat recovery followed a similar trend: 76.87% ± 3.23% was retained in the stretched curd, and 15.90% ± 1.73% was recovered in ricotta cheese, with only 6.94% ± 2.10% remaining in the whey. Total solids recovery in the stretched curd was 47.62% ± 2.86%, with 8.34% ± 1.06% in ricotta cheese and 43.82% ± 2.18%

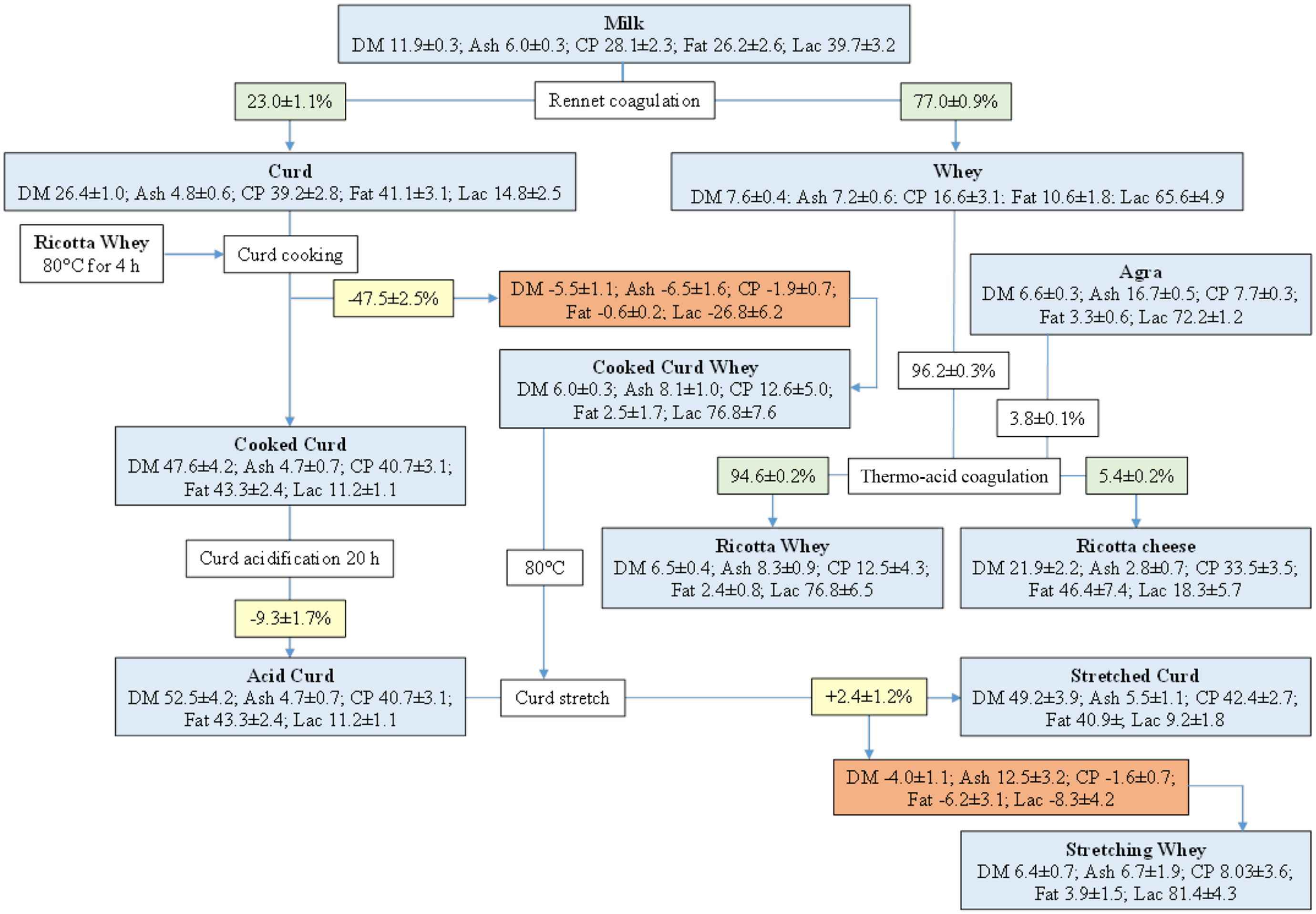


**Figure 1.** Diagram of the cheesemaking process of Caciocavallo Palermitano cheese. LAC = lactose. Blue boxes show the centesimal composition, percentage of the DM of the different products and by-products (mean of 3 cheesemaking plants ± SD). Green boxes show the distribution (%) of the initial mass following the process step (mean ± SD). Yellow boxes show the variation (%) of the fresh weight of the product following the process step (mean ± SD). Orange boxes show the variation in the centesimal composition (on DM basis), calculated from the initial composition and relative to the fraction migrated from the solid to the liquid phases.

