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Effects of the detailed protein composition of milk on curd yield and composition measured by model micro-cheese curd making of individual milk samples

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ABSTRACT

The effect of the contents of casein (CN) and whey protein fractions on curd yield (CY) and composition was estimated using 964 individual milk samples. Contents of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, glycosylated κ-CN (Gκ-CN), unglycosylated κ-CN, β-LG, and α-LA of individual milk samples were measured using reversed-phase HPLC. Curd yield and curd composition were measured by model micro-cheese curd making using 25 mL of milk. Dry matter CY (DMCY) was positively associated with all casein fractions but especially with α_{S1}-CN and β-CN. Curd moisture decreased at increasing β-CN content and increased at increasing γ-CN and Gκ-CN content. Due to their associations with moisture, Gκ-CN and β-CN were the fractions with the greatest effect on raw CY, which decreased by 0.66% per 1-standard deviation (SD) increase in the content of β-CN and increased by 0.62% per 1-SD increase in the content of Gκ-CN. The effects due to variation in percentages of the casein fractions in total casein were less marked than those exerted by contents. A 1-SD increase in β-CN percentage in casein (+3.8% in casein) exerted a slightly negative effect on DMCY ($\beta = -0.05\%$). Conversely, increasing amounts of α_{S1}-CN percentage were associated with a small increase in DMCY. Hence, results suggest that, at constant casein and whey protein contents in milk, the DMCY depends to a limited extent on the variation in the α_{S1}-CN:β-CN ratio. κ-Casein percentage did not affect DMCY, indicating that the positive relationship detected between the content of κ-CN and DMCY can be attributed to the increase in total casein resulting from the increased amount of κ-CN and not to variation in κ-CN relative content. However, milk with increased Gκ-CN percentage in κ-CN also shows increased raw CY and produces curds with increased moisture content. Curd yield increased at increasing content and relative proportion of β-LG in whey protein, but this is attributable to an improved capacity of the curd to retain water. Results obtained in this study support the hypothesis that, besides variation in total casein and whey protein contents, variation in protein composition might affect the cheese-making ability of milk, but this requires further studies.

Key words: casein fraction, protein composition, curd yield, cheese making

INTRODUCTION

Curd yield (**CY**) is an indicator of the efficiency of the cheese-making process and of the profitability of the dairy industry, particularly in countries, such as Italy, where most of the milk produced is processed into cheese. Protein content is fundamental in determining CY, but variation in protein composition exists (Fox and McSweeney, 2003) and may affect variation in the efficiency of cheese making. Inherent characteristics of milk protein fractions contribute to variation in size, surface charge, hydrodynamic radius, hydration, and mineral content of casein micelle (Fox and McSweeney, 2003). For example, κ-CN, by means of its glycosylated domain, provides casein micelles with stability toward aggregation. The release of the polar glycomacropeptide domain from κ-CN eliminates the polar electrostatic and steric stabilization of micelles, increasing surface hydrophobicity and leading to clot formation (Fox and McSweeney, 2003). Effects of *CSN3* genotypes on coagulation properties result from differences in κ-CN:total CN ratio across genotypes (Bonfatti et al., 2010a). In addition, *CSN3* B is associated with significant high proportions of glycosylated fractions relative to the *CSN3* A variant (Bijl et al., 2014) and influ-

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ences rennet coagulation time variation (Bonfatti et al., 2014). Contents of protein fractions are expected to affect the rheological and syneresis properties of curds, the recovery of fat and protein in cheese, and, ultimately, cheese yield. Due to difficulties in measuring cheese yield and recovery rates of milk constituents, the number of studies investigating effects of milk protein composition on variation in such traits is small. Most studies focused on rennet coagulation time and curd firmness as indirect indicators of cheese yield (Jõudu et al., 2008; Bonfatti et al., 2010a), and very few (Van den Berg, 1992; Wedholm et al., 2006; Hallén et al., 2010) investigated the relationship between milk protein composition and actual cheese yield; this is because the use of large volumes of milk often limits the number of cheese-making trials that can be performed. As a consequence, the available data are usually very limited. In addition, the variability of milk composition in bulk milk is greatly reduced. These critical issues make it difficult to properly investigate the relationships between milk composition and CY or curd quality. Only 1 study has investigated the effect of protein composition on a large number of individual experimental cheeses (Cipolat-Gotet et al., 2018) to maximize variability in protein composition, but the amount of milk required (1.5 L/cow) limits the number of animals that can be sampled on farm.

The aim of this study was to estimate the effects of the detailed protein composition of milk on CY and curd composition using model micro-cheese curd making using 25 mL of milk, thus allowing the collection and processing of many samples while enabling the chemical analysis of the curds. The proportions in casein and in κ-CN of the main κ-CN fractions (glycosylated and unglycosylated) were also investigated.

