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# The use of Brix refractometer as a simple and economic device to estimate the protein content of sheep milk

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ABSTRACT

In this study, 737 individual sheep milk samples were collected 2 times from morning milkings to evaluate the relationships between Brix refractometer measurements and milk constituents— protein and fat percentages—to verify its ability to predict milk constituents. The Pearson's simple (rSP) and partial (rPP) correlations between milk constituents were calculated, and several first- and second-order regressions were tested to predict the protein and fat percentages. The rSP coefficients between the Brix refractometer measurement and fat (rSP = 0.46) and protein (rSP = 0.87) contents were different from the rPP coefficients, particularly for fat percentage (rPP = 0.04 and rPP = 0.82, for fat and protein percentages, respectively). The results of the forecasts can be considered satisfactory only for the first order regression that predicted the percentage of milk protein, while the regression that predicted the percentage of fat + milk protein presented a weak forecasting capacity.


## 1. Introduction

Dairy sheep management varies greatly with the breed, production system, and country. The most important dairy sheep in the European Mediterranean countries (France, Greece, Italy, Spain, and Turkey) produce 65% of the total European sheep milk, and most dairy sheep are raised under extensive and semi-extensive systems (Sitzia et al., 2015). The main use of sheep milk in the world is that of making cheese. In Mediterranean and Eastern European countries, milk is often processed at the small farm level or in small local dairies.

From a chemical point of view, cheese is mainly composed of proteins and fats, as well as water, lactose and mineral salts. Therefore, adequate fat and protein percentages in milk utilized for cheese production are particularly important in its chemical definition, as they have a decisive effect on cheese yield (Pirisi, Lauret, & Dubeuf, 2007). Cheese yield prediction formulas have evolved from one from 1895 that was based only on casein and fat, but the percentage of fat and, above all, that of milk protein are the main factors responsible for cheese yield (Emmons & Modler, 2010). In a recent paper on sheep milk cheese (Pazzola et al., 2023), the best performance in predicting cheese yield was obtained by utilizing the total solids in milk. The same authors reported that the predictions for estimating cheese yield by using the milk components—proteins and fats—were lower, thus highlighting the complexity of the relationships between milk nutrients and their recovery in curds.

In any case, knowledge of the main components of sheep milk, especially fat and protein, has become a significant aid even in small artisan dairies that cannot afford expensive tools. In fact, the use of rapid analytical methods in the dairy industry has become essential to control milk adulteration, microbial contamination and the influence of animal nutrition. However, the long wait for official analytical methods, as well as the high cost, may not be effective. In contrast, rapid analytical techniques, such as near-infrared spectroscopy (NIRS), have gained prominence and proven to be efficient tools that provide immediate results, but their cost is often not affordable for small dairy farms, so cheaper equipment could be used at the expense of estimation accuracy. In this regard, the Brix refractometer could be an easy-to-use tool whose cost is easily accessible even for a small farm.

Percent Brix is a measure of sucrose concentrations in a liquid, while when used in liquids not containing sucrose, the Brix measurement approximates the percentage of total solids (TSs) (Quigley, Lago, Chapman, Erickson, & Polo, 2013). A Brix refractometer was used to estimate TSs in waste milk with satisfactory results (Moore, Taylor, Hartman, & Sischo, 2009) when evaluating the IgG concentration in the maternal colostra of sheep (Santiago et al., 2020), horses and jennies (Turini, Nocera, Bonelli, Mele, & Sgorbini, 2020; McCue, 2021), and cattle (Buczinski &

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Vandeweerd, 2016; Lokke, Engelbrecht & Wiking, 2016). Even in porcine colostrum, the Brix refractometer has proven to be an economical, fast and satisfactory tool for estimating immunoglobulin concentration, allowing the differentiation between good and poor quality colostrum (Hasan, Junnikkala, Valros, Peltoniemi, & Oliviero, 2016).

In a previous study on the colostra of Valle del Belice sheep, a high and positive correlation ($r = 0.90$) was found between the Brix value and the colostrum protein percentage (Todaro, Maniaci, Gannuscio, Pampinella, & Scatassa, 2023a); similar results ($r = 0.72$) were found in Merino sheep colostra that were sampled 24 h after lambing (Agenbag, Swinbourne, Petrovski, & van Wettere, 2023). Finally, Floren, Sischo, Crudo & Moore (2016) reported that a Brix refractometer can be a useful tool for estimating total solids concentration in reconstituted milk to help the farmer monitor its quality in milk replacement feeding.

Therefore, the objective of this study was to establish the relationship between Brix refractometer readings and the percentage of milk fat and/or protein in order to easily estimate milk composition with a simple farm-level device.

