Association of casein micelle size and enzymatic curd strength and dry matter curd yield

Denise Ribeiro de Freitas¹, Fernando Nogueira de Souza¹, Jamil Silvano de Oliveira², Diêgo dos Santos Ferreira³, Cristiane Viana Guimarães Ladeira¹,⁴, Mônica Maria Oliveira Pinho Cerqueira⁵

¹Programa de Pós-graduação, Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), Avenida Antônio Carlos, 6627, Pampulha, 31270-901, Belo Horizonte, MG, Brasil. E-mail: deniseribeirof@yahoo.com. *Corresponding author.
²Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brasil.
³Faculdade de Farmácia, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brasil.
⁴Empresa de Pesquisa Agropecuária de Minas Gerais, Belo Horizonte, MG, Brasil.
⁵Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brasil.

ABSTRACT: The aim of the present study was to explore the association between milk protein content and casein micelle size and to examine the effects of casein micelle size on enzymatic curd strength and dry matter curd yield using reduced laboratory-scale cheese production. In this research, 140 bulk tank milk samples were collected at dairy farms. The traits were analyzed using two linear models, including only fixed effects. Smaller micelles were associated with higher κ-casein and lower αs-casein contents. The casein micellar size (in the absence of the αs-casein and κ-casein effects) did not affect the enzymatic curd strength; however, smaller casein micelles combined with higher fat, lactose, casein and κ-casein contents exhibited a favorable effect on the dry matter curd yield. Overall, results of the present study provide new insights into the importance of casein micelle size for optimizing cheese production.

Key words: caseins, cheese, whey proteins.

INTRODUCTION

Milk caseins (CN), i.e., αs-CN, β-CN, and κ-CN, aggregate into spherical micelles with average diameters ranging from 150 to 200 nm (DE KRUIF, 1998). Thus far, the detailed micellar structure is not completely known. κ-CN predominates on the outer surface, influencing the physico-chemical stability of micelles in milk, while other CNs are located inside the sphere (FOX & MCSWEENEY, 2003). Variations in the CN and whey protein contents, particularly higher κ-CN contents or higher degrees of κ-CN glyclosylation in milk, have been associated with smaller micelles (DEVOLD et al., 2000). Variations in casein micelle size (CMS) appear to influence the effect of κ-CN content and glyclosylation degree on milk gelation properties and cheese production (DZIUBA & MINKIEWICZ, 1996). Consequently, CMS represents a potential indicator trait for exploration in animal breeding to enhance the technological quality of milk, particularly in cheese production (GLANTZ et al., 2010).

Aside from the κ-CN genotype, content and degree of glyclosylation, associations between CMS and milk protein composition have received little attention (BIJL et al., 2014). In addition, as smaller micelles are associated with increased κ-CN and CN contents, it is not yet clear whether the effect of CMS on milk gelation reflects differences in milk protein content/composition or whether the effect is...
directly due to variations in the CMS. Studies on the effect of CMS associated with other factors affecting dry matter cheese yield, such as fat and protein contents, are scarce (GLANTZ, et al., 2010). Moreover, previous studies have demonstrated that gel strength is not always associated with cheese yield (BONFATTI et al., 2014), and the production of model cheeses through laboratory cheese-making processes can more appropriately indicate cheese yields in comparison to milk coagulation properties (BONFATTI et al., 2014; CIPOLATT-GOTET et al., 2014).

The aims of the present study were i) to investigate the associations between CMS and milk composition and ii) to evaluate the association between CMS and enzymatic curd and dry matter curd yield, as measured in laboratory-scale cheese production.

MATERIALS AND METHODS

Bulk tank milk samples were obtained from 140 crossbreed (Holstein x Zebu) dairy herds located in the state of Minas Gerais, Brazil. For each sample, two aliquots of milk were collected: one aliquot was collected in a 150-mL sterilized flask for laboratory-scale cheese production, and the second aliquot was collected in a 50-mL tube, mixed with a preservative (Bronopol, 2-bromo-2-nitropropane-1,3-diol, 0.6:100 v/v) and analyzed for gross composition, protein composition, and somatic cell counting. Samples were stored at 4°C during transport to the laboratory.