distributed across the whey streams. Mineral partitioning was higher in the whey (53.02% ± 8.13%) than in the stretched curd (43.12% ± 7.43%), whereas lactose predominantly followed the whey stream (86.61% ± 2.54%). For all components, total system-level recoveries exceeded 98.7%, with deviations within ±1.04%.

**Table 1.** Dry matter content of all mass streams of the cheesemaking system (%)[1]

| Item | Cp 1 | Cp 2 | Cp 3 | RMSE | *P*-value |
|---|---|---|---|---|---|
| Milk | 11.67[b] | 11.72[b] | 12.33[a] | 0.098 | 0.011 |
| Curd | 25.15[b] | 26.75[a] | 27.32[a] | 0.314 | 0.013 |
| Whey | 7.89[a] | 7.14[b] | 7.71[ab] | 0.154 | 0.034 |
| Agra | 6.30 | 6.68 | 6.76 | 1.030 | 0.286 |
| Ricotta cheese | 23.58 | 22.03 | 20.09 | 2.036 | 0.358 |
| Ricotta whey | 6.96 | 6.14 | 6.49 | 0.237 | 0.091 |
| Cooked curd | 42.97[c] | 47.58[b] | 52.37[a] | 0.499 | 0.001 |
| Cooked curd whey | 6.39[a] | 5.81[b] | 5.90[b] | 0.070 | 0.007 |
| Acid curd | 48.15[c] | 52.18[b] | 57.24[a] | 1.123 | 0.009 |
| Stretched curd | 46.01 | 49.67 | 51.87 | 4.794 | 0.895 |
| Stretching whey | 6.97 | 5.90 | 6.29 | 0.737 | 0.446 |

[a–c]Within rows, different superscripts indicate significant differences ($P \leq 0.05$).

[1]Values are expressed as mean of 3 replicates. Cp = cheesemaking plant; RMSE = root mean square error.

**Table 2.** Ash content of all mass streams of the cheesemaking system (% on DM basis)[1]

| Item | Cp 1 | Cp 2 | Cp 3 | RMSE | $P$-value |
|---|---|---|---|---|---|
| Milk | 6.08 | 5.96 | 5.86 | 0.326 | 0.811 |
| Curd | 4.89 | 5.06 | 4.46 | 0.673 | 0.688 |
| Whey | 7.15 | 6.97 | 7.39 | 0.699 | 0.842 |
| Agra | 17.07 | 16.15 | 16.76 | 0.788 | 0.840 |
| Ricotta cheese | 2.64 | 3.18 | 2.57 | 0.856 | 0.736 |
| Ricotta whey | 8.37 | 7.79 | 8.73 | 1.010 | 0.679 |
| Cooked curd | 4.80 | 5.07 | 4.43 | 0.825 | 0.761 |
| Cooked curd whey | 8.28 | 7.52 | 8.57 | 1.177 | 0.688 |
| Stretched curd | 6.16 | 5.36 | 4.88 | 1.200 | 0.609 |
| Stretching whey | 5.94 | 6.86 | 7.20 | 2.391 | 0.869 |

[1]Values are expressed as mean of 3 replicates. Cp = cheesemaking plant; RMSE = root mean square error.

**Table 3.** Crude protein content of all mass streams of the cheesemaking system (% on DM basis)[1]

| Item | Cp 1 | Cp 2 | Cp 3 | RMSE | $P$-value |
|---|---|---|---|---|---|
| Milk | 42.24 | 38.70 | 36.70 | 1.521 | 0.077 |
| Curd | 29.95 | 27.87 | 26.54 | 2.256 | 0.424 |
| Whey | 18.93 | 15.51 | 15.45 | 3.240 | 0.542 |
| Agra | 7.85 | 8.02 | 7.38 | 3.047 | 0.592 |
| Ricotta cheese | 36.14[a] | 34.87[ab] | 29.36[b] | 1.650 | 0.050 |
| Ricotta whey | 15.23 | 10.43 | 11.87 | 4.848 | 0.642 |
| Cooked curd | 43.60[a] | 41.44[ab] | 37.20[b] | 1.206 | 0.029 |
| Cooked curd whey | 15.31 | 10.05 | 12.48 | 5.698 | 0.687 |
| Stretched curd | 44.64 | 42.93 | 39.75 | 2.074 | 0.201 |
| Stretching whey | 10.96 | 6.50 | 6.64 | 3.689 | 0.481 |

[a,b]Within rows, different superscripts indicate significant differences ($P \leq 0.05$).

