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Article

# The Use of a Digital Brix Refractometer to Estimate Cheese-Making Constituents in Ewes' Milk

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**Abstract:** In this study, 737 individual sheep milk samples were collected to evaluate the relationships between Brix refractometer measurements and milk constituents—particularly, protein and fat percentages—with the aim of verifying the ability of the refractometer 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 results of the forecasts can be considered satisfactory only for the simple regression that predicted the percentage of milk protein through the measurements read with the Brix refractometer ( $PRT = -2.996 + 0.639 \cdot \text{Brix}$ ), while the regression that predicted the percentage of fat + milk protein presented a weak forecasting capacity, which was probably due to the absence of partial correlations between the Brix refractometer measurements and the fat percentage.

**Keywords:** sheep milk constituents; Brix refractometer; milk protein percentages; refractometer forecasting ability

## 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 [1]. The main use of sheep milk in the world is that of making cheese. In Mediterranean and Southeastern European countries, this is usually conducted at the farm level or in small local dairies.

From a quantitative point of view, cheese is mainly composed of proteins and fats, as well as minerals, water, etc. Therefore, an adequate protein and fat content in the milk that is used as a raw material for cheese making is of particular importance in defining the product's chemical quality, as it has a determining effect on the cheese yield [2]. 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 [3]. In a recent paper on sheep cheese [4], 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 remaining milk characteristics—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 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 indispensable. This is due to documented evidence of adulteration, microbial contamination, and the influence of livestock feed on the quality of milk and dairy

products. Because of the delays involved in the use of wet chemistry methods during the evaluation of these products, rapid analytical techniques, such as near-infrared spectroscopy (NIRS), have gained prominence and have been proven to be efficient tools that provide instant results. This technique is rapid, nondestructive, precise, and cost-effective in comparison with other laboratory techniques. Portable devices are easily used on farms to perform quality control measurements, but their cost is often not affordable for small dairy farms, so cheaper equipment could be used at the expense of estimation accuracy.

The Brix percentage is a measure of sucrose concentrations in a liquid such as fruit juice, molasses, and wine. When used in non-sucrose-containing liquids, the Brix percentage approximates the percentage of total solids (TSs) [5]. A Brix refractometer was used to estimate TSs in waste milk with satisfactory results [6] when evaluating the IgG concentration in the maternal colostrum of sheep [7], horses and jennies [8, 9], and cattle [10]. In swine, the Brix refractometer has been shown to be a cheap, fast, and satisfactory instrument for estimating Ig concentration, allowing differentiation between good- and poor-quality colostrum [11].

The Brix refractometer has also been proven to be a valuable tool for estimating the total protein concentration in sheep [7] and cow [12] colostrum samples. In fact, in a previous study on the colostrum of Valle del Belice sheep, a high and positive correlation ( $r=0.90$ ) was found between the Brix value and the colostrum protein percentage [13]; similar results ( $r=0.72$ ) were found in Merino sheep colostrum that were sampled 24 h after lambing [14]. Finally, Floren et al. [15] reported that a Brix refractometer can be beneficial in estimating the concentration of total solids in milk replacement mixes to help monitor the milk replacement feeding consistency.

Therefore, the objective of the present study was to establish the relationships between Brix refractometer readings and the fat and protein percentages of sheep milk to help dairies rapidly assess potential cheese yield.

## 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 carried out on a dairy farm that typically raised 500 Valle del Belice dairy ewes. This farm was located in the territory of Santa Margherita di Belice in the province of Agrigento (Sicily, Italy). We carried out our study 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 thawed at room temperature (20–25 °C), vortexed for 10 s to ensure adequate homogeneity, warmed at 42 °C in a water bath, and analyzed for their lactose, fat, 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  $\pm 0.2\%$  Brix at 20 °C.

The data were checked for unlikely values. For each detected milk parameter, Student's *t* for skewness and kurtosis and the Chi-square for heterogeneity of variance were calculated, and they had a normal distribution, with the exception of the SCC. Therefore, this variable was transformed into  $\text{Log}_{10}$  to normalize it before the analysis. Moreover, cheese-making constituents (SCUs) 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 ( $r_{SP}$ ) and partial correlations ( $r_{PP}$ ) 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 multiple stepwise regression model was run, with the level of significance set to 0.15. With the aim of predicting the milk protein and SCU percentages, first- and second-degree regression models were fitted. The adequacy of fit of the predictive regression models was assessed by comparing the actual and predicted values. The criteria for the comparison were Pearson and rank correlations between the actual and predicted values, the difference between their standard deviations, the standard deviations of differences between the actual and predicted values (mean square error predicted = MSEP), the prediction bias, and the Wilkink test, which corresponded to 100 times the ratio between the standard deviation of differences between the actual and predicted values and the mean value [16].

