Original scientific paper
*Оригинални научни рад*
UDC 637.12:637.146
DOI 10.7251/AGREN2502153D


University of Banjaluka, Faculty of Agriculture
Agro-knowledge Journal

# Milk coagulation properties of three cattle breeds

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## Abstract

Milk coagulation is a crucial process in cheese making, as it directly affects the texture and quality of cheese. This study aimed to determine the influence of breed on milk rennet coagulation properties. The present research included 138 individual milk samples from 3 breeds. The research has shown that there are no statistically significant differences in milk coagulation properties among the breeds. However, the results indicate that the coagulum firmness of the Brown Swiss breed is better than that of the Holstein Friesian and Simmental breeds. Still, to confirm these differences more samples must be analyzed.

***Key words:*** milk, coagulation, breed.

## Introduction

In Serbia, around 1.4 billion litres of cow's milk are produced annually. A significant amount of milk is used for cheese production. According to the Statistical Office of the Republic of Serbia data (2023), approximately 70 different types of cheese are produced in the dairy industries in Serbia each year, totalling around 38,000 tonnes. In comparison, according to the Statistical Office of the Republic of Slovenia (2024), approximately 558.9 million litres of cow's milk were collected in 2023 and Slovenian dairies produced around 15,700 tonnes of cheese and 49,800 tonnes of fermented milk products, indicating a diverse dairy production sector. Cheese yield (% CY), expressed as the percentage ratio between the cheese produced and the milk processed, is of global economic importance, while daily cheese yield (dCY), expressed in kilograms of cheese produced daily per cow, is the final direct or indirect production target of many dairy farmers (Stocco et al., 2017). Although all milk can coagulate, only milk with suitable coagulation properties (MCPs) is considered appropriate for cheese production (Summer et al., 2002). They can be measured with direct rheological methods measuring differences in viscosity, such as computerized renneting meters, or indirect optical methods using different spectroscopic approaches (Bittante et al., 2012). The key milk properties for cheesemaking are good reactivity with rennet, a high degree of curd firming capacity, and good syneresis ability and whey expulsion (De Marchi et al., 2007). Traditionally, MCPs are expressed with the following parameters: rennet coagulation time (RCT), time to curd firmness of 20 mm (k20), and curd firmness at 30 min after enzyme addition (a30) (Bittante et al., 2012). These parameters describe the milk coagulation process: the enzymatic or primary phase after rennet addition, the non-enzymatic or secondary phase involving the aggregation of degraded casein micelles, and the final phase corresponding to gel structure formation (De Marchi et al., 2007). In addition to genetic factors, several other elements influence MCP, including casein content (Van Hooydonk and Walstra, 1987; Caron et al., 1997), κ-casein genetic variants (Okigbo et al., 1985; Davoli et al., 1990; Ikonen et al., 1997; Tyrisevä et al., 2003; 2004), β-LG genotypes (Kübarsepp et al., 2005), titratable acidity (Summer et al., 2002), pH levels (Lindström et al., 1984; Ikonen et al., 2004), and calcium content (Tervala et al., 1985; Kübarsepp et al., 2005). Milk proteins, particularly caseins, play a critical role in milk coagulation by forming a three-dimensional network that entraps water and fat globules. However, some molecular mechanisms involved in this process are still not fully understood (Zhang et al., 2023). The total concentration of milk protein in bovine milk in the total population in Vojvodina given by Main breeding organization (2024) was about 3.43% for Brown Swiss (BS), 3.27% for Holstein- Friesian (HF) breed, and 3.31% for Simmental (SIM) breed in 2023. The milk protein in bovine milk can be mainly classified as caseins (~80%), whey proteins (~16%), and milk fat globule membrane proteins (1–4%) (D'Alessandro et al., 2011). The important roles of caseins in milk coagulation have been widely studied. For example, lower κ-casein (κ-CN) content — or lower proportion of κ-CN relative to total casein — in poorly coagulating and non-coagulating milk has been associated with a negative correlation between κ-CN content and casein micelle size (Frederiksen et al., 2011). Generally, milk coagulation starts with the hydrolysis of κ-CN by chymosin, the principal enzyme in animal rennet, although microbial and fermentation-produced rennet is also widely used (Amalfitano et al., 2019). When the hydrolyzation degree of κ-CN reaches ~80%, the formed paracasein micelles start to aggregate in the presence of ionic calcium to form a coagulum, in which the fat globules are trapped (Guinee, 2003). Subsequently, the increasing number of secondary interactions within the curd result in its contraction and partial expulsion of whey over time (Zhang et al. 2023). Optimal values for RCT for milk intended for cheese production typically range between 10–18 minutes, as shorter coagulation times enable a faster and more efficient cheese-making process. However, excessively short RCT values may result in a fragile gel structure, which can complicate curd handling and reduce cheese yield. Ikonen et al. (2004) found longer coagulation times (15-25 minutes) in HF cows compared to other breeds, due to the genetic milk characteristics of this breed. The coagulation time for BS cows usually ranges between 10-18 minutes, making them more suitable for cheese production. De Marchi et al. (2008) indicated that SIM has better coagulation characteristics (12-20 minutes) than HF but slightly worse than BS. On the other hand, optimal k20 values for commercial cheese production typically range between 1.5 and 3 minutes, reflecting a rapid formation and firming of the curd. These values facilitate efficient cheese production by enabling quick firming of the milk mass. Wedholm et al. (2006) have concluded that Holstein has values around 2-4 minutes for k20, which is higher compared to other breeds. The optimal k20 values are similar for BS (1.5-3 minutes) and for the SIM breed (1.8-3 minutes) (De Marchi et al., 2008). The value that De Marchi et al. (2007) cite as optimal for curd firmness (a30) after 30 minutes is usually between 25-35 mm, as these values indicate a firm curd suitable for further processing in the cheese industry. The a30 values for Holstein are typically between 15-25 mm, which indicates a softer curd and may affect cheese quality (Wedholm et al., 2006). In contrast, values ranging from 20-35 mm in the BS breed indicate a firmer curd (De Marchi et al., 2008), as do values between 20-30 mm for the SIM, making them suitable for the cheese industry. Different cow breeds have proven suitable for the production of various dairy products due to variations in the chemical composition of their milk, which directly affects processing properties and the quality of the final product. Holstein milk is characterized by a lower fat and protein content compared to other breeds (Ikonen et al., 2004), but it has a higher overall milk yield. Because of these characteristics, Holstein milk is most suitable for producing liquid dairy products, such as pasteurized milk and yogurt (De Marchi et al., 2008), where high milk output and consistent production are beneficial for processing efficiency and overall productivity. Lower values for curd firmness (a30) and a slower coagulation process (RCT) often reduce efficiency in the production of hard cheeses, although the milk can be used for softer cheeses or fermented products where curd firmness and coagulation time have less impact on quality (Wedholm et al., 2006). Brown Swiss milk is rich in proteins and has a high casein content, which contributes to better coagulation characteristics. BS milk has optimal values for RCT and k20, as well as curd firmness (a30) that falls within a range suitable for cheese production (De Marchi et al., 2008). This breed has proven to be ideal for the production of hard and semi-hard cheeses, where stronger coagulation characteristics of the milk are required. The high protein content further contributes to a greater cheese yield, making BS milk valuable for cheese production. Simmental milk balances between Holstein and Brown Swiss in terms of fat and protein content, as well as coagulation properties. Simmental milk exhibits a sufficiently high protein and fat content, making it suitable for products such as butter and cream, where a high fat content is desirable (De Marchi et al., 2008).