[1]Values are expressed as mean of 3 replicates. Cp = cheesemaking plant; RMSE = root mean square error.

**Table 4.** Fat content of all mass streams of the cheesemaking system (% on DM basis)[1]

| Item | Cp 1 | Cp 2 | Cp 3 | RMSE | $P$-value |
|---|---|---|---|---|---|
| Milk | 39.39[b] | 39.07[b] | 44.96[a] | 1.140 | 0.023 |
| Curd | 24.04 | 25.43 | 29.12 | 1.525 | 0.091 |
| Whey | 10.30 | 9.84 | 11.79 | 1.957 | 0.631 |
| Agra | 3.55 | 3.20 | 3.27 | 4.564 | 0.957 |
| Ricotta cheese | 48.28 | 42.32 | 48.66 | 8.613 | 0.736 |
| Ricotta whey | 2.80 | 1.47 | 2.83 | 1.746 | 0.705 |
| Cooked curd | 41.25[b] | 42.25[b] | 46.33[a] | 0.568 | 0.006 |
| Cooked curd whey | 2.89 | 2.39 | 2.09 | 2.016 | 0.702 |
| Stretched curd | 40.47 | 41.58 | 40.67 | 0.949 | 0.114 |
| Stretching whey | 4.78 | 4.65 | 4.65 | 1.636 | 0.652 |

[a,b]Within rows, different superscripts indicate significant differences ($P \leq 0.05$).

[1]Values are expressed as mean of 3 replicates. Cp = cheesemaking plant; RMSE = root mean square error.

**Table 5.** Lactose content of all mass streams of the cheesemaking system (% on DM basis)[1]

| Item | Cp 1 | Cp 2 | Cp 3 | RMSE | $P$-value |
|---|---|---|---|---|---|
| Milk | 39.93 | 40.74 | 38.49 | 3.956 | 0.854 |
| Curd | 13.4 | 17.17 | 13.89 | 2.203 | 0.323 |
| Whey | 63.62 | 67.68 | 65.37 | 5.819 | 0.797 |
| Agra | 72.10 | 71.50 | 73.09 | 5.176 | 0.619 |
| Ricotta cheese | 11.05 | 15.71 | 16.81 | 3.166 | 0.079 |
| Ricotta whey | 74.43 | 81.39 | 78.41 | 7.487 | 0.700 |
| Cooked curd | 10.35 | 11.24 | 12.05 | 1.116 | 0.425 |
| Cooked curd whey | 73.53 | 80.94 | 75.76 | 8.808 | 0.717 |
| Stretched curd | 1.58 | 1.56 | 2.50 | 2.093 | 0.758 |
| Stretching whey | 75.84 | 81.33 | 81.18 | 4.650 | 0.573 |

[1]Values are expressed as mean of 3 replicates. Cp = cheesemaking plant; RMSE = root mean square error.

**Table 6.** Recovery (%) of milk components across the cheesemaking system (mean ± SD; n = 18)[1]

| Item | Stretched curd | Ricotta cheese | Stretching whey | Total |
|---|---|---|---|---|
| Weight | 11.23 ± 0.32 | 4.33 ± 0.18 | 83.74 ± 0.26 | 99.29 ± 0.38 |
| Water | 6.12 ± 0.87 | 3.57 ± 0.12 | 89.01 ± 1.20 | 98.70 ± 0.45 |
| Total solids | 47.62 ± 2.86 | 8.34 ± 1.06 | 43.82 ± 2.18 | 99.79 ± 0.86 |
| Ash | 43.12 ± 7.43 | 3.72 ± 0.89 | 53.02 ± 8.13 | 99.86 ± 0.97 |
| Protein | 72.04 ± 6.87 | 11.55 ± 1.48 | 16.04 ± 7.61 | 99.62 ± 1.04 |
| Fat | 76.87 ± 3.23 | 15.90 ± 1.73 | 6.94 ± 2.10 | 99.71 ± 0.67 |
| Lactose | 10.53 ± 1.61 | 2.76 ± 1.13 | 86.61 ± 2.54 | 99.90 ± 0.46 |

[1]Recovery (%) was calculated as the ratio of each component in the specified matrix to its initial mass in milk. Total recovery represents the sum of recoveries in stretched curd, ricotta cheese, and stretching whey.

