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β-Casein A1 and A2: Effects of polymorphism on the cheese-making process

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ABSTRACT

Of late, "A2 milk" has gained prominence in the dairy sector due to its potential implications in human health. Consequently, the frequency of A2 homozygous animals has considerably increased in many countries. To elucidate the potential implications that beta casein (β-CN) A1 and A2 may have on cheese-making traits, it is fundamental to investigate the relationships between the genetic polymorphisms and cheese-making traits at the dairy plant level. Thus, the aim of the present study was to evaluate the relevance of the β-CN A1/A2 polymorphism on detailed protein profile and cheese-making process in bulk milk. Based on the β-CN genotype of individual cows, 5 milk pools diverging for presence of the 2 β-CN variants were obtained: (1) 100% A1; (2) 75% A1 and 25% A2; (3) 50% A1 and 50% A2; (4) 25% A1 and 75% A2; and (5) 100% A2. For each cheese-making day (n = 6), 25 L of milk (divided into 5 pools, 5 L each) were processed, for a total of 30 cheese-making processes. Cheese yield, curd nutrient recovery, whey composition, and cheese composition were assessed. For every cheese-making process, detailed milk protein fractions were determined through reversed-phase HPLC. Data were analyzed by fitting a mixed model, which included the fixed effects of the 5 different pools, the protein and fat content as a covariate, and the random effect of the cheese-making sessions. Results showed that the percentage of κ-CN significantly decreased up to 2% when the proportion of β-CN A2 in the pool was ≥25%. An increase in the relative content of β-CN A2 (≥50% of total milk processed) was also associated with a significantly lower cheese yield both 1 and 48 h after cheese production, whereas no effects were observed after 7 d of ripening. Concordantly, recovery of nutrients reflected a more efficient process when the inclusion of β-CN A2 was ≤75%. Finally, no differences in the final cheese composition obtained by the different β-CN pools were observed.
Key words: protein variant, cheese yield, curd, dairy cow, efficiency

INTRODUCTION

Cheese manufacture is a worldwide practice, and is particularly widespread in European countries and those populated by European immigrants (Fox et al., 2017). In Italy, more than 75% of the milk produced is destined for cheese production (CLAL, 2022). Thus, the quantity and quality of cheese, in terms of both volume of milk and grams of protein in the milk, are key factors for the economic outcome of the dairy industry (Wedholm et al., 2006). Monitoring all the relationships between quality and cheese-making, such as cheese yield and recovery of milk constituents in the curd, is a crucial step to define efficiency across the entire process (Banks, 2007). Particularly, this approach would also include gathering information on the milk protein fractions and their genetic polymorphisms, which have been widely associated with composition, production traits, and technological properties of milk (Wedholm et al., 2006). For example, the favorable effects of the B allele of κ-CN over the A and E alleles on milk composition and coagulation properties have been documented (Caroli et al., 2000), as well as the effect of the B allele of β-LG in increasing the CN number compared with the A variant (Lundén et al., 1997). Similarly, β-CN exerts an important role during cheese-making due to its positive correlation with actual cheese yield (Marziali and Ng-Kwai-Hang, 1986; Cipolat-Gotet et al., 2018). The β-CN accounts for more than 35% of the total milk protein and has 15 different genetic variants (Daniloski et al., 2022), 7 of which have been identified, mostly in European cattle breeds (A1, A2, A3, B, C, I, and E; Sebastiani et al., 2022). The A1 and the ancestral A2 variants of β-CN occur most frequently in cattle populations, and dif-