### 3. Results and Discussion

The simple statistics of the physico-chemical parameters of the individual milk samples are reported in Table 1.

**Table 1.** Physicochemical parameters of ewe's milk.

Parameters	n.	Mean value	Standard deviation	Minimum value	Maximum value
Brix	737	13.91	0.86	12.50	17.00
Fat (%)	737	5.96	1.57	3.42	9.80
Protein (%)	737	5.89	0.63	4.46	8.05
Cheese-making constituents, SCU (%)	737	11.85	1.97	6.87	17.48
Lactose (%)	737	4.57	0.35	2.64	5.28
Urea (mg/dL)	737	39.67	9.22	16.16	73.32
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 (mmol/L)	737	0.096	0.143	0.01	1.04
β-hydroxybutyrate, BHB (mmol/L)	737	0.093	0.104	0.01	1.70

SCU: fat + protein percentages.

The milk fat and protein percentages presented high variability, which was probably due to the individual data—in particular, the average fat percentage was lower than the standard in this breed of ewes [17]. This was likely due to the morning milking, which resulted lower in fat. The milk urea level had an average of  $39.67 \pm 9.22$  mg/dL, which was higher than the mean value reported by Todaro et al. [17] and the value (35 mg/dL) that is considered acceptable for dairy ewes [18]. This fact was explained by the sampling in the spring (April and May), which is famously characterized by a high availability of green forage [19, 20]. The somatic cell count (SCC) had an average logarithmic value of 2.38, which corresponded to 240,000 somatic cells per milliliter of milk. Surprisingly, this value was lower than those reported in previous studies on individual samples of the same breed [21, 22].

The milk freezing point (MFP) had an average of  $-0.532$  °C, which was lower than that of bulk sheep milk produced in Sicily [23], but it is well known that the MFP is heavily dependent on water-soluble compounds, which are lower in morning milk.

The concentrations of milk acetone and β-hydroxybutyrate (BHB) were similar—0.096 and 0.093, respectively—and slightly lower than the values reported in the literature on cow milk [24, 25], 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 concentration of non-esterified FAs (NEFAs) in blood plasma. As the supply of NEFAs is overloaded, the production of ketone bodies (acetoacetic acid, acetone, and BHB) in the liver increases [26]. Therefore, the aforementioned blood metabolites may serve as reliable indicators for a cow's energy status. Grelet

et al. [25] 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, thereby providing rapid and cost-effective tools for managing ketosis and negative energy balance on dairy farms.

In Table 2, a matrix of the correlation coefficients is shown.

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

	Brix	Fat	Protein	SCU	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 (%)	0.46***	1	-0.34***	nd	-0.88***	0.10**	0.24***	-0.39***	0.02	-0.13***
Protein (%)	0.87***	0.52***	1	nd	-0.49***	0.26***	0.20***	-0.19***	0.09**	-0.14***
Cheese-making constituents (%), SCU	0.64***	0.96***	0.73***	1	nd	nd	nd	nd	nd	nd
Lactose (%)	0.01	0.50***	-0.36***	-0.51***	1	0.22***	0.12***	-0.51***	0.21***	-0.22***
Urea (mg/dL)	0.21***	-0.06	0.22***	0.02	0.10**	1	-0.18***	0.39***	-0.02	-0.04
Somatic Cell Count (Log <sub>10</sub> ), 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 (mmol/L)	-0.38***	-0.27***	-0.32***	-0.32***	-0.29***	-0.21***	-0.03	-0.16***	1	0.61***
β-hydroxybutyrate (mmol/L), BHB	-0.48***	-0.27***	-0.32***	-0.32***	-0.29***	-0.21***	0.30***	0.07	0.64***	1

SCU: Cheese-making constituents (fat + protein percentages); \* P<0.05; \*\* P<0.01; \*\*\* P<0.001; nd: not determined.