The fat, total protein, CN, lactose, total solids, non-fat solids, and milk urea nitrogen contents (IDF, 2000) and somatic cell count were determined using a Bactocount ICB 150 unit (Bentley Instruments, Chaska, USA) equipped with a TA-XT2 Texture Analyzer (Stable Micro Systems, Malvern, UK) with a He-Ne laser set to 632.8nm.

Cheese production was simulated in a small-scale method devised by MELILLI et al. (2002), with slight modifications. Raw milk samples (25g) were poured into 50-mm beakers, and 300µL of diluted acetic acid (1:1:10 v/v) was added for acidification, followed by agitation for 20 s and incubation in a water bath at 35°C for 10min. Subsequently, the acidified milk was mixed with 230µL of diluted rennet (HA-LA®, Chr. Hansen) (1:10 v/v), agitated for 20s, and incubated in a water bath at 35°C for 30min. Curd strength was measured using a TA-XT2 Texture Analyzer (Stable Micro Systems, Reading, UK) equipped with a TA-10 1/2” diameter AOAC cylinder probe moving downward at 1mm/s, and the strength (g) was measured at a depth of 4mm. Subsequently, the curd sample was cut into 4 uniform pieces through the y-axis, transferred into a 50-mL tube, and centrifuged (1,100 x g, 30min, 10°C). The supernatant containing whey was carefully poured into a tube, whereas the precipitated gel was poured onto metal plates, oven dried (100°C ± 2°C, 4 h), and weighed. The dry matter curd yield was calculated as

Ciência Rural, v.49, n.3, 2019.
the percentage ratio of the dry matter weight over the raw milk weight.

**Statistical analysis**

Associations between the average CMS and milk composition were investigated through estimates of Pearson’s product-moment correlation between the traits and the effects of the milk protein composition on the average CMS estimated using a linear model, with αs-CN, β-CN and κ-CN contents as independent variables. The CN content was expressed in g/L, and the CN levels were grouped according to three ranges: class 1 (concentration < $x$ – 0.5 SD), class 2 ($x$ – 0.5 SD ≤ concentration ≤ $x$ + 0.5 SD), and class 3 (concentration > $x$ + 0.5 SD). Although, a cause-effect relationship between average milk CMS, milk urea nitrogen, and milk pH has been reported, these variables were not included in the model to avoid multicollinearity, as these parameters presented a high correlation with κ-CN content.

The effect of CMS on the gel strength and on the production of cheese dry matter were estimated using linear regression. The fat, lactose, total CN, % $\alpha_s$-CN, % β-CN, and % κ-CN contents and average CMS, without the effect of $\alpha_s$-CN or κ-CN, were used as independent variables. However, the original values of the micelle size were not used, rather the estimated residue was obtained using a model of linear regression, with the average CMS as a dependent variable and % $\alpha_s$-CN and % κ-CN as independent variables. The estimated residue was not correlated ($P>0.05$) with the other variables of the model. All variables were included in the model as continuous variables. The total CN content was included in the model with respective fractions to investigate the potential effects of individual CNs, in the absence of quantitative effects, as this parameter has been reported to exert a significant influence on cheese dry matter production. The somatic cell and total bacteria counts were not included in the model as these values were not significant. Statistical analyses were performed using the Statistical Analysis System, SAS 9.2 (SAS Institute Inc.; Cary, NC).

**RESULTS**

Pearson’s correlation was used to estimate the association of CMS and κ-CN with composition and other milk quality parameters. The CMS variations were associated with the protein (0.23; $P<0.05$), casein (0.17; $P<0.05$), urea (-0.40; $P<0.01$), $\alpha_s$-CN (0.46; $P<0.001$), κ-CN (-0.52; $P<0.001$), β-LG (-0.37; $P<0.001$) and $\alpha$-LA (-0.15; $P<0.05$) contents, CN number (-0.22; $P<0.001$) and milk pH (0.21; $P<0.05$). κ-CN variations were associated with milk pH (-0.31; $P<0.01$).

The association of milk protein composition with CN micelles, based on the least square means for each class, is presented in Table 1. The β-CN content did not affect the CMS, but smaller micelles were detected in milk samples with lower $\alpha_s$-CN and higher κ-CN contents. The average micelle size for milk samples with contents below 2.5 g/L (class 1) was 178.79 ± 1.47nm (average ± SD), while for samples with contents higher than 3.43 g/L (class 3), the average size was 177.35 ± 1.50nm.