## 2. Materials and methods

Milk samples were collected as part of routine animal milk collection in breeding farms and as part of a non-experimental veterinary practice. No animal discomfort was caused during the sample collection for the purpose of this study. Directive 2010/63/EU of the European Parliament and Council and the Italian D. Lgs. 26/2014 do not apply to non-experimental practices. An ethical review by the animal welfare body was, therefore, not required.

The study was conducted on a farm in the province of Agrigento (Sicily, Italy) that raises Valle del Belice dairy sheep. Milk sampling was done during the spring, with two sampling sessions that were conducted in April and May. During the morning milking, all of the lactating ewes were hand-milked, and individual samples of 50 mL of milk were collected and immediately refrigerated at +5 °C. Altogether, 737 individual milk samples were collected.

All milk samples were sent to the Experimental Zooprophylactic Institute of Sicily, Italy, where they were analyzed after vortexing for 10 s to ensure adequate homogeneity and then heated to 42 °C in a water bath, and analyzed for their lactose, fat, crude protein, urea, acetone, and beta-hydroxybutyrate concentrations, freezing point, and somatic cell count via the infrared method (Combi-Foss 6000, Foss Electric, Hillerød, Denmark). Moreover, the refractive index was measured with an optical Brix refractometer (Manual Refractometer MHRB-40 ATC, Mueller Optronic, Erfurt, Germany). The refractometer was equipped with a Brix scale ranging from 0 to 40 % Brix; the accuracy of the instrument was ±0.2 % Brix at 20 °C.

The analytical data were checked to exclude any outliers. For each milk component analyzed, the Student's t for skewness and kurtosis and the chi-square for the heterogeneity of the variance were calculated, verifying the normality of the distribution, with the exception of the SCC (somatic cell count). Therefore, this variable was transformed into $Log_{10}$ to normalize it before the analysis. Moreover, cheese-making constituents (fat + protein) were calculated as fat plus protein percentages.

Milk constituents were analyzed by using MEAN, CORR, FACTOR, and REG procedures of SAS *v.* 9.1.2. To investigate the relationships between the Brix refractometer values and milk constituents, the Pearson simple (rSP) and partial correlations (rPP) among all variables determined were calculated. To evaluate the forecasting abilities of the protein and fat milk percentages according to the Brix refractometer measurements, a stepwise multiple regression model was employed, with the significance level set at 0.15. With the aim of predicting the milk protein percentage and/or the sum of fat plus protein percentages, first and second order regression models defined by Equations (1)–(4) were fitted.

$$CP = a + b \times Brix \tag{1}$$

$$CP = a + b \times Brix + c \times Brix^2 \tag{2}$$

$$Fat + CP = a + b \times Brix \tag{3}$$

$$Fat + CP = a + b \times Brix + c \times Brix^2 , \tag{4}$$

where: CP = crude protein content (%, m $m^{-1}$); Brix = the Brix value; Fat = fat content (%, m $m^{-1}$); a, b, c = coefficients of the fitted equations.

The adequacy of fit of the predictive regression models was assessed by comparing actual and predicted values. The criteria for comparison were the Pearson and rank correlations between the actual (from IR analysis) and predicted values, the difference between their standard deviations, the standard deviations of the differences between the actual and predicted values (predicted mean square error = MSEP), the prediction bias and the Wilmink test, which corresponded to 100 times the ratio between the standard deviation of the differences between the actual and predicted values and the mean value (Macciotta, Cappio-Borlino, & Pulina, 2000).

## 3. Results and discussion

The simple statistics of the physico-chemical parameters of the individual milk samples are reported in Table 1. The milk fat and protein percentages presented high variability, likely due to the individual data—in particular, the average fat percentage was lower than the standard in this breed of ewes (Todaro, Gannuscio, Mancuso, Ducato, & Scatassa, 2023b). This is probably due to the fact that we only used the milk obtained from the morning milking, which was less fatty than the daily milk production. The milk urea level had an average of 39.67 ± 9.22 mg $dL^{-1}$, which was slightly higher than the mean value reported by Todaro et al. (2023b) and the value of 35 mg $dL^{-1}$, that is considered acceptable for dairy ewes (Cannas, Pes, Mancuso, Vodret, & Nudda, 1998). This fact was explained by the sampling in the spring (April and May), which is characterized by a high availability of green forage (Molle, Decandia, Cabiddu, Landau, & Cannas, 2008; Todaro, Bonanno, & Scatassa, 2014). The SCC had an average logarithmic value of 2.38, which corresponded to 240,000 somatic cells $mL^{-1}$ of milk. Surprisingly, this value was lower than those reported in previous studies on individual milk samples of the Valle del Belice breed (Riggio, Portolano, Bovenhuis, & Bishop, 2010; Tolone, Riggio, & Portolano, 2013).

