Effects of κ-CN Glycosylation on Rennet Coagulation Properties of Milk in Simmental Cattle

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Summary

Contents of casein fractions are known to affect coagulation properties and cheese yield of milk, but studies on the effects of κ-CN composition on variation of coagulation properties of milk are still very scarce. Effects exerted by κ-CN composition on variation of milk coagulation properties (MCP) were investigated using 2,084 individual milk samples of Simmental cows. Rennet coagulation time (RCT), and curd firmness (A_{30}) were measured using a computerized renneting meter. Milk protein composition and genotypes at *CSN2*, *CSN3* and *BLG* were obtained by reversed-phase HPLC. The percentage ratios of κ-CN (κCN%), of Glycosylated-κ-CN (G-κCN%), and Unglycosylated-κ-CN (U-κCN%) to total casein were measured. The degree of glycosylation (GD) was measured as the percentage ratio of glycosylated-κ-CN to total κ-CN. A difference of 1.7 min (corresponding to 0.37 SD of the trait) was observed for the average RCT of the two extreme classes of κCN% content. RCT decreased when κCN% and G-κCN% increased, whereas U-κCN% exhibited a slightly unfavourable effect on the onset of the coagulation process. A slight decrease of RCT was also observed for high GD, although this effect was less clear than that of G-κCN%. A favourable effect of κCN%, G-κCN% and GD on $A_{30}$was also detected.

Key words

κ-CN, glycosylation, milk coagulation time, curd firmness, casein

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Received: March 30, 2013 | Accepted: June 26, 2013

Aim

Most research concerning factors affecting technological properties of milk has focused on κ-CN because it is the target of chymosin, because of its stabilizing properties toward casein micelles, and because the associations between *CSN3* B, the content of κ-CN and improved milk coagulation properties have been well established (Caroli et al., 2009). Little is known about the role of κ-CN glycosylation for variation of milk technological properties. Glycosylation is known to affect several functions of κ-CN, such as the stabilizing activity for the other caseins, the effect on the micelle size and hydrophobicity, and susceptibility to chymosin proteolysis. It has been hypothesized that the variation of the glycosylation degree of κ-CN might influence the variation of milk coagulation properties (MCP). The aim of this study was to estimate the effects exerted by the degree of glycosylation of κ-CN on rennet coagulation properties of individual milk samples of Italian Simmental cows.

Material and methods

The study involved 2,084 individual milk samples of Simmental cows reared in 47 commercial herds. Milk sampling occurred once per animal, concurrently with the monthly milk recording of the dairy herd. Cows were the offspring of 207 sires. Contents of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, glycosylated-κ-CN (**G-κCN**) and unglycosylated-κ-CN (**U-κCN**), α-LA, and β-LG of individual milk samples were measured by RP-HPLC using the method of Bonfatti et al. (2008). Total CN content (**TCN**, g/L) was computed as the sum of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, and total κ-CN (i.e., the sum of G-κCN and U-κCN). The percentage ratios of κ-CN to TCN (**κCN%**), G-κCN to TCN (**G-κCN%**), and U-κCN to TCN (**U-κCN%**) were calculated. Glycosylation degree (**GD**) was defined as the proportion of G-κCN to total κ-CN. Genotypes of cows at *CSN2*, *CSN3*, and *BLG*, were also obtained by RP-HPLC (Bonfatti et al., 2008).

Measures of MCP of individual milk samples were recorded by using the Computerized Renneting Meter (CRM-48, Polo Trade, Monselice, Italy). Within 3 h after sample collection, samples (10 mL) were preheated at 35 °C and an amount of 200 μL of rennet (Hansen standard 160, 80% chymosin, 1:14,900, Pacovis Amrein AG, Bern, Switzerland), diluted to 1.6% (vol/vol) in distilled water, was added to milk. A measure of rennet coagulation time (RCT) and of curd firmness at 30 min after rennet addition (A_{30}) was obtained per each sample. Non-coagulating milk (i.e., milk that did not coagulate within 30 min after rennet addition) was not considered in the statistical analysis. Measurement of milk pH was carried out immediately before MCP analysis.