MATERIALS AND METHODS

Animals and Milk Sampling

The data used in this study were from a large sampling initiative, enrolling 964 Simmental cows reared in 20 commercial herds in the north of Italy, which collected individual milk samples from February 2013 to June 2014. All cows were milked twice a day and fed a TMR. Herd size ranged between 30 and 125 cows. Cows enrolled in the study were between 5 and 484 DIM, and their parity ranged from 1 to 9. Milk sampling occurred once per animal, during the morning milking, concurrently with the monthly milk recording of the herd. A preservative (bronopol, 2-bromo-2-nitropropane-1,3-diol; 0.6:100 vol/vol) was added to the milk immediately after collection, and the milk was transported in portable coolers to the laboratory for model micro-cheese curd making and reversed-phase HPLC analysis of detailed protein composition.

Milk Composition

Fat, protein, and lactose contents of the milk were determined by mid-infrared spectroscopy (MilkoScan FT6000, Foss Electric, Hillerød, Denmark), and SCC was analyzed using a FossSomatic FC (Foss Electric), according to the standard procedure used in the national milk recording program. Somatic cell score was computed as $\log_2(\text{SCC} \times 10^3) + 3$. The Italian Simmental cattle breeders association (ANAPRI, Udine, Italy) provided milk yield records and pedigree information for the cows involved in the study.

Milk Protein Composition

Contents (expressed as g/L of skim milk) of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, glycosylated κ-CN (**Gκ-CN**), unglycosylated κ-CN (**Uκ-CN**), β-LG, and α-LA in milk were measured by reversed-phase HPLC (Bonfatti et al., 2008). As the analytical method showed very high repeatability and reproducibility (Bonfatti et al., 2008), no replicates were needed. The content of total κ-CN was obtained as the sum of Gκ-CN and Uκ-CN, whereas the content of total casein was computed as the sum of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, and κ-CN. The content of total whey protein was defined as the sum of β-LG and α-LA. Percentages of single protein fractions were calculated from their contents. Milk protein content determined during the routine milk recording (i.e., on whole milk) was not used in the calculation of the absolute or relative contents of the single protein fractions because protein profile obtained by HPLC was measured on skim milk.

Compared with the protein composition measured in a former experiment on Simmental cows (Bonfatti et al., 2010b), casein content was lower due to a significantly lower content of β-CN. This may be explained partly by the different sampling conditions used in this study. Protein composition was measured in milk used for curd making (i.e., samples were not frozen after milking but rather transported to the laboratory in portable coolers and then kept at room temperature for ~2 h before curd making). In addition, the farms sampled in the current study overlap only marginally with those sampled in the study of Bonfatti et al. (2010b) and variations in β-CN allele frequency were detected, with alleles that are associated with higher amounts of β-CN (e.g., A1 and B) scarcely present (data not reported in tables).

CY and Composition

Curds were obtained by model micro-cheese curd-making procedures carried out within 5 h after sample collection. Twenty-five milliliters of raw milk was heated for 15 min at an internal temperature of 35°C; then, 500 μL of rennet solution (1.2% vol/vol of rennet Naturen Standard 215, Chr. Hansen, Hørsholm, Denmark; 215 IMCU/mL, in distilled water) was added, and the milk was stirred briefly. Milk was left to coagulate at 35°C and, after 30 min, the curd was cross-cut into 4 parts with a spatula and let to rest at 35°C for 15 min. At the end of curd healing, samples were centrifuged at 3,202 × *g* for 20 min at 10°C. After centrifugation, the supernatant was removed without compressing the curd. Each curd was then weighted and preserved at −20°C until the assessment of curd gross composition. Micro-cheese making was carried out simultaneously for a maximum of 16 milk samples, which constituted a batch.

Repeatability and reproducibility of the method were tested by measuring raw CY in several replicates. In particular, 4 individual milk samples were analyzed 4 consecutive times (batches) in the same day, in 4 replicates for each batch (n = 64). Repeatability (%) was measured as

$$\text{repeatability} = \frac{\sigma^2_{\text{sample}} + \sigma^2_{\text{batch}}}{\sigma^2_{\text{sample}} + \sigma^2_{\text{batch}} + \sigma^2_e} \times 100,$$

where σ^2_{sample} is the variance of the individual milk sample, σ^2_{batch} is the variance of the batch, and σ^2_e is the residual variance. Reproducibility (%) was calculated as

$$\text{reproducibility} = \frac{\sigma^2_{\text{sample}}}{\sigma^2_{\text{sample}} + \sigma^2_{\text{batch}} + \sigma^2_e} \times 100.$$

Repeatability and reproducibility were 89.8 and 82.6, respectively, in line with other comparable methods (Cipolat-Gotet et al., 2016).

Curds were analyzed for the contents (%) of water by vacuum oven at 100°C (method 926.08; AOAC International, 2003), total protein by macro-Kjeldahl (method 2001.14; AOAC International, 2002), and fat by an accelerated extraction method (ASE, Thermo Scientific Dionex, Sunnyvale, CA).