The simple correlation (rSP) are shown below the diagonal, and the partial correlation coefficients (rPP) are shown above the diagonal. Similarly, to what was found for sheep colostrum [13], 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 [27], so it is likely that the correlation between Brix refractometer measurements and fat percentage was 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 ( $r_{SP}=-0.18$ ;  $P<0.001$ ) and between the Brix refractometer measurements and FP ( $r_{SP}=-0.18$ ;  $P<0.001$ ) 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 for the chemical composition of the milk of Valle del Belice breed ewes [17].

On the basis of this strong correlation between the Brix refractometer measurements and the milk protein percentage, in Table 3, we report 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 that predicted the percentage of milk proteins ( $PRT = -2.996 + 0.639 \cdot \text{Brix}$ ); the determination coefficient was equal to 0.75. The difference between the real mean and forecasted mean was zero; the correlation between the actual and predicted data was 0.75, and the rank correlation (0.87) was slightly higher. Nevertheless, the Wilmink test value, which measured the validity of the prediction, was lower and, therefore, better than the range of 8.0–8.5, according to which the forecasting ability of the model can be considered sound [16]. The use of squared regression ( $PRT = 8.338 - 0.965 \cdot \text{Brix} + 0.057 \cdot \text{Brix}^2$ ) did not improve the forecasting ability; therefore, its use does not seem justified.

However, when we used the values from the Brix refractometer for the prediction of dairy constituents ( $SCU = \text{percentages of fat} + \text{protein}$ ), the results of the predictions were not satisfactory with either the first-degree regression or the second-degree regression. The determination coefficients varied from 41.3 to 44.8% for the first- and second-degree 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.

**Table 3.** Predictions of milk protein (PRT, %) and cheese-making constituents (SCU=fat + protein, %) according to first and second order regression models.

	$PRT = a + b \cdot \text{Brix}$	$PRT = a + b \cdot \text{Brix} + c \cdot \text{Brix}^2$	$SCU = a + b \cdot \text{Brix}$	$SCU = a + b \cdot \text{Brix} + c \cdot \text{Brix}^2$
a	-2.996	8.338	-9.6176	66.996
SE	0.188	2.393	0.903	11.268
<i>P-value</i>	<0.001	<0.001	<0.001	<0.001
b	0.639	-0.965	1.4720	-9.374
SE	0.013	0.338	0.065	1.592
<i>P-value</i>	<0.001	<0.004	<0.001	<0.001
c		0.057		0.382
SE		0.012		0.056
<i>P-value</i>		<0.001		<0.001
R <sup>2</sup> (%)	75.2	75.9	41.3	44.8
Y (mean)	5.89	5.89	10.85	10.85

<sup>4</sup> Y <sup>^</sup> (mean)	5.89	5.98	10.85	10.80
Pearson correlation (Y, Y <sup>^</sup> )	0.87	0.87	0.54	0.57
Rank correlation	0.84	0.84	0.46	0.46
<sup>5</sup> $\sigma$ Y <sup>^</sup>	0.55	0.56	1.27	1.31
<sup>6</sup> $\sigma$ Y - $\sigma$ Y <sup>^</sup>	0.08	0.07	0.70	0.66
<sup>7</sup> Bias	0.00	-0.09	0.00	0.06
<sup>8</sup> $\sigma$ (Y - Y <sup>^</sup> ) (MSEP)	0.32	0.31	1.66	1.63
<sup>9</sup> ( $\sigma$ (Y - Y <sup>^</sup> ) / Y)*100	5.36	5.28	15.34	14.99

<sup>1</sup>SE: standard error. <sup>2</sup>A: intercept. <sup>3</sup>B: angular coefficient. <sup>4</sup>Mean of predicted values. <sup>5</sup>Standard deviation of predicted values. <sup>6</sup>Difference between standard deviation of actual and predicted values. <sup>7</sup>Bias: mean of the differences. <sup>8</sup>Standard deviation of differences between actual and predicted values (MSEP = mean square error predicted). <sup>9</sup>Wilink test: 100 times the ratio between the standard deviation of differences between actual and predicted values and the mean value [16].

## 5. Conclusions

The digital Brix refractometer is a promising and easy-to-use tool for measuring colostrum quality in sheep, thus enabling fast and real-time results. Similarly, to what was found for sheep colostrum, this study showed a strong 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.

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