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fer only by a single substitution at residue 67, with a proline (Pro^{67}) in A2 β-CN milk and a histidine (His^{67}) in A1 β-CN milk (Sebastiani et al., 2022). Due to the difference in the amino acid residue at position 67, the two β-CN variants differently affect the pattern of enzymatic cleavage during digestion, potentially leading to different possible effects on human health. Daniloski et al. (2021) reviewed the available in vivo investigations concerning the hypothesis that β-CN A1 may be a risk factor for several noncommunicable diseases. Their conclusions are in line with those of the European Food Safety Authority (Noni et al., 2009), according to which there are not enough evidence-based studies to merit recommendations by public health authorities related to the consumption and health associations of either A1 or A2 β-CN milk. Regardless of the scientific evidence, "A2 milk" continues to gain prominence in the dairy sector, and the frequency of homozygous A2 animals has considerably increased in many countries in the last 25 years, especially for Holstein Friesian cows (Hohmann et al., 2021). For this reason, investigations to understand any potential implications that A1 or A2 β-CN may have on milk technological properties are required. In this regard, several studies have been carried out on the effects of β-CN genetic polymorphism on milk clotting properties, but their focus was limited to the coagulation traits due to the labor-intensive nature of cheese-making trials (Cipolat-Gotet et al., 2018). In addition, the role of the genetic variants of milk proteins in the cheese-making process have been studied at an individual level (Wedholm et al., 2006; Cipolat-Gotet et al., 2018; Bisutti et al., 2022), but knowledge concerning the role of the relative contents of β-CN variants in bulk milk on cheese-making processes is still lacking. Indeed, milk processed at the dairy plant level comes from a large number of cows. Thus, the aim of this study was to estimate the relevance of the β-CN A1/A2 polymorphism on the cheese-making process in field conditions by evaluating cheese yields, curd nutrient recoveries, and curd chemical composition.

MATERIALS AND METHODS

Cows selected for the present study belonged to a commercial herd, and the milk was collected during routine milking procedure and was not invasive; therefore ethical approval was not required.

Sampling Procedure and Experimental Design

A commercial Holstein Friesian herd located in the Veneto region of Italy and associated with the Latteria Soligo dairy company (Soligo, Italy) was selected for the study. First, the gross milk composition of all available early-lactation cows was determined using a MilkoScan FT7 (Foss Electric A/S) in the laboratory of the Breeders Association of Veneto Region (Vicenza, Italy). Concurrently, milk samples were analyzed for the determination of protein polymorphisms using reversed-phase HPLC (Vigolo et al., 2022a). A total of 6 β-CN A1 and 6 β-CN A2 homozygous cows with similar milk compositions were selected for the study. As κ-CN and β-LG have known effects on milk composition and cheese quality, all cows were also selected for a balanced κ-CN and β-LG phenotype, to keep their effect constant across all samples. On 6 occasions between May and July 2022, 10 L of milk were collected from each individual cow during the evening milking and successively bulked together according to the β-CN genotype, obtaining "A1 milk" and "A2 milk" bulk samples (milk was from 3 β-CN A1 and 3 β-CN A2 homozygous cows, chosen alternately from the 12 available to obtain a similar fat-to-protein ratio). Subsequently, the A1 milk and A2 milk bulk samples were further mixed to create 5 different pools according to the mixes: (1) 100% A1 milk; (2) 75% A1 milk and 25% A2 milk; (3) 50% A1 milk and 50% A2 milk; (4) 25% A1 milk and 75% A2 milk; and (5) 100% A2 milk (Figure 1). Approximately 6 L of each pool was put aside and stored at 4°C for the cheese-making trials conducted the day after sampling. Moreover, 50 mL of each pool was stored aside at 4°C for subsequent traditional milk composition analysis and detailed milk protein fraction determination.

Cheese-Making Procedure

For each day (n = 6), 5 cheese-making trials were carried out (one for each pool). Five liters of milk were used for each cheese-making session. Milk was slowly heated to 40°C by water circulation in a heating jacket using a water bath (SB24, Falc Instruments). The pH was measured once the milk reached 40°C, and starter cultures of mixed *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (microMilk) were added to the milk as indicated by the producer. After 10 min of gentle mixing, 2.5 mL of commercial liquid calf rennet [75:25 chymosin:bovine pepsin, 110 international milk clotting units (IMCU), De Longhi Michele and Co.] in aqueous solution (1:10) was added to the milk. The added rennet was blended thoroughly and then left to coagulate under quiescent conditions for 16 min at 39°C. Then, the coagulum was cut, 150 g of salt was added, and the whole was gently blended for 10 min. After draining for a further 10 min, the curd was extracted from the vat and filled into circular cheese molds, and 50 mL of each pool's whey was stored aside at 4°C for subsequent composition analysis and detailed protein fraction de-