## Yield Estimation

Results of the MLR analysis evaluating the effects of milk protein, fat, and urea concentrations on cheese yield are summarized in Table 7. Out of 156 total observations, 151 were used for the final model (5 were excluded due to missing values). The MLR model was highly significant ($P < 0.0001$) and exhibited strong explanatory power ($R^2 = 0.822$; adjusted $R^2 = 0.813$).

Although the initial model considered 11 milk compositional variables, only protein (%), fat (%), and urea (mg/dL) were significant ($P < 0.05$). Together, these 3 parameters accounted for more than 81% of the observed variability in actual cheese yield. The RMSE was 0.1769, with a coefficient of variation of 1.58%, indicating high predictive precision relative to the mean yield (11.13%). The final MLR model predicting cheese yield was highly significant ($P < 0.0001$) and is expressed by the following equation:

$$\text{Yield (\%)} = 4.16 + 1.22 \times \text{Protein (\%)} + 0.40 \times \text{Fat (\%)} + 0.051 \times \text{Urea (mg/dL)},$$

where the intercept is the model constant and the regression coefficients represent the estimated change in cheese yield (%) associated with a one-unit increase in each predictor, with all other predictors held constant.

Multicollinearity analysis showed VIF values of 1.56 for protein, 1.08 for fat, and 1.47 for urea, confirming the absence of redundant predictors. Protein content exerted the strongest influence (regression coefficient [**β**] = 1.22; t-statistic [**t**] = 17.25; standardized β = 0.73), followed by fat (β = 0.40; t = 9.33; standardized β = 0.36) and urea (β = 0.051; t = 8.22; standardized β = 0.33).

Correlation analysis revealed a moderate association between protein and urea (r = 0.407; $P < 0.0001$) and a weaker correlation between fat and protein (r = −0.368; $P = 0.0083$). Residual analysis indicated that production season did not significantly influence yield variability ($F = 1.70$; $P = 0.174$), accounting for only 5.4% of the residual variance.

Cross-validation confirmed the model's robustness, yielding RMSE of 0.183 and a strong correlation between observed and predicted values (r = 0.898), corresponding to a cross-validated $R^2 \approx 0.81$. The MAE was 0.147, whereas the MBE was negligible ($5.3 \times 10^{-15}$), indicating no systematic bias. Bland–Altman analysis showed limits of agreement of ±0.343, representing ~3% of the mean yield, which confirms high concordance between experimental and predicted values.

Figure 2 shows the relationship between experimentally observed and model-predicted cheese yield values. Predicted values are distributed close to the 1:1 reference line across the entire range of observations.

Figure 3 illustrates the residuals, calculated as the differences between observed and predicted cheese yield values. The residuals are randomly dispersed around the horizontal zero line, and the residual variance remains stable across the entire range of predicted values. This random distribution confirms that the model is well-specified and that the error term follows a normal distribution without systematic bias.

**Table 7.** Multiple linear regression model predicting cheese yield (n = 151)[1]

| Variable | β (estimate) | SE | t | *P*-value | 95% CI | β standardized | VIF | Partial $R^2$ |
|---|---|---|---|---|---|---|---|---|
| Intercept | 4.1607 | 0.2971 | 14.01 | <0.0001 | 3.5736–4.7478 | — | — | — |
| Protein (%) | 1.2180 | 0.0706 | 17.25 | <0.0001 | 1.0785–1.3576 | 0.7319 | 1.4867 | 0.672 |
| Fat (%) | 0.4010 | 0.0430 | 9.33 | <0.0001 | 0.3161–0.4860 | 0.3625 | 1.2453 | 0.375 |
| Urea (mg/dL) | 0.0506 | 0.0062 | 8.22 | <0.0001 | 0.0384–0.0627 | 0.3302 | 1.3317 | 0.318 |

[1]Model statistics: $R^2 = 0.822$; adjusted $R^2 = 0.813$; RMSE = 0.1769; $F_{(3,145)} = 226.92$; $P < 0.0001$.