**Table 1**
Descriptive statistics of physicochemical parameters of ewe's milk.

| Parameters | n | Mean value | Standard deviation | Minimum value | Maximum value |
|---|---|---|---|---|---|
| Brix value | 737 | 13.91 | 0.86 | 12.50 | 17.00 |
| Fat (% m $m^{-1}$) | 737 | 5.96 | 1.57 | 3.42 | 9.80 |
| Crude protein (% m $m^{-1}$) | 737 | 5.89 | 0.63 | 4.46 | 8.05 |
| Fat + crude protein (% m $m^{-1}$) | 737 | 11.85 | 1.97 | 6.87 | 17.48 |
| Lactose (% m $m^{-1}$) | 737 | 4.57 | 0.35 | 2.64 | 5.28 |
| Urea (mg $dL^{-1}$) | 737 | 39.7 | 9.2 | 16.2 | 73.3 |
| Somatic Cell Count (Log10) | 737 | 2.38 | 0.73 | 1.38 | 4.59 |
| Freezing point (°C) | 737 | −0.532 | 0.007 | −0.552 | −0.491 |
| Acetone (mm) | 737 | 0.096 | 0.143 | 0.01 | 1.04 |
| β-hydroxybutyrate, BHB (mm) | 737 | 0.093 | 0.104 | 0.01 | 1.70 |

**Table 2**
Pearson correlations below the diagonal and partial correlation coefficients above the diagonal.[a]

| | Brix | Fat | CP | Fat + CP | Lactose | Urea | SCC | FP | Acetone | BHB |
|---|---|---|---|---|---|---|---|---|---|---|
| Brix | **1** | 0.04 | 0.82*** | nd | 0.07* | −0.06 | −0.16*** | −0.12*** | 0.00 | −0.01 |
| Fat (% m m$^{-1}$) | 0.46*** | **1** | −0.34*** | nd | −0.88*** | 0.10** | 0.24*** | −0.39*** | 0.02 | −0.13*** |
| Crude protein (% m m$^{-1}$), CP | 0.87*** | 0.52*** | **1** | nd | −0.49*** | 0.26*** | 0.20*** | −0.19*** | 0.09** | −0.14*** |
| Fat + crude protein (% m m$^{-1}$) | 0.64*** | 0.96*** | 0.73*** | **1** | nd | nd | nd | nd | nd | nd |
| Lactose (% m m$^{-1}$) | 0.01 | 0.50*** | −0.36*** | −0.51*** | **1** | 0.22*** | 0.12*** | −0.51*** | 0.21*** | −0.22*** |
| Urea (mg dL$^{-1}$) | 0.21*** | −0.06 | 0.22*** | 0.02 | 0.10** | **1** | −0.18*** | 0.39*** | −0.02 | −0.04 |
| Somatic Cell Count ($Log_{10}$), SCC | −0.18*** | 0.26*** | 0.09** | 0.24*** | −0.60*** | −0.21*** | **1** | 0.10** | −0.10** | 0.13*** |
| Freezing point (°C), FP | −0.18*** | 0.14*** | −0.03 | −0.26*** | −0.38*** | 0.23*** | 0.21*** | **1** | −0.04 | −0.06 |
| Acetone (mM) | −0.38*** | −0.27*** | −0.32*** | −0.32*** | −0.29*** | −0.21*** | −0.03 | −0.16*** | **1** | 0.61*** |
| β-hydroxybutyrate (mM), BHB | −0.48*** | −0.27*** | −0.32*** | −0.32*** | −0.29*** | −0.21*** | 0.30*** | 0.07 | 0.64*** | **1** |

[a] *P < 0.05; **P < 0.01; ***P < 0.001; nd: not determined.

The average value of the milk freezing point (MFP) was −0.532 °C, slightly lower than that reported for sheep's milk produced in Sicily (Scatassa et al., 2017), but it is well known that the MFP is heavily dependent on water-soluble compounds, which are lower in morning milk.