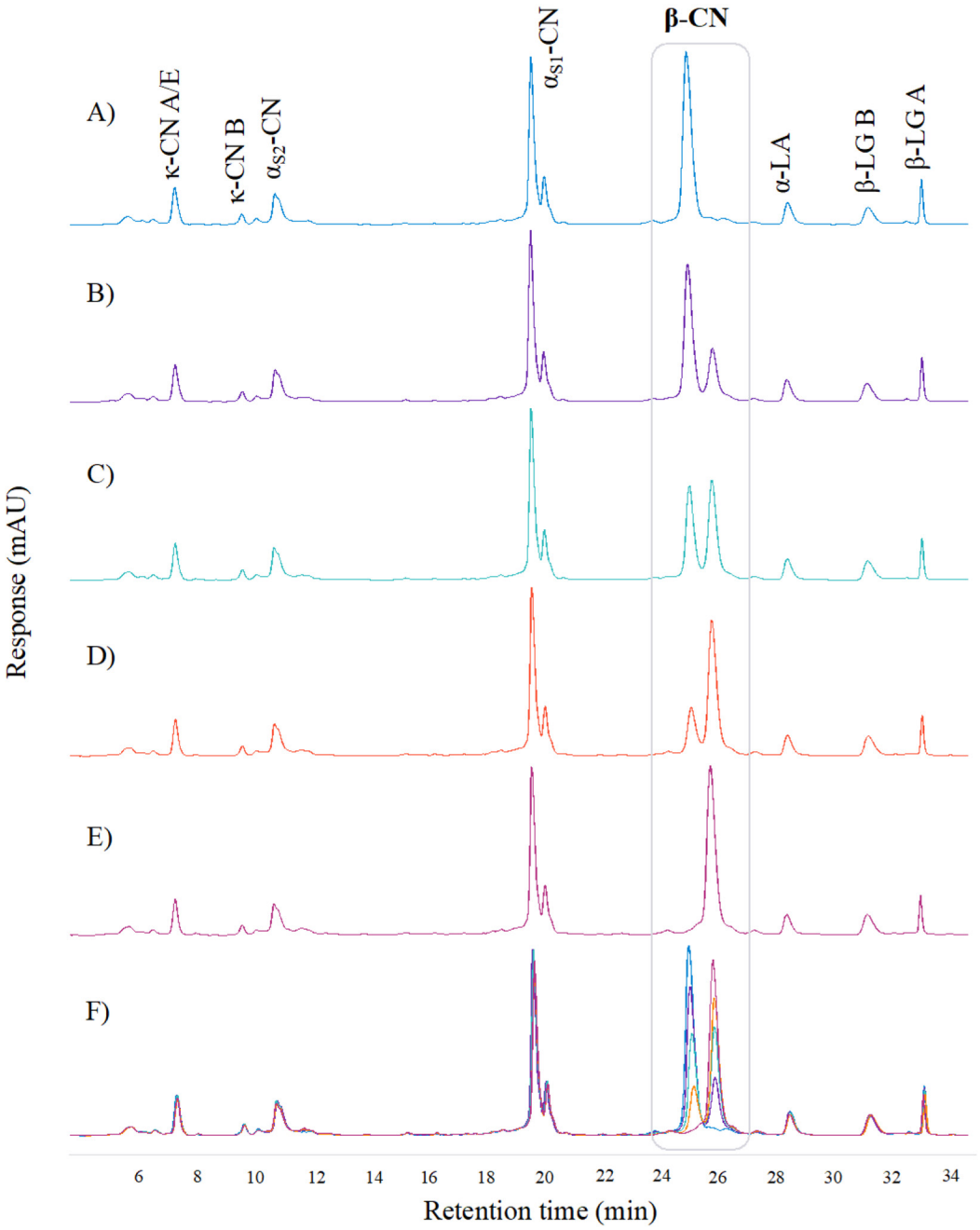

Figure 1. Reversed-phase HPLC chromatograms of bulk milk samples of 5 tested milk pools containing different proportions of β-CN A1 and A2. (A) 100% A1 milk; (B) 75% A1 milk and 25% A2 milk; (C) 50% A1 milk and 50% A2 milk; (D) 25% A1 milk and 75% A2 milk; (E) 100% A2 milk; (F) aforementioned chromatograms overlapped. A1 and A2 milks were extracted from cows that were A1A1 or A2A2 for the β-CN genotype, respectively. mAU = milli-absorbance units.

termination. The curd was first weighed (time $\mathbf{t_0}$) and then stored for 7 d in the ripening cell at +4°C and 78% relative humidity. The curd was further weighed 1, 48, and 168 h after extraction ($\mathbf{t_1}$, $\mathbf{t_{48}}$, and $\mathbf{t_{168}}$, respectively). The experimental design is summarized in Figure 2.