Effect of CMS on gel strength and cheese dry matter production was evaluated using a linear regression model with CMS (without the effect of $\alpha_s$-CN or κ-CN) and fat, lactose, and total CN contents as independent variables. Results are reported in Table 2. CMS did not affect the gel strength when the effect of the residue CMS was examined using the statistical model.

Smaller micelles of casein (without the effect of $\alpha_s$-CN and κ-CN) exhibited favorable effect on dry matter cheese yield. Conversely content

---

Table 1 - Least squares means (LSMEANS) and standard error (SE) of the effect of casein micelle size grouped according to concentration levels of its fractions.

| Effect   | Protein fraction class* | LSMEANS | SE  | LSMEANS | SE  | LSMEANS | SE  |
|----------|-------------------------|---------|-----|---------|-----|---------|-----|
| $\alpha_s$-casein | Class 1 | 178.79$^a$ | 1.47 | 185.58$^b$ | 1.37 | 186.08$^c$ | 1.60 |
| $\alpha_s$-casein | Class 2 | 182.93 | 1.41 | 184.46 | 1.37 | 183.07 | 1.53 |
| $\alpha_s$-casein | Class 3 | 189.83$^d$ | 1.50 | 183.26$^e$ | 1.40 | 177.35$^f$ | 1.50 |

*aDifferent superscripted letters within a row indicate significant differences ($P \leq 0.05$) among the values. 
*bCasein fraction was classified as follows: class 1 (concentration < $x$ – 0.5 SD), class 2 ($x$ – 0.5 SD ≤ concentration ≤ $x$ + 0.5 SD), class 3 (concentration > $x$ + 0.5 SD).
of fat, lactose, casein and κ-CN was positively associated with dry matter cheese yield. Increases in dry matter cheese yield were 0.53, 0.25, 0.17 and 0.61 percentage points for 1-SD unit of fat, lactose, casein and κ-CN, respectively.

**DISCUSSION**

The positive correlation of pH with CMS reflects the influence of milk acidity on the CMS. Indeed, as found here, MCDERMOTT et al. (2016) also reported a negative correlation between pH and protein fractions, such as κ-CN, which in turn affect CMS. GLANTZ et al. (2010) reported similar results, demonstrating that pH reduction results in colloidal calcium phosphate migration to the whey phase and affects the micellar surface and/or alter the stability of κ-CN layer. Thus, micellar aggregation or dissociation into sub-micellar particles are resulted from environmental alterations, such as pH, which in turn disturb micelle stability as a consequence of the lack of rigid three-dimensional ternary conformation in casein micelles (WALSTRA, 1990). In addition, VASBINDER & DE KRUIF (2003) showed that small alterations in pH had a great influence on whey protein denaturation and gelation properties in milk.

Results of the relationship between αs-casein and CN micelle size have not been described in previous studies (DALGLEISH et al., 1989; BIJL et al., 2014). CN micelles have dynamic structures that can be disrupted or reorganized into smaller micelles, with CN loss or solubilization to the whey phase (LIU & GUO, 2008). Our research showed that micellar dissociation may be associated with pH variation and urea content; therefore, micellar reorganization or CN loss might affect the content of αs-CN in CN micelles.

DALGLEISH et al. (1989) and DALGLEISH (2011) reported a similar correlation between κ-CN levels and CMS. The κ-CN outer layer, particularly the glycosylated molecules, is primarily responsible for the steric and electrostatic repulsive forces between micelles and is a major factor for CMS variations. Animals that are homozygous for κ-CN variant B produce milk with a higher ratio of glycosylated κ-CN compared with animals homozygous for variant A (DALGLEISH, 2011; BIJL et al., 2014).