The concentrations of acetone and β-hydroxybutyrate (BHB) in milk were similar—0.096 mM and 0.093 mM, respectively—and slightly lower than the values reported in the literature on cow milk (De Roos, Van Den Bijgaart, Hørlyk, & De Jong, 2007; Grelet et al., 2016), but no references were found for sheep milk. It is well known that with a negative energy balance, the adipose tissue of lactating females is mobilized; this determines an increase in the blood plasma concentration of non-esterified FAs (NEFAs). As the supply of NEFAs is overloaded, the production of ketone bodies (acetoacetic acid, acetone, and BHB) in the liver increases (Chilliard et al., 2000). Therefore, the ketone bodies may serve as reliable indicators for a cow's energy status. Grelet et al. (2016) demonstrated the potential of Fourier transform mid-infrared spectrometry (Combi-Foss 6000, Foss Electric) for predicting citrate content with good accuracy and for supplying indications of the contents of BHB and acetone in milk, thus allowing rapid and economical indications to manage ketosis and negative energy balance in dairy cattle farms.

In Table 2, a matrix of the correlation coefficients is shown. Simple correlation coefficients (rSP) are reported below the diagonal, while partial correlation coefficients (rPP) are reported above the diagonal. Similarly to what was found for sheep colostra (Todaro et al., 2023a), the Brix refractometer measurements presented the highest positive correlations with the milk protein percentage (rSP = 0.87; P < 0.001; rPP = 0.82; P < 0.001). Conversely, the comparative analysis of the simple and partial correlations between the Brix refractometer measurements and the milk fat percentage showed a positive correlation when considering rSP (rSP = 0.46; P < 0.001), while no correlation was found when considering rPP (rPP = 0.04; ns). Simple correlation analysis shows whether there is a relationship between two variables and how strong that relationship can be, while a partial correlation analysis allows the estimation of the association between two quantitative variables after eliminating the influence of other variables (Vargha, Bergman, & Delaney, 2013); so it is likely that the correlation between Brix refractometer measurements and fat percentage could be affected by other variables.

The differences between the simple and partial correlation coefficients were highlighted in terms of both the correlation between the Brix measurements and acetone and the correlation between the Brix measurements and BHB. The analysis of the simple correlations showed negative and significant correlation coefficients, which canceled out if the partial correlation coefficients were considered. The simple correlation coefficients between the Brix refractometer measurements and the SCC (rSP = −0.18; P < 0.001) and between the Brix refractometer measurements and FP (rSP = −0.18; P < 0.001) were weak and negative. Similar values were found for the partial correlation coefficients, demonstrating a real negative correlation between the Brix measurements and the SCC and between the Brix measurements and FP.

The presentation and discussion of correlation coefficients between other milk constituents are not the objects of this study, even though their variations were similar to those reported by Todaro et al. (2023b).

On the basis of this significant correlation between the Brix refractometer measurements and the milk crude protein percentage, reported in Table 3 is the forecasting ability of Brix measurements when estimating the protein percentage and the sum of the fat and protein percentages, which are responsible for cheese yield.

Overall, the results of the forecasts can only be considered satisfactory for the simple regression defined in Equation (5) that predicted the percentage of milk crude proteins (Fig. 1A):

$$CP = -2.996 + 0.639 \times Brix \quad (5)$$

**Table 3**
Predictions of milk crude protein (CP; %, m m$^{-1}$) and fat + CP (%, m m$^{-1}$) according to first and second order regressions.

| | Equation (1) | Equation (2) | Equation (3) | Equation (4) |
|---|---|---|---|---|
| [a]a | −2.996 | 8.338 | −9.6176 | 66.996 |
| [b]SE | 0.188 | 2.393 | 0.903 | 11.268 |
| *P-value* | *<0.001* | *<0.001* | *<0.001* | *<0.001* |
| [c]b | 0.639 | −0.965 | 1.4720 | −9.374 |
| [b]SE | 0.013 | 0.338 | 0.065 | 1.592 |
| *P-value* | *<0.001* | *<0.004* | *<0.001* | *<0.001* |
| c | | 0.057 | | 0.382 |
| [b]SE | | 0.012 | | 0.056 |
| *P-value* | | *<0.001* | | *<0.001* |
| $R^2$ (%) | 75.2 | 75.9 | 41.3 | 44.8 |
| Y (mean) | 5.89 | 5.89 | 10.85 | 10.85 |
| [d]Y^ (mean) | 5.89 | 5.98 | 10.85 | 10.80 |
| Pearson correlation (Y, Y^) | 0.87 | 0.87 | 0.54 | 0.57 |
| Rank correlation | 0.84 | 0.84 | 0.46 | 0.46 |
| [e]σ Y^ | 0.55 | 0.56 | 1.27 | 1.31 |
| [f]σ Y - σ Y^ | 0.08 | 0.07 | 0.70 | 0.66 |
| [g]Bias | 0.00 | −0.09 | 0.00 | 0.06 |
| [h]σ (Y−Y^) (MSEP) | 0.32 | 0.31 | 1.66 | 1.63 |
| [i] (σ (Y−Y^)/Y)*100 | 5.36 | 5.28 | 15.34 | 14.99 |

[a] a: intercept.
[b] SE: standard error.
[c] b: angular coefficient.
[d] Mean of predicted values.
[e] Standard deviation of predicted values.
[f] Difference between standard deviation of actual and predicted values.
[g] Bias: mean of the differences.
[h] Standard deviation of differences between actual and predicted values (MSEP = mean square error predicted).
[i] Wilmink test: 100 times the ratio between the standard deviation of differences between actual and predicted values and the mean value (Macciotta et al., 2000).