Analysis of Milk Composition and Cheese-Making Traits

The 5 assessed cheese-making traits were based on the weights of the wheels and milk, and the classical formula for actual cheese yield ($\mathbf{CY_{CURD}}$, %) was used:

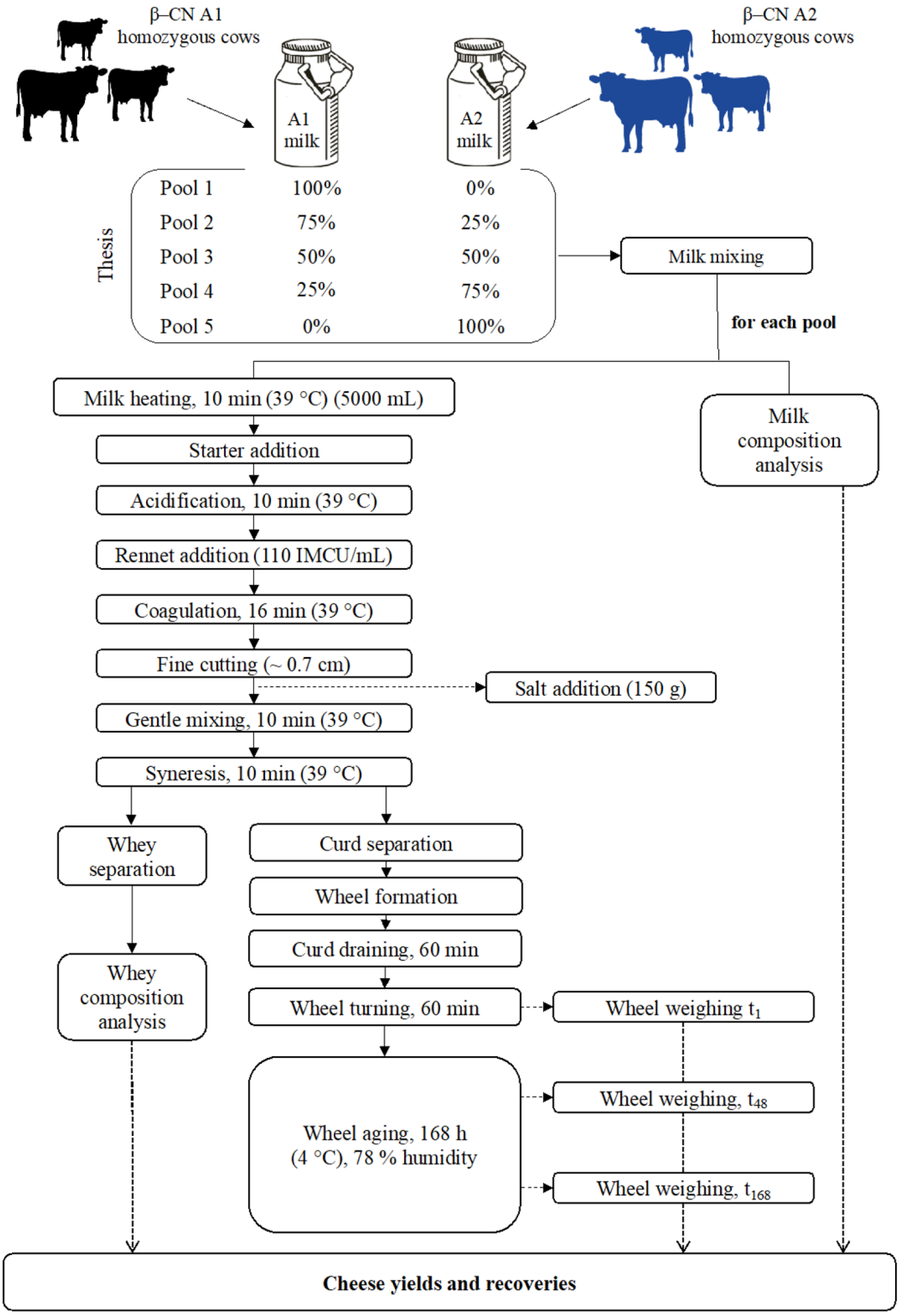

Figure 2. Experimental design to evaluate the effects of the β-casein polymorphism on the cheese-making process. A1 and A2 milks were extracted from cows that were A1A1 or A2A2 for the β-CN genotype, respectively. IMCU = international milk clotting units; t_1, t_{48}, and t_{168} = curd weights at 1, 48, and 168 h after extraction, respectively.