WEDHOLM et al., (2006) and BONFATTI et al. (2010) reported positive associations between the κ-CN content and gel strength. In the present study, Pearson’s correlation coefficient between gel strength and κ-CN content was 0.35 ($P<0.001$), indicating that the association between smaller CN micelles and higher gel strength may partially reflect the higher content of κ-CN. DZIUBA & MINKIEWICZ (1996) reported that a higher level of κ-CN glycosylation, associated with smaller and more hydrophobic micelles, favors firmer rennet gels, reflecting increased κ-CN hydrolysis through chymosin and a closer packing arrangement (and aggregation between) of para-CN micelles forming the basic building blocks (para-CN aggregates) of the gel matrix. Thus, animals carrying the CNS3 B allele produce milk with a higher degree of κ-CN glycosylation, smaller micelles and enhanced cheese gel strength in comparison to animals carrying the A allele (WEDHOLM et al., 2006; BIJL et al., 2014; BONFATTI et al., 2014).

Smaller CN micelles (without the effect of αs-CN or κ-CN) exhibited favorable effects on dry matter cheese yield.

---

**Table 2** - Regression coefficients and standard error (SE) of the effects of milk composition and average casein micelle size on gel strength and cheese dry matter production (the magnitude of these effects is expressed in SD units of traits).

| Trait                  | Mean | SE   | P value | Mean  | SE   | P value |
|------------------------|------|------|---------|-------|------|---------|
| Fat (g 100 g⁻¹)        | -0.15| 0.44 | $P>0.05$| 0.53  | 0.13 | $P<0.001$|
| Lactose (g 100 g⁻¹)    | -0.05| 1.26 | $P>0.05$| 0.25  | 0.37 | $P<0.001$|
| Casein (g 100 g⁻¹)     | 0.25 | 1.29 | $P<0.05$| 0.17  | 0.38 | $P<0.01$ |
| αs-casein, %            | -0.07| 0.04 | $P>0.05$| -0.07 | 0.01 | $P>0.05$ |
| β-casein, %             | -0.04| 0.05 | $P>0.05$| -0.05 | 0.01 | $P>0.05$ |
| κ-casein, %             | 0.24 | 0.08 | $P<0.05$| 0.61  | 0.02 | $P<0.001$|
| Casein micelle size, a  | 0.12 | 0.02 | $P>0.05$| -0.14 | 0.00 | $P<0.01$ |

*Residue of the statistical model: Casein micelle size = αs-casein + κ-CN: k-casein + error.
matter cheese yield. Moreover, the fat, lactose, CN and κ-CN contents were positively associated with the dry matter cheese yield. Consistent with the study of VERDIER-METZ et al. (2001), the relationship between fat and CN contents and cheese yield was positive and linear. Hence, the effect of fat was considerably greater than the effect of CN. Generally, fat and CN represent approximately 94% of the dry matter of cheese (LUCEY & KELLY, 1994).

The CMS was considered in the model as a residue; hence, it was difficult to interpret the regression coefficient generated based on these results. In other words, it was not possible to quantify the effect of CMS on the dry matter cheese yield based on each unit of size decrease. However, a key finding of the present study was that milk samples with smaller CN micelles and higher proportions of fat, CN and κ-CN might lead to an optimized production of dry matter cheese yield. This result suggested that the highest κ-CN content would be associated with the smallest micelles, independent of the cause-effect relationship between these variables, and might be beneficial to the gel structure. WALSH et al. (1998) showed that milk samples from animals homozygous for the κ-CN B gene were associated with smaller CN micelles and produced cheese with smaller pores, implying that compact micelles form more interactions between molecules during gel formation. ZHAO et al. (2014) reduced the CMS using ultrasonification and observed that the gel structure presented smaller and more uniform pores, likely contributing to the retention of more milk components in cheeses with better yield (HALLEN et al., 2010).

Results of the present study provided novel insights into the positive effects of small CN micelles and higher fat, CN and κ-CN contents on dry matter cheese yield, indicating that the effect of CMS on dry matter cheese yield does not result from a different milk protein content/composition, but is rather an effect directly resulting from variations in the CMS. It is likely that small micelles exert two favorable effects during the initial cheese processing. First, small CN micelles have more surface area than large CN micelles, likely increasing the number of junctions between micelles during the initial cheese processing and increasing the incorporation of micelles into the gel network. Consequently, this effect facilitates a more compact and uniform arrangement of the gel network, likely reducing losses in whey through improved entrapping. Second, small CN micelles may reduce the coefficient of diffusion between the enzyme molecules and the CN micelles, potentially further decreasing the rennetime and consequently enhancing cheese curd firmness and overall cheese yield.