The effects of κ-CN composition on MCP were estimated through linear mixed models using the MIXED procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC). The models included: the random effect of herd-test day, the fixed effects of the parity of the cow (first, second, third and fourth or later parities), DIM class (11 classes of 30-d intervals, with the exception of the last class, which included samples collected at DIM 300 or greater), *CSN2* genotype (A^1A^1, A^1A^2, A^1B, A^1I, A^2A^2, A^2B, A^2I, BB, BI, II), *CSN3* genotype (AA, AB, BB), *BLG* genotype (AA, AB, BB), the fixed effect of the class of κCN%, G-κCN%, U-κCN% or GD, the linear effect of milk pH, and the random effect of the sire of the cow. As in a preliminary analysis non-linear effects were observed, the content of each protein fraction and GD was considered as a class variable. Variables were classified as follows: class C-- (content < $\overline{x}$ – SD), class C- ($\overline{x}$ - SD ≤ content < $\overline{x}$ – 0.5SD), class C0 ($\overline{x}$ – 0.5SD ≤ content < $\overline{x}$ + 0.5SD), class C+ ($\overline{x}$ + 0.5SD ≤ content < $\overline{x}$ + SD), and class C++ (content ≥ $\overline{x}$ + SD).

Results and discussion

Descriptive statistics for the contents of the major protein fractions, as well as for genotype and allele frequencies, for the data used in this study can be found in Bonfatti et al. (2010). Descriptive statistics for casein and κ-CN composition and MCP are reported in Table 1. U-κCN accounted for approximately 54% of κ-CN, in agreement with Vreeman et al. (1986) and Mollé and Léonil (1995). Both the overall amount of G-κCN in milk and the proportion of G-κCN in κ-CN varied, in agreement with Coolbear et al. (1996). A great variability in the content of *N*-acetylneuraminic acid of κ-CN have been also reported (Robitaille et al. ,1991). However, recent studies (Holland et al., 2004, 2005; Jensen et al., 2012a) suggested a more consistent pattern of κ-CN post-translational modifications than that reported by the above-mentioned studies.

In total, 6.3% of samples were noncoagulating milk. These samples were excluded from the statistical analysis because RCT was unknown. Mean (SD) of RCT and A_{30} for samples that coagulated was 16.5 (4.6) min and 29.1 (7.5) mm, respectively.

Estimates of the class effect of κCN%, G-κCN%, U-κCN% and GD on RCT and A_{30} are reported in Table 2. RCT decreased when the content of κCN% in milk increased. A difference of 1.7 min for RCT (corresponding to 0.37 SD of the trait) was observed

Table 1. Descriptive statistics[1] of protein composition and milk coagulation properties (N = 2,084)

Trait[2]	Abbreviation	Mean	CV	P1	P99
Proportions on total casein					
κ-CN, %	κCN%	10.43	18.48	6.14	14.92
Glyco-κ-CN, %	G-κCN%	4.86	27.67	2.45	8.96
Unglyco-κ-CN, %	U-κCN%	5.57	21.67	2.84	8.27
Glycosylation degree, %	GD	46.37	17.01	30.45	69.55
Rennet coagulation time, min	RCT	16.54	27.77	7.60	28.07
Curd firmness, mm	A_{30}	29.07	25.77	9.00	42.00

[1]P1 = 1st percentile; P99 = 99th percentile; [2]Total casein was computed as the sum of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN and κ-CN; κ-CN was computed as the sum of Glyco-κ-CN and Unglyco-κ-CN; Glycosylation degree was computed as: Glyco-κ-CN/κ-CN*100.