$$CY_{CURD}\,(\%) = \frac{\text{weight of wheel}\,(g)}{\text{weight of milk}\,(g)} \times 100.$$

The CY_{t1}, CY_{t48}, and CY_{t168} were assessed by substituting the weights obtained at different times (i.e., 1, 48, and 168 h after extraction) into the numerator of the formula. Milk and whey samples of each cheese-making process for the different pools were delivered to the laboratory of the Breeders Association of Veneto Region (Padova, Italy) for determination of gross composition using a MilkoScan FT7 (Foss Electric A/S). Somatic cell count (SCC, cells/μL) was determined using a Fossomatic (Foss Electric A/S) and transformed to somatic cell score (SCS) according to the formula, $SCS = \log_2(SCC/100) + 3$. Further, considering the chemical composition of milk and whey, the recoveries (**REC**, %) of milk protein and fat in the curd were calculated according to Cipolat-Gotet et al. (2013):

$$REC_{PROTEIN}\,(\%) = \frac{\text{milk protein}\,(g) - \text{whey protein}\,(g)}{\text{milk protein}\,(g)} \times 100;$$

$$REC_{FAT}\,(\%) = \frac{\text{milk fat}\,(g) - \text{whey fat}\,(g)}{\text{milk fat}\,(g)} \times 100.$$

After 7 d of ripening, the wheels were grated and analyzed for chemical composition (protein, fat, humidity, and DM content) using a FoodScan Dairy Analyzer (Foss Electric A/S).

Detailed Protein Fraction Determination

Detailed milk protein composition of each pool used for the cheese-making process and the subsequent whey obtained was determined through HPLC, as detailed in Vigolo et al. (2022a). Briefly, the HPLC equipment consisted of an Agilent 1260 Infinity II LC system (Agilent Technologies) equipped with a quaternary pump (Agilent 1260 Infinity II, G7111B) and a diode array detector (Agilent 1260 Infinity II, G7115A). For protein separation, a reversed-phase analytical column C8 (Zorbax 300SB-C8 RP, Agilent Technologies) with silica-based packing (3.5 μm, 300 Å, 150 × 4.6 i.d.) and gradient elution carried out with a mixture of solvent A (0.1% trifluoroacetic acid in water) and solvent B (0.1% trifluoroacetic acid in acetonitrile) was used. The method allows for the discrimination of the κ-CN, β-CN, and β-LG polymorphisms. The results are expressed as total κ-CN, β-CN, and β-LG, and are calculated by combining the area underneath the peaks that identify the polymorphisms of the same fraction (Figure 2). Quantification of different milk protein fractions was carried out using external standards of α-CN, κ-CN, β-CN, α-LA, β-LG A, and β-LG B (Merck KGaA) at the highest available purity levels. Total CN content was computed as the sum of α_{S1}-CN, α_{S2}-CN, β-CN, and κ-CN, whereas total whey protein (**WP**) content was calculated as the sum of α-LA and β-LG.

Statistical Analysis

Outlier values for the investigated traits (i.e., values outside the range mean ± 3 SD) were treated as missing. The effects of different proportions of β-CN A1/A2 variants on CY_{CURD}, REC, and curd chemical composition were estimated by fitting a linear mixed model in SAS software v. 9.4 (SAS Institute Inc.). The model was as follows:

$$y_{ijk} = \mu + M_i + T_j + \beta_1 p_{ijk} + \beta_2 f_{ijk} + \varepsilon_{ijk},$$

where y_{ijk} is the response variable; μ is the overall intercept of the model; M_i is the fixed effect of the ith pool of milk (i = 1 to 5); T_j is the random effect of the jth cheese-making session (j = 1 to 6); β_1 is the fixed effect of linear regression coefficient of y_{ijk} on protein concentration (p); β_2 is the fixed effect of linear regression coefficient of y_{ijk} on fat concentration (f); and ε_{ijk} is the random residual ~ $N\left(0, \sigma^2_\varepsilon\right)$, where σ^2_ε is the residual variance associated with observation ijk. Data are presented as least squares means (LSM) ± standard error. Multiple comparisons among LSM of the fixed effects were performed through Bonferroni post-hoc test. Statistical significance was established at $P < 0.05$.