CONCLUSION

Smaller micelles increase cheese dry matter production, without affecting the cheese gel strength. Although, influence of CMS on cheese yield should be further investigated, these findings provide new insights into the combined effects of small micelles and higher fat, lactose, CN and κ-CN contents on cheese production, suggesting that the selection of smaller CN micelles would aid in optimizing cheese production.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful for financial support from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG Project n° 02074/12) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). DFR is also grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to her fellowship.

AUTHORS’ CONTRIBUTIONS

The authors contributed equally to the manuscript.

REFERENCES

BIJL, E. et al. Factors influencing casein micelle size in milk of individual cows: genetic variants and glycosylation of κ-casein. *International Dairy Journal*, v.34, p.135-141, 2014. Available from: <https://doi.org/10.1016/j.idairyj.2013.08.001>. Accessed: Jan. 18, 2018. doi: 10.1016/j.idairyj.2013.08.001.

BONFATTI, V. et al. Effects of β-κ-casein (CSN2-CSN3) haplotypes, β-lactoglobulin (BLG) genotypes, and detailed protein composition on coagulation properties of individual milk of Simmental cows. *Journal Dairy Science*, v.93, p.3809-3817, 2010. Available from: <https://doi.org/10.3168/jds.2009-2779>. Accessed: Jan. 18, 2018. doi: 10.3168/jds.2009-2779.

BONFATTI, V. et al. Variation in milk coagulation properties does not affect cheese yield and composition of model cheese. *International Dairy Journal*, v.39, p.139-145, 2014. Available from: <https://doi.org/10.1016/j.idairyj.2014.06.004>. Accessed: Jan. 24, 2018. doi: 10.1016/j.idairyj.2014.06.004.

CIPOLAT-GOTET, C. et al. Factors affecting variation of different measures of cheese yield and milk nutrient recovery from an individual model cheese-manufacturing process. *Journal Dairy Science*, v.96, p.7952-7965, 2013. Available from: <https://doi.org/10.3168/jds.2013.2379>.
LIU, Y.; GUO, R. pH-dependent structures and properties of casein micelles. *Biophysical Chemistry*, v.136, p.67-73, 2008. Available from: <https://doi.org/10.1016/j.bpc.2008.03.012>. Accessed: Jan. 24, 2018. doi: 10.1016/j.bpc.2008.03.012.

LUCEY, J.; KELLY, J. Cheese yield. *International Journal of Dairy Technology*, v. 47, p.1-14, 1994. Available from: <https://doi.org/10.1111/j.1471-0307.1994.tb01264.x>. Accessed: Jan. 24, 2018. doi: 10.1111/j.1471-0307.1994.tb01264.x.

MCDERMOTT, A. et al. Prediction of individual milk proteins including free amino acids in bovine milk using mid-infrared spectroscopy and their correlations with milk processing characteristics. *Journal of Dairy Science*, v. 99, p. 3171-3182, 2016. Available from: <https://doi.org/10.3168/jds.2015-9747>. Accessed: Jan. 24, 2018. doi: 10.3168/jds.2015-9747.

WALSH, C.D. et al. Influence of kappa-casein genetic variant on rennet gel microstructure, cheddar cheesemaking properties and casein micelle size. *International Dairy Journal*, v.8, p.707-714, 1998. Available from: <https://doi.org/10.1016/S0958-6946(98)00103-4>. Accessed: Jan. 24, 2018. doi: 10.1016/S0958-6946(98)00103-4.

WEDHOLM, A. et al. Effect of protein composition on the cheese-making properties of milk from individual dairy cows. *Journal of Dairy Science*, v. 89, p.3296-3305, 2006. Available from: <https://doi.org/10.3168/jds.S0022-0302(06)72366-9>. Accessed: Jan. 24, 2018. doi: 10.3168/jds.S0022-0302(06)72366-9.

ZHAO, L. et al. Effect of ultrasound pretreatment on rennet-induced coagulation properties of goat’s milk. *Food Chemistry*, v.165, 167-174, 2014. Available from: <https://doi.org/10.1016/j.foodchem.2014.05.081>. Accessed: Jan. 24, 2018. doi: 10.1016/j.foodchem.2014.05.081.