Fat separation by ASE was carried out according to guidelines suggested by Thermo Scientific Dionex for fat cheese extraction conditions (Dionex application note 3-345). For curd, sample preparation consisted of placing a cellulose filter into a 10-mL extraction cell before loading the sample. Two grams of ASE Prep DE was added to the cell, and 1 g of curd was homogenized and incorporated in the ASE Prep DE. Fat extraction was carried out with petroleum ether and isopropyl alcohol (3:2 vol/vol) at a temperature of 120°C. When the extraction was complete, solvent was evaporated with an N_2 stream. Each sample was then oven dried (120°C for 1 h). Finally, vials were allowed to reach room temperature and reweighted.

Raw CY was calculated as the ratio of the weight of the curd to the total weight of milk plus the weight of rennet solution. Dry matter CY (**DMCY**, %), water yield (%), protein yield (%), and fat yield (%) were calculated as raw CY multiplied by the percentage of curd DM, water, protein, and fat, respectively. Percentages of protein and fat in the curd expressed on a DM basis were also calculated.

Statistical Analysis

The effects of protein composition on CY and curd composition were estimated in a set of Bayesian analyses using 4 different linear models. All models included the effect of the herd-test day (20 levels) and model micro-cheese curd-making batch (83 levels, nested within herd-test day class), the linear effects of milk fat and lactose content, SCS, milk pH, and the additive genetic effect of the cow. In addition, the 4 models accounted for the linear effect of the following covariates: casein and whey protein (model 1); whey protein, the content of 1 specific casein fraction at a time (i.e., α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, κ-CN, Gκ-CN, or Uκ-CN) and the cumulative content of the remaining casein fractions (models 2 to 8, respectively); whey protein, the contents of Gκ-CN and Uκ-CN, and the cumulative content of the casein fractions other than κ-CN (model 9); and casein and the content of β-LG and α-LA (model 10).

In all Bayesian analyses, bounded uniform distributions, indicating vague prior knowledge of the estimated parameters, were used as prior densities for the effects of herd-test day, micro-cheese-making batch, and all covariates. Additive genetic effects and residual were assumed to be normally distributed. The pedigree file included all animals owning phenotyping records and all their known ancestors (9,204 animals). In each model, parameters of interest were the slopes of linear regressions of the covariates, related to the milk protein composition and accounted for by the 4 models, on the traits measured in the micro-cheese-making procedures. For such parameters, marginal posterior densities were estimated using a single chain of 500,000 Gibbs samples.

After a burn-in of 50,000 iterations, samples were saved every 250 iterations. Model convergence was checked by visual inspection of the chains.

The focus of this study was on variations in the relative content of casein fractions at constant casein. Accounting for both the effects of one casein fraction at a time and casein in the statistical model would have caused a high degree of collinearity due to spurious correlations between the content of each casein fraction and casein. We included in the model the cumulative content of all other fractions as an alternative to casein because correlations between a protein fraction and the sum of the others were much weaker and because model solutions allowed us to estimate the net effect of variations in protein composition.

Solutions obtained for model 1 were estimates of the effects exerted by variations in casein at constant whey protein and vice versa, which can be interpreted as variation in the casein index. Results from models 2 to 8 were estimates of the effect of variation in a given casein fraction at constant content of all the other casein fractions and whey protein. Hence, such estimates are the effects of variations in casein determined by the variation in a specific casein fraction. Model 9 estimated the effect of variations in Gκ-CN (Uκ-CN) at constant content of Uκ-CN (Gκ-CN) and at constant content of all other casein fractions except κ-CN. This can be interpreted as the net effect of variations in total κ-CN content at constant casein and whey protein due to a variation either in Gκ-CN or, alternatively, in Uκ-CN. Model 10 evaluated the effect of variations in β-LG and α-LA at constant casein (i.e., the effect of variations in whey protein due to the variation in a single whey protein fraction).

Starting from model solutions, we estimated the effects exerted only by variations in the relative content of a single protein fraction. In particular, solutions of models 2 to 8 were used to estimate the effect due to an increase in the relative content of a single casein fraction at constant casein (i.e., the effect of a change in casein composition at constant casein) by computing the expected change in the investigated traits resulting from an increased content of a single casein fraction and a concurrent equal decrease in the content of the other casein fractions. Likewise, using solutions from model 9, we estimated the effect due to a change in the rate of casein glycosylation (i.e., the relative content of Gκ-CN in κ-CN) at constant κ-CN content and, using the solution of model 10, the effect due to a change in whey protein composition at constant whey protein. Such estimates were computed for a change in the content of a single protein fraction resulting in a 1-SD increase in the percentage of that protein fraction in casein (for caseins) or whey protein (for whey proteins).

The median of the marginal density of the posterior probability was used as a point estimate of parameters. The posterior probability for an estimate of being greater (for positive estimates) or lower (for negative estimates) than 0 ($\boldsymbol{P_0}$) was calculated. Estimates were considered to be statistically relevant when P_0 was greater than 95%. The lower (for positive estimates) and upper (for negative estimates) bound of the confidence interval with 95% posterior probability was also obtained from the marginal densities of the estimated parameters. Methodological details and a comprehensive discussion on advantages of Bayesian techniques compared with classical statistics can be found in Blasco (2005).