RESULTS AND DISCUSSION

Descriptive Statistics

Overall, means of milk composition traits (Table 1) were consistent with average values reported in literature for Italian bulk milk (Benedet et al., 2018; Vigolo et al., 2022b). The mean pH value (6.69) was within the normal range (6.5–7.0) for this trait (Fox et al., 2017). Among traditional traits, SCS and urea showed the greatest coefficient of variation (CV; 41.75% and 16.85%, respectively), whereas the other traits exhibited much lower CV, ranging from 0.23% (pH) to 4.93% (casein percentage). As the bulk milk used for the present study was sampled during a short period of time and the cows were specifically selected for similar milk compositions, the low variability was expected. The α-CN (as the sum of α_{S1}-CN and α_{S2}-CN) was the predominant casein fraction (13.76 mg/mL), represent-

Table 1. Descriptive statistics of bulk milk composition traits and detailed milk protein composition, expressed as amount (mg/mL) and percentage (%)

Trait[1]	n	Mean	CV (%)	Minimum	Maximum
Milk composition					
Protein (%)	29	3.37	4.00	3.19	3.65
Casein (%)	29	2.67	4.93	2.49	2.92
Fat (%)	29	3.62	1.77	3.54	3.76
Lactose (%)	29	4.90	1.34	4.78	5.04
Total solids (%)	29	12.38	1.02	12.10	12.71
Urea (mg/dL)	29	22.79	16.85	16.70	30.20
SCS (units)	29	2.63	41.75	0.94	4.79
pH	30	6.69	0.23	6.67	6.72
Protein fraction (mg/mL of milk)					
Total CN	30	32.94	5.53	28.73	36.06
κ-CN	30	7.00	6.42	6.02	8.13
α_{S1}-CN	29	3.93	6.29	3.36	4.44
α_{S2}-CN	30	9.83	5.96	8.24	10.96
β-CN	30	12.25	6.25	10.47	13.46
Total WP	30	6.15	7.54	5.38	7.21
α-LA	30	1.48	4.85	1.33	1.62
β-LG	30	4.67	8.60	4.02	5.61
Protein fraction (% of total protein in milk)					
Total CN	30	84.26	1.27	81.22	85.57
κ-CN	29	17.75	3.45	16.67	18.89
α_{S1}-CN	29	10.05	2.64	9.57	10.54
α_{S2}-CN	29	25.23	1.49	24.55	26.55
β-CN	29	31.39	1.43	30.30	32.30
Total WP	30	15.74	6.80	14.43	18.78
α-LA	29	3.78	3.11	3.61	4.17
β-LG	30	11.95	7.93	10.67	14.61

[1]SCS = $\log_2$(SCC/100) + 3. WP = whey protein.

ing 35.20% of total protein, followed by β-CN (12.25 mg/mL), which accounted for 31.34% of total protein (Table 1). The remaining part was composed of κ-CN, with an average value of 7 mg/mL (17.91%), and WP (15.73%), represented by α-LA (1.48 mg/mL) and β-LG (4.67 mg/mL), with the latter exhibiting the greatest CV (8.60%). Overall, Vigolo et al. (2022c) obtained similar results on bulk milk samples for α-CN, β-CN, and β-LG, and lower κ-CN and α-LA. The CN index (ratio between the sum of CN fractions and the sum of all milk protein fractions), calculated using results of the chromatographic analysis, was greater compared with mid-infrared spectroscopy predictions (84% and 79%, respectively). Such overestimation is likely due to (1) the inability of HPLC to quantify minor WP fractions and nonprotein nitrogen, causing potential distortion in the estimation of the ratios of the different fractions (Niero et al., 2016), and (2) the preparation of milk samples involving a skimming step before chromatographic analysis (Bonfatti et al., 2011).