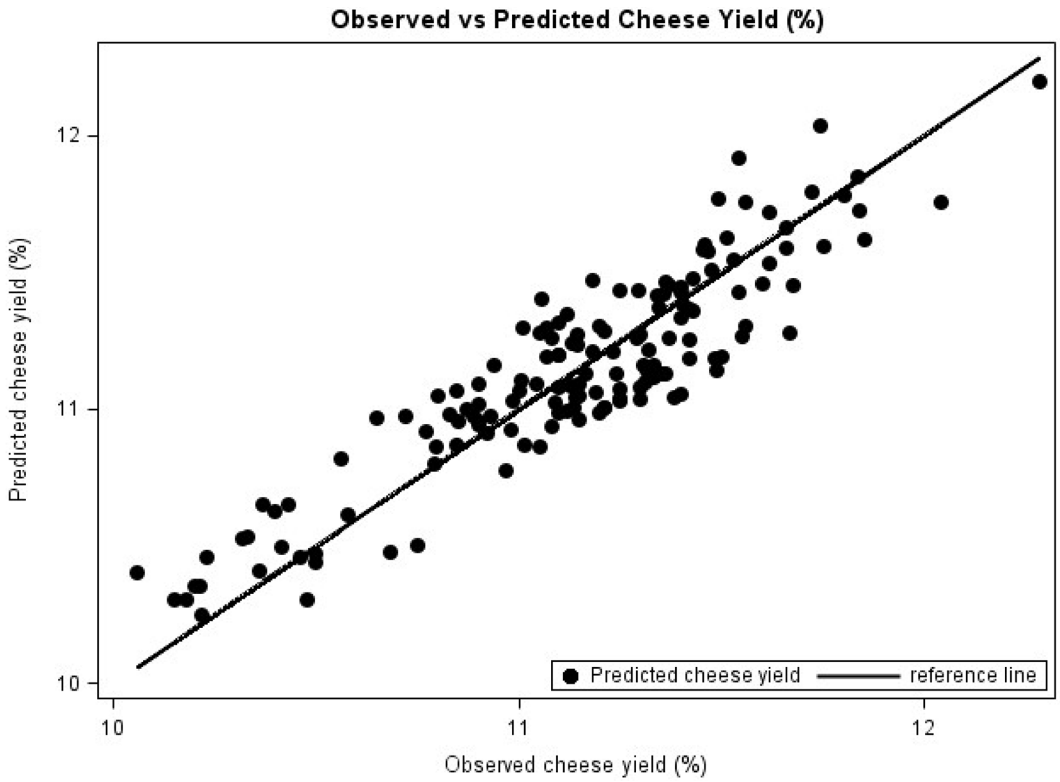


**Figure 2.** Relationship between observed and predicted cheese yield (%) of Caciocavallo Palermitano cheese based on the multiple linear regression model (n = 151). The solid line represents the line of identity (y = x).

## DISCUSSION

### *Mass Balance Across Cheesemaking Stages*

The mass balance analysis highlights that the initial stages of cheesemaking, particularly rennet coagulation and whey expulsion, dictate the distribution of the primary milk components. Proteins and fat are concentrated within the curd, whereas lactose and minerals predominantly partition into the liquid fraction, confirming the selective partitioning typical of stretched-curd cheese processing (Fox et al., 2017; Bergamaschi and Bittante, 2018; Parhi et al., 2025).

Interplant differences in milk DM are reflected in both the curd and the whey, demonstrating that even relatively small variations in raw milk composition can be amplified throughout the production process. In Cp 3, the higher DM of the initial milk and curd indicates superior solids retention. Conversely, in Cp 1, the elevated DM in the whey suggests greater losses during syneresis, likely attributable to technological factors such as curd cutting size, stirring intensity, or drainage duration (Perreault et al., 2017; Mirzakulova et al., 2025). Similarly, differences in DM trends observed in cooked and acid curds highlight variability among cheesemaking plants in the management of the cooking and acidification phases, with consequent effects on curd dehydration and protein matrix contraction (Walstra et al., 2006; Everard et al., 2011; Parhi et al., 2025). Overall, the variability observed among cheesemaking plants reflects the intrinsic heterogeneity of artisanal production systems, where differences in raw material composition and technological practices contribute to variability in the final product. In particular, studies on traditional cheesemaking systems have shown that, even under comparable technological procedures, differences in ingredient quality and process management can lead to significant variations in product composition and characteristics (Busetta et al., 2023; Mangione et al., 2023b).