RESULTS AND DISCUSSION

Characteristics of the Model Micro-Curds

Numerous attempts have been made to mimic in a laboratory setting and on a small scale the complex process of cheese making. Several procedures, comprehensively reviewed by Cipolat-Gotet et al. (2013), have been proposed. These procedures can be divided into very small (1.7 to 100 mL of milk processed) and small-scale or pilot experiments (200 mL to 30 L of milk processed). The first allow for the sampling and processing of many samples, but the latter enable researchers to analyze individual model cheeses. Even though it is expected that larger milk volumes provide higher repeatability, direct comparisons between different techniques, as well as their relevance for industrial cheese making, have not been investigated.

In our study, a new procedure processing 25 mL of milk was used as a method to process a large number of samples while providing a sufficient amount of curd for subsequent chemical analyses. Descriptive statistics for the investigated traits are reported in Table 1. Average raw CY obtained in the present study was approximately 2 times higher than that observed by Marziali and Ng-Kwai-Hang (1986) and Cipolat-Gotet et al. (2013). As average DMCY was similar to estimates reported in those studies, such difference is attributable to differences in water retention of curds caused by different features of the experimental cheese-making process (i.e., the presence or absence of milk acidification by starter cultures before renneting). Average raw CY was similar to that observed by Hurtaud et al. (1995) and Hallén et al. (2010), who also processed small volumes of milk (from 10 to 30 mL) using micro-cheese-making methods similar to the one used in our study. In agreement with Cipolat-Gotet et al. (2013), water yield exhibited greater variability (CV = 27%) than did DMCY (CV = 14%). This indicates that the

Table 1. Descriptive statistics of the investigated traits (n = 964)[1]

Trait	Mean	SD	1st percentile	99th percentile
Milk composition				
Protein, %	3.55	0.39	2.79	4.67
Fat, %	3.86	0.69	2.28	5.83
Lactose, %	4.79	0.23	4.14	5.18
pH	6.74	0.08	6.54	6.93
Protein fractions, g/L				
Casein	31.65	3.50	24.59	40.78
α_{S1}-CN	13.51	1.49	10.56	17.91
α_{S2}-CN	4.16	0.64	2.87	5.70
β-CN	9.75	1.81	6.13	14.23
γ-CN	0.66	0.22	0.36	1.36
κ-CN	3.56	0.73	2.20	5.47
Unglycosylated κ-CN	1.87	0.41	1.05	2.86
Glycosylated κ-CN	1.68	0.51	0.92	3.35
Whey protein	5.44	0.79	3.82	7.63
β-LG	4.09	0.71	2.70	6.13
α-LA	1.35	0.24	0.88	2.06
Curd yield, %				
Raw curd yield	26.42	6.00	16.95	44.25
DM yield	7.53	1.09	5.17	10.43
Water yield	18.89	5.24	11.11	34.56
Protein yield	2.86	0.40	2.09	4.08
Fat yield	3.62	0.80	1.41	5.58
Curd composition				
Moisture, %	70.85	3.93	64.00	80.48
Protein, % in DM	38.33	4.60	29.47	53.05
Fat, % in DM	47.78	7.00	22.33	61.48

[1] Contents of all protein fractions were measured by reversed-phase HPLC on skim milk. Casein = α_{S1}-CN + α_{S2}-CN + β-CN + γ-CN + κ-CN; whey protein = β-LG + α-LA; κ-CN = unglycosylated κ-CN + glycosylated κ-CN.

variation in the amount of water retained in the curd is more relevant, as a source of variation for raw CY, than the variation in the recovery rates of milk solids. This was expected because the micro-cheese-making method used in this study produced very soft curds, for which whey retention contributes to a large extent to variation in raw CY. Soft curds resulted from the absence of acidification before rennet addition and from cutting the curd in particles of relatively large size.

Estimates of the effects of casein and whey protein on the investigated traits, obtained using model 1, are reported in Table 2. Variations in curd composition due to variations in the contents of each casein fraction, at constant cumulative content of the other caseins, are reported in Table 3. Results are to be interpreted as the effect of a variation in the relative content of a given fraction in casein but also the effect of a variation in casein that is due to the variation of that given fraction. Estimates of the net effects exerted by variations in the relative proportions of the casein fractions, at constant casein content, calculated from solutions of models 2 to 8, are reported in Table 4. In addition to the effect of total κ-CN content in casein, the proportions in casein and in κ-CN of the main κ-CN fractions (glycosylated and unglycosylated) were investigated, and results are reported in Table 5.

Variation in Raw CY, Water Yield, and Curd Moisture

Due to the experimental conditions, variations in raw CY were tightly related to variations in water yield and curd moisture. Indeed, the direction of the effect of each protein fraction on these traits was identical with few exceptions: increasing amounts of total casein and α_{S1}-CN (grams and %) were associated with an increased raw CY and water yield but with a reduced or constant curd moisture, respectively, meaning that their effect was not attributable to greater water retention of the curds. Conversely, Uκ-CN percentage in casein was associated with increased curd moisture, which did not result in a higher CY.