Descriptive statistics of cheese-making traits are reported in Table 2. The CY_{CURD} calculated 1, 48, and 168 h after drainage is expressed as a percentage of the total milk processed. Values decreased by more than 3% over 1 wk, from 19.05% (CY_{t1}) to 15.74% (CY_{t168}), as a consequence of moisture loss. Likewise, CV of CY_{CURD} traits linearly decreased from 8.69% (CY_{t1}) to 6.63% (CY_{t168}), suggesting greater stability to wheying-off over time (Fox et al., 2017). Overall, after 7 d of ripening, wheels lost approximately 30% of their initial weight. Because the CY formula used in the present study does not account for external effects of variation (e.g., differences in plant throughput and cheese-making procedure, such as the size into which the rennet curd was cut, the cooking temperature, or the quantity of salt added to the curd, initial milk composition, or ripening conditions), a meaningful comparison with literature was not possible. Nevertheless, average values of the current study were consistent with those reported by Sturaro et al. (2015) in similar plant conditions. The average values of $REC_{PROTEIN}$ and REC_{FAT} indicated that the cheese-making process resulted in the loss of 19.78% protein and 13.78% fat (Table 2), which is in agreement with the findings of Cipolat-Gotet et al. (2018) and Stocco et al. (2018) in individual milks. The whey obtained from the cheese-making process had average contents of protein, fat, and lactose of 0.67%, 0.50%, and 4.97%, respectively, with fat presenting the greatest CV (18.83%). Average whey total solids (7.15%) was consistent with values retrieved from literature and indicated a standard cheese-making procedure where only about half of the solids initially present in milk are incorporated into cheese (Fox et al., 2017). Even if whey composition is highly

Table 2. Descriptive statistics of cheese yield (CY_{CURD}), curd nutrient recoveries (REC), whey composition, and curd chemical composition traits

Trait[1]	n	Mean	CV (%)	Minimum	Maximum
CY_{CURD} (%)					
CY_{t1}	30	19.05	8.69	16.21	21.52
CY_{t48}	29	17.29	7.53	15.08	19.27
CY_{t168}	30	15.74	6.63	13.69	17.56
REC (%)					
$REC_{PROTEIN}$	29	80.22	1.70	77.26	82.76
REC_{FAT}	28	86.22	3.14	82.14	92.29
Whey composition (%)					
Protein	30	0.67	9.96	0.55	0.83
Fat	29	0.50	18.83	0.29	0.65
Lactose	29	4.97	1.51	4.82	5.12
Total solids	29	7.15	1.51	6.84	7.36
Cheese composition (%)					
Protein	30	16.83	4.90	15.30	18.32
Fat	30	20.09	5.44	18.44	22.25
Dry matter	29	41.41	3.19	39.16	44.11
Moisture	30	58.71	2.32	55.89	60.93
Salt	30	0.91	15.28	0.63	1.25
pH	30	5.30	0.66	5.22	5.37

[1]CY_{t1} = weight of curd after 1 h as a percentage of weight of the milk processed; CY_{t48} = weight of curd after 48 h as a percentage of weight of the milk processed; CY_{t168} = weight of curd after 168 h as a percentage of weight of the milk processed; $REC_{PROTEIN}$ = protein of the curd as a percentage of the protein of the milk processed; REC_{FAT} = fat of the curd as a percentage of the fat of the milk processed.

dependent on different cheese-making procedures (e.g., temperature and time adopted in different steps), results of the present study were consistent with findings of Bland et al. (2015) in Holstein Friesian bulk milk. Curd composition after 7 d of ripening exhibited average protein, fat, DM, and moisture content of 16.83%, 20.09%, 41.41%, and 58.71%, respectively, with CV ranging from 2.32% for moisture to 5.44% for fat (Table 2). Greater percentages were reported by Cipolat-Gotet et al. (2013) for all the curd composition traits.

Effects of β-CN Variants on Detailed Milk Protein Fractions

Results reported in Table 3 demonstrate that the effect of β-CN pools significantly contributed to the variation of milk protein profiles by altering quantities and relative proportions. The only exceptions are α_{S1}-CN and α-LA, which remained constant regardless of the β-CN pool. The effects of protein contents also significantly affected the amount (except for κ-CN) and proportion (except for α_{S1}-CN) of casein fractions, whereas the effect of fat content accounted for most of the variation in WP, with highest *F*-values for α-LA and β-LG in regard to both amount and proportion.