The aim of this study was to determine influence of breed on milk rennet coagulation properties.

## Material and Methods

The study included 3 dairy farms located in Slovenia with a total of 64 Holstein cows (29 from Bovec and 35 from Cerklje na Gorenjskem), 39 Brown Swiss cows from Bovec, and 35 Simmental cows from Ljubljana. Individual milk samples were collected between July and August. Individual milk samples were analyzed using Milkoscan 6000 FT, according to the standard ISO 9622/IDF 141 method (ISO/IDF, 2013) and for somatic cell count (SCC) using Fossomatic 5000 according to ISO 13366-2/IDF 148-2 (ISO/IDF, 2006). Analyses of milk quality traits were performed in the accredited milk laboratory of the Institute of Dairy Science and Probiotics at the Biotechnical faculty in Slovenia. Milk coagulation properties were evaluated using a computerized renneting meter (CRM) (Polo Trade, Monselice, Italy). The analysis involved measuring the rennet coagulation time (RCT), which is the interval from the addition of rennet to the start of milk gelation, automatically identified by the instrument as the first point of probe resistance; the curd-firming rate (k20), defined as the time from the beginning of gelation until the curve's width reaches 20 mm, determined by the CRM software; and curd firmness at 30 minutes (a30), represented by the width of the curve at a specific time after rennet was added. First, 10 mL aliquots of milk were heated to 35 °C in an aluminum block and then 200 µl of enzyme solution (NATUREN® Premium 225 (Chr. Hansen, Denmark)), diluted to 1.2% (w/v) in distilled water to achieve a final concentration of 0.0516 international milk clotting units (IMCU) per ml of milk, was added. The samples were mixed for 30 seconds and subjected to 30- minute analysis. MCPs were analysed using a PROC GLM procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). The following general linear model was applied:

$$Y = \mu + R_i + L_j + D_k + b_{M,P,L,U,SCC}(x_{ijkl} - \bar{x}) + e_{ijklm}$$

Where:
Y = parameter studied (RCT, k20, or a30)
μ = intercept of the statistical model
$R_i$= fixed effect of breed (i = Holstein-Friesian, Simmental, Brown Swiss)
$L_j$= fixed effect of parity (j = 1–8)
$D_k$= fixed effect of lactation stage (k = 1–5)
$b_{M,P,L,U,SCC}(x_{ijkl} - \bar{x})$= linear regression coefficients for milk fat (M), protein (P), lactose (L), urea (U), and somatic cell count (SCC), with individual values centered around their means
$e_{ijklm}$= residual error

The Pearson correlation coefficients between MCP traits were calculated using the PROC CORR procedure.

## Results and Discussion

The average results for milk fat, protein, lactose, solids- non- fat (SNF), urea, freezing point, and somatic cell count (SCC) are presented in Table 1.

Tab. 1 Average milk composition of different breeds

| Breed | Milk fat (%) | Protein (%) | Lactose (%) | SNF (%) | Milk urea (mg/ml) | SCC (*1000/ml) |
|---|---|---|---|---|---|---|
| HF | 3.83[a] | 3.29[b] | 4.46[a] | 8.48[a] | 27.58[a] | 445.38[a] |
| SIM | 4.15[a] | 3.44[ab] | 4.18[a] | 8.29[a] | 12.91[b] | 206.54[a] |
| BS | 3.95[a] | 3.65[a] | 4.43[a] | 8.8[a] | 33.78[c] | 492.9[a] |
| Mean | 3.98 | 3.46 | 4.36 | 8.52 | 24.76 | 381.6 |

SNF- solid- non-fat; SCC- somatic cell count; Values in the same column with the same lower-case letters do not differ statistically significantly (p>0.05); Between arithmetic means in the same column, with different labels (a, b, c, d), there is a statistically significant difference (p<0.05)

The observed differences in milk components such as fat, lactose, solids-non-fat (SNF), and somatic cell count (SCC) are not statistically significant, in contrast to milk urea and proteins which are statistically significant. The lack of significance for SCC is likely due to the high variation within breeds, which is typical for this trait given its sensitivity to various environmental and physiological factors. Mean values, determined in this study, for milk fat (3.98%) were lower and protein contents (3.46%) were approximately the same as average values for the total amount of cow’s milk collected in Slovenia from January to May, 2023, (4.25% milk fat, 3.45% protein) provided by the Statistical

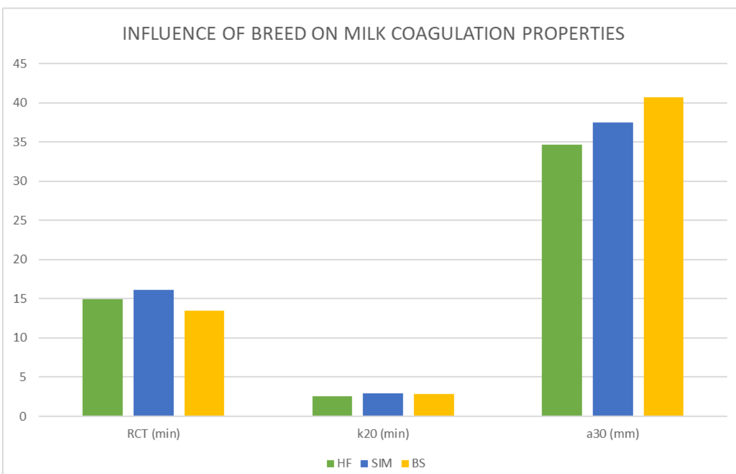
Office of the Republic of Slovenia (SURS). Furthermore, the protein content varied among breeds, with Brown Swiss cows exhibiting the highest protein content (3.65%). This finding aligns with previous studies indicating that Brown Swiss cows are often selected for higher protein yields, making their milk more suitable for dairy products requiring substantial protein content (De Marchi et al., 2008).