Table 2. Least square estimate (± SE) for the class effect of total κ-CN, κ-CN fractions, and glycosylation degree of κ-CN, compared to the intermediate class (C0), on rennet coagulation time (RCT) and curd firmness (A_{30})

Trait	Effect[2]	Class				
		C--	C-	C0	C+	C++
RCT						
	κCN%	0.99 ± 0.30^{a}	0.32 ± 0.26^{b}	0^{b}	-0.26 ± 0.23^{b}	-0.71 ± 0.35^{c}
	G-κCN%	1.00 ± 0.26^{a}	0.44 ± 0.22^{b}	0^{c}	-0.37 ± 0.24^{c}	-1.04 ± 0.25^{d}
	U-κCN%	-0.66 ± 0.25^{a}	-0.37 ± 0.23^{ab}	0^{bc}	0.34 ± 0.23^{c}	0.50 ± 0.26^{d}
	GD	0.06 ± 0.24^{a}	0.25 ± 0.20^{a}	0^{a}	-0.70 ± 0.23^{b}	-1.23 ± 0.23^{c}
A_{30}						
	κCN%	-2.61 ± 0.55^{a}	-0.76 ± 0.47^{b}	0^{b}	0.16 ± 0.41^{b}	0.43 ± 0.62^{b}
	G-κCN%	-1.29 ± 0.47^{a}	-0.85 ± 0.40^{a}	0^{bc}	-0.40 ± 0.43^{ab}	0.56 ± 0.46^{c}
	U-κCN%	-0.60 ± 0.45	-0.11 ± 0.41	0	-0.44 ± 0.41	-0.32 ± 0.47
	GD	0.31 ± 0.44^{a}	0.22 ± 0.36^{a}	0^{a}	0.71 ± 0.43^{ab}	0.94 ± 0.42^{b}

[1]kCN%, G-kCN%, U-kCN%, and GD (y) were classified as follows: class C-- (y < $\bar{x}$ − SD), class C- ($\bar{x}$ - SD ≤ y < $\bar{x}$ − 0.5SD), class C0: ($\bar{x}$ − 0.5SD ≤ y < $\bar{x}$ + 0.5SD), class C+: ($\bar{x}$ + 0.5SD ≤ y < $\bar{x}$ + SD), and class C++ (y ≥ $\bar{x}$ + SD); [2] κ-CN% was the κ-CN to total CN ratio, G-κCN% was the Glycosylated-κ-CN to total CN ratio, U-κCN% was the Unglycosylated-κ-CN to total CN ratio, GD was computed as G-κCN/κ-CN*100; estimates with different superscripts differ at $P < 0.05$.

between the two extreme classes of κCN% content. A shorter RCT was measured for increasing G-κCN%, whereas U-κCN% exhibited a slightly unfavourable effect on the time required for the onset of coagulation. Based on these results, it is possible to state that the favourable effect of κ-CN on RCT has to be entirely ascribed to the glycosylated fraction. A slight decrease of RCT was also observed at high GD of κ-CN, although this effect was less marked than that of G-κCN%. It is possible to hypothesize that the time required for the onset of coagulation is affected by both the overall amount of G-κCN in milk and the extent of κ-CN glycosylation.

The effects exerted by the content of protein fractions and the extent of glycosylation on A_{30} were less pronounced, albeit still significant. As for RCT, a favourable effect of κCN%, G-κCN% and GD was observed.

A favourable effect of G-κCN on MCP has been observed by Jensen et al. (2012b), but it was in contrast with results obtained by Jensen et al. (2012a). According to the latter study, no significant variability in κ-CN post-translational modifications across samples having good or poor coagulation was observed. However, in that study no measure of total κ-CN glycosylation was available and only measures of the content of the major glycosylated κ-CN isoforms, with up to 3 glycans attached and accounting for no more than 34 to 36% of total κ-CN, were obtained.

Chromosomal regions underlying non-coagulating milk in Finnish Ayrshire include 2 potential candidate genes, a serine/threonine kinase on chromosome 2 and a sialyl transferase, which catalyses the last step of glycosylation of κ-CN, on chromosome 18 (Tyriseva et al., 2008). This finding supports the role, detected in this study, of post-translational modifications of the caseins as one of the possible underlying causes for poor milk coagulation.

Conclusions

This study indicates that variation of specific κ-CN fractions affects rennet coagulation time of milk and that the favourable effect exerted by the content of κ-CN on milk coagulation properties has to be ascribed to the glycosylated fraction of κ-CN. Although the role of κ-CN composition in variation of cheese yield needs further investigations, modification of the relative content of specific κ-CN fractions can relevantly influence the behaviour of milk during the coagulation process.

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