Although to our knowledge the effect of κ-CN composition on CY and composition has never been reported, the results on casein and α_{S1}-CN are in agreement with the literature. Gilles and Lawrence (1985) reported that curds containing high amounts of total casein tend to expel more water due to a strengthened gel contraction and more intense syneresis.

Other studies (Creamer et al., 1982; Cipolat-Gotet et al., 2018) seem to suggest that α_{S1}-CN does not favor curd moisture. α_{S1}-Casein is strongly hydrophobic and provides the protein with marked self-association and aggregation tendencies (Creamer et al., 1982). Further,

Table 2. Estimated regression coefficients (β; units of the trait per SD of the explanatory variable) for the effects of casein and whey protein contents (g/L of milk) on the investigated traits[1]

	Casein			Whey protein		
Trait	β	P_0	T_{95}	β	P_0	T_{95}
Curd yield, %						
Raw curd yield	0.84	100	0.56	0.24	93	−0.02
DM yield	0.37	100	0.31	0.02	74	−0.02
Water yield	0.47	100	0.21	0.22	94	−0.01
Protein yield	0.23	100	0.21	0.03	99	0.02
Fat yield	0.01	70	−0.02	−0.03	86	0.01
Curd composition						
Moisture, %	−0.45	100	−0.29	0.23	98	0.06
Protein, % in DM	1.16	100	0.87	0.42	99	0.15
Fat, % in DM	−2.18	100	−1.72	−0.43	93	0.06

[1] P_0 (%) = the posterior probability of a positive (for positive estimates) or negative (for negative estimates) regression coefficient; T_{95} = the bound of the estimate interval T_{95}, $+\infty$ (for positive estimates) or $-\infty$, T_{95} (for negative estimates) corresponding to a 95% marginal posterior probability.

the domain of α_{S1}-CN has 3 mol of glutamate, which is expected to contribute to intra- and intermolecular calcium bridges. These properties of α_{S1}-CN in cheese lead to extensive cross-linking of paracasein molecules (Creamer et al., 1982), which might result in greater nutrient retention in the curd (Cipolat-Gotet et al., 2018).

All the protein fractions positively affected raw CY (and water yield) except for β-CN, Uκ-CN, and α-LA. Total casein was the variable with the greatest effect on raw CY (+0.84%), but whey protein also positively affected raw CY (+0.24%) because of its effect on curd moisture. Positive relationships between casein and whey protein and raw CY were also detected by Marziali and Ng-Kwai-Hang (1986), Wedholm et al. (2006), and Cipolat-Gotet et al. (2018).

α_{S1}-CN and κ-CN. In agreement with Cipolat-Gotet et al. (2018), effects of α_{S1}-CN and κ-CN on raw CY were greater than those of other casein fractions: a 1-SD increase in the content of α_{S1}-CN and κ-CN was associated with a 0.60 and 0.54% increase in raw CY, respectively. The effect exerted by α_{S1}-CN might be related to its self-association properties, as discussed above, whereas the effect of κ-CN might be related to variations in casein micelle size. High contents of κ-CN have been associated with smaller micelles and higher calcium content, resulting in more compact and uniform arrangement of the gel network, which may reduce losses in whey as a consequence of an improved entrapping ability (Walsh et al., 1998). In our study, the amount of κ-CN was also positively associated with moisture, and this contributed to amplifying its effect on raw CY. The positive effect of κ-CN on water yield was also observed by Macheboeuf et al. (1993) but not by Cipolat-Gotet et al. (2018). The use of different laboratory cheese-making techniques, involving or not involving centrifugation for whey expulsion, might explain the inconsistent results.

β-CN. The lack of a positive association between the content of β-CN and raw CY detected in our study can be ascribed to the negative relationship between β-CN and curd moisture (−0.66% per SD of β-CN). The proportion of β-CN in casein exerted even a more pronounced negative effect on raw CY (β = −0.61%; P_0 = 100%; T_{95} = −0.41%) than the absolute amount of β-CN. This is in agreement with previous studies reporting that curd tension increases significantly with β-CN fortification (St-Gelais and Haché, 2005). As enrichment in β-CN fraction occurs when casein increases (Bonfatti et al., 2011), this might explain why curds containing a high amount of casein tend to expel more water. Conversely, Cipolat-Gotet et al. (2018) reported increased water retention in the curd at increasing amounts of β-CN.