Although differences in MCP parameters were observed among breeds, these differences were not statistically significant ($p > 0.05$). This could be due to the limited sample size or other variables influencing MCPs, such as feeding conditions or individual variations among cows. The average results for RCT, k20, and a30 are presented in Table 2.

Tab. 2 Coagulation parameters of different breeds

| Parameter | HF | SIM | BS | Mean |
|---|---|---|---|---|
| RCT (min) | 14.97[a] | 16.10[a] | 13.48[a] | 14.85 |
| k20 (min) | 2.54[a] | 2.97[a] | 2.80[a] | 2.77 |
| a30 (mm) | 34.67[a] | 37.51[a] | 40.67[a] | 37.62 |

Between arithmetic means in the same row, with the same labels (a) there is not any statistically significant difference (p>0.05).




Graph 1 Influence of breed on milk rennet coagulation properties

The rennet coagulation time (RCT) varied, with BS cows showing the shortest average time (13.48 minutes), while Simmental cows had the longest (16.10 minutes). This difference suggests that the coagulation properties of milk can vary significantly by breed, which may influence the cheese-making process.

Notably, Holstein cows exhibited the fastest curd-firming rate (k20 at 2.54 minutes) and the highest curd firmness at 30 minutes was found for BS breed (a30 at 40.67 mm), indicating their milk can be particularly suited for cheese production. Mean values, determined in this study, for RCT (HF: 14.97; SIM: 16.10; BS: 13.48) were lower than reported by M. De Marchi et al. (2007) (HF: 18.0; SIM: 16.2; BS: 16.1) (Graph 1).

Table 3 shows the correlations between the average values of rennet coagulation parameters and milk ingredients.

Tab. 3 Correlations between coagulation parameters and milk composition

| | Proteins | Milk fat | Lactose | SNF | Urea | SCC |
|---|---|---|---|---|---|---|
| RCT | -0.023 | -0.002 | -0.066 | -0.052 | -0.267* | 0.057 |
| k20 | 0.048 | 0.175* | 0.107 | 0.085 | -0.061 | -0.003 |
| r30 | 0.009 | 0.105 | 0.052 | 0.036 | 0.068 | -0.084 |

SNF- solid non-fat; SCC- somatic cell count; Significant difference *p<0.05

Based on the results shown in Table 3, it can be seen that RCT has shown a significant negative correlation with urea (r =-0.2667), suggesting that higher urea levels might be associated with shorter coagulation times. Conversely, k20 demonstrated a significant positive correlation with milk fat (r = 0.1752), indicating that higher fat content could enhance curd-firming rates. These correlations suggest that the milk's compositional attributes and coagulation properties are interrelated, highlighting the complexity of milk quality assessment.

These results may have practical implications for herd selection depending on the final product, such as cheese. Breeds like Brown Swiss, which demonstrate better MCPs, could be more beneficial for producing cheese with specific characteristics.

## Conclusion

From the results of this study, it can be concluded that no statistically significant differences were found between the breeds, observing the parameters of milk quality and coagulation properties. However, the results indicate that the coagulum firmness of the Brown Swiss breed is better than that of the Holstein Friesian and Simmental breeds, but more samples need to be analyzed to confirm these differences. The variations in milk composition and coagulation parameters among the three breeds highlight the importance of breed selection based on specific dairy production goals. Breeders and dairy farmers may consider these differences when formulating feeding strategies and selecting breeds that align with their production objectives, whether that be higher fat, protein, or overall milk yield.

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# Коагулационе особине млека код три расе говеда

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## Сажетак

Коагулација млека представља кључни процес у производњи сира, јер директно утиче на текстуру и квалитет крајњег производа. Циљ овог истраживања био је да се утврди утицај расе на коагулационе особине млека. Истраживање је обухватило 138 индивидуалних узорака млека три различите расе. Резултати су показали да не постоје статистички значајне разлике у коагулационим особинама млека међу испитиваним расама. Ипак, подаци указују на то да је чврстоћа груша код расе браун- свис боља у поређењу са расама холштајн-фризијска и сименталска. Да би се ове разлике са сигурношћу потврдиле, потребно је анализирати већи број узорака.

*Кључне ријечи:* млеко, коагулација, раса.

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*Received*: March, 17, 2025
*Accepted*: May 29, 2025