Regarding the by-products, the higher DM in ricotta cheese from Cp 2 and Cp 3 compared with Cp 1 could be associated with differences in whey drainage management during manual handling of the ricotta cheese in the thermo-coagulation phase (Salvatore et al., 2014). Conversely, ash content remained uniform across all matrices, indicating that mineral distribution, particularly calcium, was relatively consistent. This stability is an es-

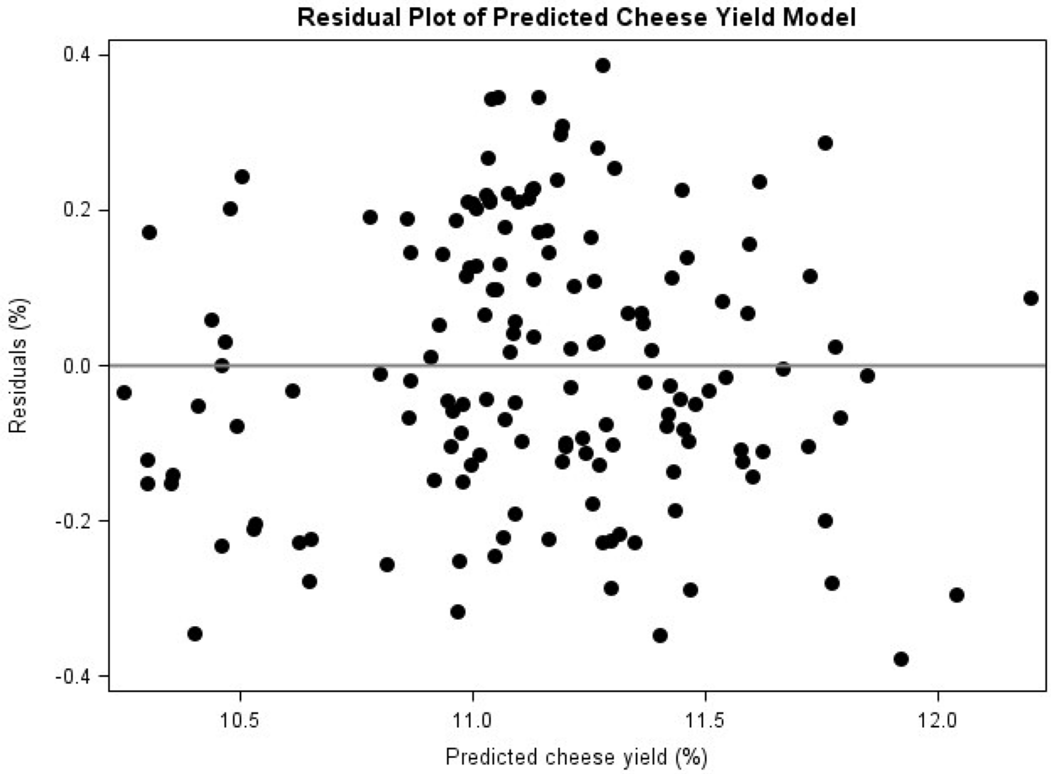


**Figure 3.** Residuals (observed − predicted cheese yield, %) plotted against predicted cheese yield values for the multiple linear regression model (n = 151). Each black circle represents one individual observation, and the horizontal solid line represents zero residual.

sential factor for maintaining the necessary curd plasticity during the subsequent stretching phase (Sheehan and Guinee, 2004; Gonçalves and Cardarelli, 2021).

Crude protein content also showed significant interplant variations. Interestingly, in ricotta cheese, Cp 1 exhibited higher CP values than Cp 3, suggesting that the efficiency of protein recovery from whey directly affects both the overall process yield and the nutritional density of the by-products (Salvatore et al., 2014; Pires et al., 2021; Mangione et al., 2023a; Selmi et al., 2025). These findings underscore the critical role of ricotta cheese production in valorizing the dairy chain, transforming what would otherwise be a whey by-product into a high-value product, in line with established concepts of whey valorization in dairy processing systems (Smithers, 2008; Prazeres et al., 2012) and traditional Sicilian ricotta production (Mangione et al., 2023b).

Lipid content in the early stages of cheesemaking reflects both milk composition and operational conditions; Cp 3 exhibited higher fat levels in the curd and cooked curd, highlighting how temperature and cooking management modulate lipid retention within the protein matrix, with direct consequences for yield and functionality (Guinee et al., 2007). Interestingly, the convergence of lipid values observed in the final stretched curd indicates that the stretching stage tends to homogenize the protein structure, thereby mitigating the initial differences between cheesemaking plants (Lucey, 2002).