κ-CN Fractions. Raw CY, water yield, and curd moisture increased with the content of Gκ-CN, its proportion in casein, and its proportion in κ-CN. Conversely, the estimated effects of Uκ-CN content on raw CY and water yield were not statistically different from zero (P_0 < 95%). Hence, a large part of the effects of κ-CN content detected can be ascribed to its glycosylated fraction. Although associations between *CSN3* B, the content of whole κ-CN, casein micelle size, and improved milk coagulation are well known (Bijl et al., 2014), the effects of the single κ-CN fractions are still unclear. Glycosylated κ-CN increases the size of the superficial hydrophilic layer of the micelles (O'Connell and Fox, 2000) as each glucoside residue adds a negative charge to the molecule (Fox and McSweeney, 2003). Glycosylation affects κ-CN stabilizing activity toward the other caseins (Dziuba and Minkiewicz, 1996), the susceptibility to chymosin proteolysis (Doi et al.,

Table 3. Estimated regression coefficients (β; units of the trait per SD of the explanatory variable) for the effects of the content (g/L of milk) of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, and κ-CN, at constant content of the other casein fractions and whey protein, on the investigated traits[1]

	α_{S1}-CN			α_{S2}-CN			β-CN			γ-CN			κ-CN		
Trait	β	P_0	T_{95}	β	P_0	T_{95}	β	P_0	T_{95}	β	P_0	T_{95}	β	P_0	T_{95}
Curd yield, %															
Raw curd yield	0.60	100	0.32	0.35	100	0.10	−0.10	76	0.12	0.31	100	0.12	0.54	100	0.36
DM yield	0.21	100	0.17	0.08	100	0.04	0.15	100	0.11	0.05	100	0.02	0.08	100	0.05
Water yield	0.39	100	0.14	0.27	98	0.05	−0.24	98	−0.05	0.26	100	0.09	0.47	100	0.30
Protein yield	0.15	100	0.13	0.04	100	0.02	0.09	100	0.07	0.01	93	0.00	0.04	100	0.02
Fat yield	−0.01	63	0.03	0.03	92	0.00	0.00	52	−0.03	0.01	69	−0.02	0.02	83	−0.01
Curd composition															
Moisture, %	−0.14	92	0.04	0.12	89	−0.04	−0.66	100	−0.51	0.15	98	0.02	0.31	100	0.19
Protein, % in DM	0.87	100	0.60	0.23	93	−0.03	0.42	100	0.16	0.01	52	−0.20	0.17	93	−0.02
Fat, % in DM	−1.49	100	−1.07	−0.27	88	0.12	−0.83	100	−0.46	−0.36	100	−0.05	−0.31	95	0.00

[1] P_0 (%) = the posterior probability of a positive (for positive estimates) or negative (for negative estimates) regression coefficient; T_{95} = the bound of the estimate interval T_{95}, $+\infty$ (for positive estimates) or $-\infty$, T_{95} (for negative estimates) corresponding to a 95% marginal posterior probability.

Table 4. Estimated regression coefficients (β; units of the trait per SD of the explanatory variable) for the effects of the relative content (% in casein) of α_{S1}-CN, α_{S2}-CN, β-CN, γ-CN, and κ-CN in total casein, at constant total casein content and whey protein content, on the investigated traits[1]

	α_{S1}-CN			α_{S2}-CN			β-CN			γ-CN			κ-CN		
Trait	β	P_0	T_{95}	β	P_0	T_{95}	β	P_0	T_{95}	β	P_0	T_{95}	β	P_0	T_{95}
Curd yield, %															
Raw curd yield	0.22	95	0.00	0.16	90	−0.04	−0.61	100	−0.41	0.26	99	0.07	0.36	100	0.18
DM yield	0.05	99	0.02	0.01	69	−0.02	−0.05	100	−0.02	0.03	92	0.00	0.00	50	−0.03
Water yield	0.17	93	−0.03	0.15	91	−0.03	−0.56	100	−0.38	0.23	99	0.06	0.35	100	0.20
Protein yield	0.05	100	0.03	0.00	51	−0.02	−0.03	100	−0.01	0.00	51	0.01	−0.01	80	−0.02
Fat yield	−0.01	77	−0.04	0.02	90	−0.01	−0.01	72	0.02	0.01	71	−0.02	0.01	79	−0.01
Curd composition															
Moisture, %	0.05	71	−0.09	0.17	99	0.04	−0.49	100	−0.36	0.18	99	0.05	0.38	100	0.27
Protein, % in DM	0.35	99	0.11	0.01	55	−0.19	−0.21	94	0.00	−0.07	74	0.10	−0.07	74	0.10
Fat, % in DM	−0.51	99	−0.16	0.10	72	−0.21	0.34	93	−0.03	−0.23	88	0.09	0.13	78	−0.16

[1] P_0 (%) = the posterior probability of a positive (for positive estimates) or negative (for negative estimates) regression coefficient; T_{95} = the bound of the estimate interval T_{95}, $+\infty$ (for positive estimates) or $-\infty$, T_{95} (for negative estimates) corresponding to a 95% marginal posterior probability.