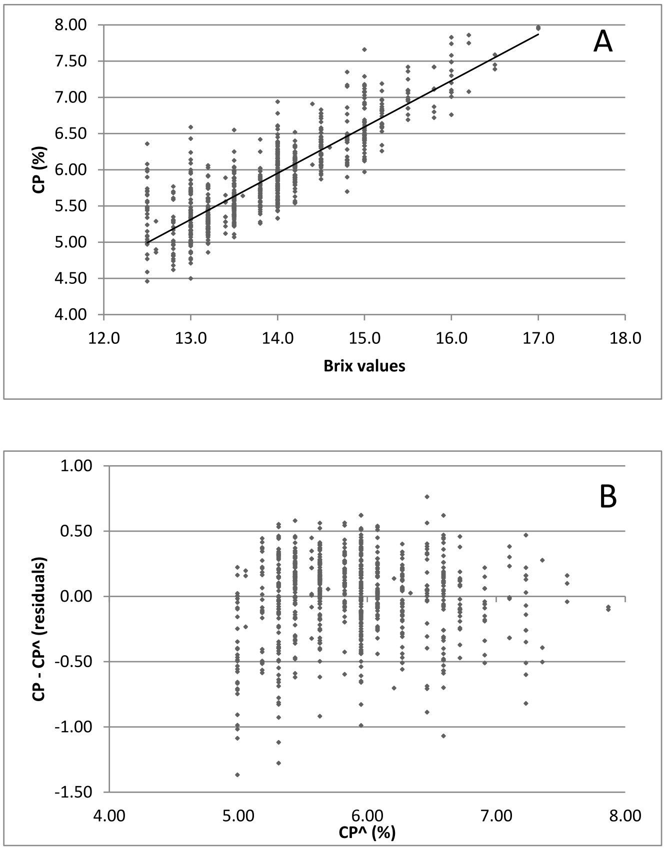


**Fig. 1.** Linear regression and residuals Equation (5).

The determination coefficient was equal to 0.75. The residuals plot shows a homogeneous distribution (Fig. 1B) and the mean of the differences (bias) was zero. The correlation between actual and predicted data was 0.75 while the rank correlation (0.87) was slightly higher. The low value of the Wilmink test highlights the solid forecasting ability of the model (Macciotta et al., 2000). The use of squared regression defined in Equation (6) did not improve the forecasting ability; therefore, its use does not seem justified.

$$CP = 8.338 - 0.965 \times Brix + 0.057 \times Brix^2 \quad (6)$$

However, when we used the values from the Brix refractometer for the prediction of dairy constituents (fat + protein), the results of the predictions were not satisfactory with either the first order regression or the second order regression. The determination coefficients varied from 41.3 to 44.8% for the first- and second order regressions. The correlations between the actual and predicted data and the rank correlations were below 0.57 and 0.46, respectively. The Wilmink test values were high—around 15 points. These results show that the forecasting capacity of these forecasting models is not satisfactory and, therefore, not feasible.

## 4. Conclusions

The digital Brix refractometer is a promising and easy-to-use tool for estimating ewes' milk constituents, thus enabling fast and real-time results. Similarly, to what was found for sheep colostra, this study showed, also for sheep milk, a significative correlation between refractometer measurements and the percentage of milk protein.

Our results demonstrated a satisfactory ability to predict the milk protein percentage through measurements read with a Brix refractometer, while the ability to predict the sum of the milk fat and protein percentages was weak, which was probably due to the absence of the partial correlation between the Brix refractometer measurements and the fat percentage. Therefore, the use of the refractometer could be a valid and very economical tool for the rapid estimation of the percentage of milk protein which could help in the milk standardization for cheesemaking in places where costly standard or reference methods are not available.

## CRediT authorship contribution statement

**M. Todaro:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **R. Gannuscio:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **I. Mancuso:** Formal analysis. **B. Ducato:** Formal analysis. **M.L. Scatassa:** Writing – review & editing, Supervision, Methodology.

## Declaration of competing interest

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

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