The absence of significant differences in lactose between plants suggests a high degree of uniformity in fermentation and stretching, with lactose removal occurring efficiently through both bacterial metabolism and whey expulsion, as described in standard dairy science literature (Walstra et al., 2006; Fox et al., 2017). Regarding protein recovery, the values observed in the stretched curd were consistent with the expected retention of casein, whereas whey proteins largely partitioned into the whey (Walstra et al., 2006). The subsequent recovery of these proteins through ricotta production confirms the effectiveness of the thermo-acid aggregation process (Maubois and Mocquot, 1975).

Fat retention in the stretched curd reflects the physical entrapment of milk fat globules within the paracasein network (Fox et al., 2017). The additional fat recovery in the ricotta cheese indicates efficient lipid capture at the system level. Overall, the production of ricotta cheese substantially improves total nutrient recovery (Salvatore et al., 2014), supporting the high efficiency of the artisanal stretched-curd system when evaluated across the entire mass balance.

### *Yield Estimation*

The regression model confirms that the yield of Caciocavallo Palermitano cheese is primarily dictated by milk intrinsic quality, with predominant contributions from the protein and fat fractions. This aligns with established literature on stretched-curd cheeses, where caseins form the structural backbone and fat contributes significantly to solids retention (Emmons et al., 1990; Pretto et al., 2013). Recent studies further support that incorporating detailed compositional fractions within advanced statistical models significantly enhances the predictive capacity for cheese yield (Mariani et al., 2022; Stocco et al., 2022; Stocco et al., 2025).

Although urea does not directly participate in curd formation, its inclusion improved the model's predictive accuracy. This reflects its role as a proxy for the cow's metabolic status and nitrogen utilization efficiency; milk urea concentration indicates the balance between dietary protein intake and ruminal microbial synthesis, which is inherently associated with variations in mammary protein synthesis and overall milk technological quality (Nousiainen et al., 2004; Spek et al., 2013).

The VIF values (<2) and the LODO cross-validation demonstrate the model's stability and absence of multicollinearity. Furthermore, the lack of a significant seasonal effect on residuals suggests that seasonality influences yield primarily through changes in milk chemical composition rather than through unmodeled process variables (Auldist et al., 1998).

The combination of high $R^2$, low MAE and RMSE, and narrow Bland–Altman limits of agreement confirms that the model is statistically robust and accurate under real artisanal production conditions.

For potential practical applications in milk valuation systems, a simplified milk yield index is proposed as an exploratory predictive equation:

$$\text{Milk yield index} = 1.26 \times \text{Protein (\%)} + 0.37 \times \text{Fat (\%)}.$$

This index should be considered a preliminary indicator of cheesemaking potential and requires further validation in independent and subsequent studies under similar production conditions before any practical application in milk payment systems (IDF and FAO, 2011; Stocco et al., 2022). Although the LODO validation showed stability across the 3 studied plants, broader external validation beyond this specific geographic area would be beneficial to confirm wider applicability. Additionally, further fractionation (e.g., casein or NPN) could provide even deeper insights into the recovery dynamics.

## CONCLUSIONS

This study demonstrates that experimentally validated closed mass balances provide an effective framework for quantifying the partitioning of milk components in artisanal stretched-curd cheesemaking. The initial processing stages, specifically coagulation and whey syneresis, are decisive for the efficient retention of protein and fat, whereas integrated ricotta production improved overall nutrient recovery. Cheese yield was primarily explained by milk composition, and the predictive model based on protein, fat, and urea showed high explanatory power and robustness, as confirmed by LODO cross-validation. The combined application of mass balance analysis and statistical modeling represents a valuable approach for characterizing traditional dairy systems and identifying key determinants of yield variability. This framework supports the optimization of dairy processes and may provide a basis for future milk payment schemes based on objective technological quality criteria. Further validation across different production contexts will be required to confirm the broader applicability of the model.

## NOTES

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**Nonstandard abbreviations used:** β = regression coefficient; Cp = cheesemaking plant; LODO = leave-one-dairy-out; MAE = mean absolute error; MBE = mean bias error; MLR = multiple linear regression; RMSE = root mean square error; t = t-statistic; VIF = variance inflation factor.

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