Table 5. Estimated regression coefficients (β; units of the trait per SD of the explanatory variable) for the effects of contents (g/L of milk), at constant content of the other casein fractions and whey protein, and relative proportions in casein (% in casein) and κ-CN (% in κ-CN) of unglycosylated (Uκ-CN) and glycosylated (Gκ-CN) κ-CN, at constant total casein content and whey protein content, on the investigated traits[1]

	Content						Proportion in casein						Proportion in κ-CN		
	Uκ-CN			Gκ-CN			Uκ-CN			Gκ-CN			Gκ-CN		
Trait	β	P_0	T_{95}	β	P_0	T_{95}	β	P_0	T_{95}	β	P_0	T_{95}	β	P_0	T_{95}
Curd yield, %															
Raw curd yield	0.15	93	−0.01	0.62	100	0.44	0.05	70	−0.12	0.45	100	0.28	0.27	100	0.11
DM yield	0.02	83	−0.01	0.08	100	0.05	−0.03	96	−0.00	0.03	94	−0.00	0.04	99	0.01
Water yield	0.13	93	−0.02	0.54	100	0.37	−0.08	80	0.07	0.42	100	0.27	0.23	100	0.09
Protein yield	0.01	92	−0.00	0.04	100	0.02	−0.02	97	−0.00	0.00	69	−0.01	0.01	95	0.00
Fat yield	0.01	79	−0.01	0.01	71	−0.02	0.01	76	−0.01	0.01	67	−0.01	0.00	56	0.02
Curd composition															
Moisture, %	0.12	96	0.01	0.35	100	0.23	0.17	100	0.06	0.38	100	0.27	0.13	98	0.03
Protein, % in DM	0.11	83	−0.06	0.09	77	−0.11	−0.03	60	0.14	−0.07	66	0.10	−0.03	61	0.15
Fat, % in DM	0.05	60	−0.23	−0.43	99	−0.10	0.30	97	0.02	−0.11	73	0.18	−0.28	96	−0.01

[1] P_0 (%) = the posterior probability of a positive (for positive estimates) or negative (for negative estimates) regression coefficient; T_{95} = the bound of the estimate interval T_{95}, $+\infty$ (for positive estimates) or $-\infty$, T_{95} (for negative estimates) corresponding to a 95% marginal posterior probability.

1979), and casein micelle size (Bijl et al., 2014). Results obtained for the effect of κ-CN glycosylation on milk coagulation properties are scarce and inconsistent (Robitaille et al., 1993; Bonfatti et al., 2014; Jensen et al., 2012a,b) and, to our knowledge, no report on the effect of κ-CN glycosylation on curd water-holding capacity is available in the literature.

γ-CN. We observed a relevant increase in water yield and moisture at increasing contents of γ-CN in milk protein, in agreement with Guinee and O'Brien (2010). Content of γ-CN originates from proteolytic degradation of β-CN (Fox and McSweeney, 2003) and is used as an indicator of the degree of proteolysis occurring in milk. An increased level of proteolysis is detrimental for cheese making because it negatively affects the content of intact casein. Guinee and O'Brien (2010) reported that the decreased content of gel-forming casein (indications of a high content of γ-CN) results in low curd firmness and slow aggregation and fusion of paracasein micelles during the cheese-making process. Relative to high curd firmness, low curd firmness is conducive to impaired syneresis capacity of the curd, increased moisture, great susceptibility of curd particles to shattering at cutting and early stages of stirring, and large losses of curd fines and milk fat.

Whey Proteins. Estimates of the effects of whey protein fractions on the investigated traits are reported in Table 6. A relevant increase ($P_0 > 95\%$) in raw CY and water yield was detected when content (and relative proportion) of β-LG increased and that of α-LA decreased, but the effect of β-LG can be ascribed to an improved capacity of the curd to retain water (moisture increased by 0.88% per 1-SD increase in the relative proportion of β-LG in whey protein). This likely explains why whey protein, mainly constituted by β-LG, had an overall positive effect on CY (Table 2). In agreement with our results, Marziali and Ng-Kwai-Hang (1986) reported a positive relationship between β-LG and cheese yield, confirmed also by Wedholm et al. (2006). However, a positive relationship between α-LA and cheese yield has also been reported (Marziali and Ng-Kwai-Hang, 1986; Cipolat-Gotet et al., 2018).

Variations in CY Not Attributable to Variations in Water Retention

As we evaluated soft curds, in which raw CY is largely dependent on variations in the amount of water retained in the curd, a better indicator of transformation efficiency of milk into curd is DMCY. Total casein content, at constant whey protein content, was positively associated with DMCY (Table 2), in agreement with Cipolat-Gotet et al. (2018). The increase in DMCY per 1-SD increase in casein was 0.37%. These results suggest that greater DMCY is to be expected at increasing values of milk casein index. In agreement with our results, Wedholm et al. (2006) reported that casein index and whey protein were positively associated and not associated, respectively, with the amount of cheese solids obtained per gram of milk protein.

As expected, DMCY increased when the content of any casein fraction increased (P_0 = 100%), but, in agreement with the literature (Marziali and Ng-Kwai-Hang, 1986; Wedholm et al., 2006), α_{S1}-CN and β-CN were the casein fraction with the greatest positive effect (Table 3). However, a 1-SD increase in β-CN percentage in casein (+3.8% in casein) exerted a slightly negative effect on DMCY (β = −0.05%; P_0 = 100%; T_{95} = −0.02%; Table 4). Conversely, increasing amounts of α_{S1}-CN percentage were associated with a small increase in DMCY (Table 4). Hence, results suggest that, at constant casein and whey protein milk content, the efficiency of the cheese-making process depends on the variation in the α_{S1}-CN:β-CN ratio. This is in contrast with Hallén et al. (2010), who concluded that variation in casein composition is not related to CY. Differences across studies are likely to be ascribed to a different sample size or characteristics of the experimental curds.

An increase in κ-CN percentage did not affect DMCY (P_0 = 50%; Table 4). This indicates that the positive relationship detected between the content of κ-CN and DMCY can be attributed to the increase in casein resulting from the increased content of κ-CN and not to variation in casein composition. In particular, a positive small effect of the Gκ-CN (content and proportion in κ-CN) on DMCY and protein yield was observed (P_0 > 95%), whereas an increase in the percentage of Uκ-CN in casein exhibited a very weak but negative association (P_0 > 95%) with DMCY and protein yield (Table 5). Hence, milk samples with similar content of protein, casein, and κ-CN may still exhibit a different cheese-making ability, depending on their κ-CN composition.

In the literature, only Robitaille et al. (1993) investigated the association between κ-CN glycosylation and cheese yield, focusing on the moisture-adjusted cheese yield (which is analogous to DMCY in our study). In contrast with our results, those authors reported that κ-CN glycosylation did not affect moisture-adjusted cheese yield. A different cheese-making process and the exclusive use of *CSN3* AA animals, which decreased the variation in the degree of κ-CN glycosylation compared with our study, might be the cause of such inconsistency.

In agreement with Cipolat-Gotet et al. (2018), α_{S2}-CN had a small effect on the cheese-making process: an increase in its content was associated with an increase in DMCY (Table 3), but no variations in DMCY were observed at increasing proportions of α_{S2}-CN in casein (Table 4). It is worth noting that every increase in DMCY observed in our study was due to an increase in protein yield, not fat yield.

Variations in Protein and Fat in Curd DM

Generally, contents of all the protein fractions were associated with a higher amount of protein and lower fat in curd DM, with the exception of γ-CN (Table 3). Being a proteolytic product of β-CN (Fox and McSweeney, 2003), γ-CN is an indicator of the content of intact casein and its amount is associated with (1) a great susceptibility of curd particles to shattering at cutting and early stages of stirring and (2) a higher

Table 6. Estimated regression coefficients (β; units of the trait per SD of the explanatory variable) for the effects of contents (g/L of milk) of β-LG and α-LA, at constant casein content, and relative proportion (%) of β-LG in total whey protein, at constant casein content and whey protein content, on the investigated traits[1]

	Content						Proportion in whey protein		
	β-LG			α-LA			β-LG		
Trait	β	P_0	T_{95}	β	P_0	T_{95}	β	P_0	T_{95}
Curd yield, %									
Raw curd yield	0.30	97	0.04	−0.18	95	0.00	1.56	100	0.44
DM yield	0.02	82	−0.02	−0.02	83	0.01	0.14	91	−0.03
Water yield	0.27	98	0.04	−0.16	95	0.00	1.41	100	0.42
Protein yield	0.04	100	0.02	−0.02	95	0.00	0.16	100	0.07
Fat yield	−0.03	90	0.01	0.00	61	0.02	−0.02	59	0.12
Curd composition									
Moisture, %	0.25	99	0.08	−0.07	83	0.05	0.88	98	0.14
Protein, % in DM	0.48	100	0.20	−0.16	90	0.04	1.79	100	0.64
Fat, % in DM	−0.47	97	−0.04	0.04	58	−0.27	−1.12	83	0.65

[1] P_0 (%) = the posterior probability of a positive (for positive estimates) or negative (for negative estimates) regression coefficient; T_{95} = the bound of the estimate interval T_{95}, +∞ (for positive estimates) or −∞, T_{95} (for negative estimates) corresponding to a 95% marginal posterior probability.

amount of other proteolytic products (i.e., small peptides). These 2 factors lead to large losses of curd fines and milk fat (i.e., lower protein and fat recovered in the curd). However, when casein and whey proteins were expressed as percentages, their effect on curd composition was weaker and in most cases not significant. The increased content of whey protein was associated with small increases in curd protein, which likely resulted from the high average amount of whey retained in the micro-curds and, as a consequence, of the whey protein retained.

CONCLUSIONS

The effect of contents and relative proportions of caseins and whey proteins, including κ-CN fractions, were extensively studied directly on CY and composition using a large number of individual milk samples. Results obtained in this study support the hypothesis that, besides variation in total casein and whey protein contents, variation in protein composition affects the cheese-making ability of milk. Different protein fractions were associated with a higher or lower water-holding capacity of the curd. Protein contents and relative percentage in casein and in whey protein also affected the amount of fat and protein retained in the curd, which is independent from water retention. In addition, different protein fractions have been demonstrated to affect curd composition. Because of the low volume of processed milk and the simple procedure for curd making, results from model curds might not be directly transferrable to actual cheese making. Experimental cheese-making trials processing milk with extreme protein composition should be performed to clarify the effects on actual cheese yield and quality.

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