The LSM of detailed milk protein fractions for the different β-CN pools are reported in Table 4. An opposite trend was observed between casein and WP fractions: pools 1 and 2 (≥75% A1 milk) reported up to 2% higher casein percentage than pools 4 and 5 (≤25% A1 milk; $P < 0.001$) and, conversely, a lower percentage of total WP (5.78% and 6.54% for pool 1 and pool 5, respectively; $P < 0.001$). This pattern was consistent with previous findings in individual milk, in which an association between β-CN genotype A2A2 and greater WP concentration was observed (Bisutti et al., 2022). More specifically, in the present study, differences in total WP (both in amount and in percentage) were attributed to the β-LG fraction, which showed a significantly greater amount ($P < 0.05$) in pool 5 (5.03 mg/mL) compared with pool 1 (4.32 mg/mL). Comparison with the literature is difficult because association of β-CN variants with detailed milk protein fractions has been reported almost exclusively for individual milk. Nevertheless, knowledge about individual genotypes is helpful in understanding the results obtained at the milk processing plant level. Among casein fractions, the effects of β-CN variants on the relative concentrations of other protein fractions are not consistent in the literature. For example, some studies report that the presence of β-CN A2 is associated with greater amounts of β-CN (Heck et al., 2009), and others have observed the same polymorphism associating with lower relative concentration (Morris et al., 2005; Bisutti et al., 2022). In the present study, no clear influence of the β-CN allele on its absolute amount was observed; however, in accordance with Morris et al. (2005) and Bisutti et al. (2022), its percentage decreased as the amount of A2 milk increased ($P < 0.001$). Moreover, a contrasting result was observed for α-CN: the greatest amount of α_{S2}-CN occurred in pool 1 and 2, and no differences between pools were observed for α_{S1}-CN (Table 4).

Table 3. *F*-values and significance of fixed effects included in the model for detailed milk protein composition, cheese yield (CY), curd nutrient recoveries (REC) traits, and curd chemical composition

	Fixed effect			Random effect	
Trait[1]	β-CN pool	Protein content	Fat content	σ^2_{trial} [2] (%)	RSD[3]
Milk protein composition (mg/mL)					
Total CN	3.37*	5.03*	1.10	25.28	1.37
κ-CN	3.53*	1.98	0.02	37.87	0.29
α_{S2}-CN	9.96***	16.79***	0.48	35.47	0.12
α_{S1}-CN	1.42	5.23*	0.70	28.80	0.41
β-CN	3.82*	6.40*	1.03	32.28	0.49
Total WP	3.01*	0.03	7.16*	76.47	0.19
α-LA	1.01	0.08	1.98	66.60	0.04
β-LG	3.73*	0.11	8.50**	78.54	0.15
Milk protein composition (%)					
Total CN	8.13***	0.03	12.53**	0.00	0.29
κ-CN	9.87***	10.36**	2.83	12.59	0.10
α_{S2}-CN	16.06***	23.71***	0.79	10.40	0.10
α_{S1}-CN	3.37*	0.24	0.31	0.00	0.09
β-CN	6.38**	4.97*	2.64	0.00	0.09
Total WP	8.13***	0.03	12.53**	9.53	0.40
α-LA	5.00**	2.21	14.15**	4.24	0.09
β-LG	8.05***	0.41	13.36**	10.46	0.32
CY_{CURD} (%)					
CY_{t1}	13.18***	28.33***	5.34*	45.22	0.62
CY_{t48}	5.33**	22.45***	6.17*	32.80	0.68
CY_{t168}	3.58*	17.37***	3.01	30.69	0.61
Whey composition (%)					
Protein	5.58**	11.98**	0.76	39.69	0.02
Fat	6.20**	9.89**	2.57	15.06	0.06
Lactose	16.72***	2.16	17.39***	61.24	0.03
REC (%)					
$REC_{PROTEIN}$	5.95**	0.58	0.32	41.97	0.66
REC_{FAT}	6.41**	10.08**	4.83*	14.61	1.57
Cheese composition (%)					
Protein	1.18	7.99*	0.53	19.45	0.67
Fat	1.75	26.45***	10.28**	28.01	0.61
Dry matter	1.36	13.73**	4.98*	20.50	0.98
Moisture	1.65	15.94***	6.38*	21.28	0.95
Salt	0.48	2.09	0.05	36.66	0.11
pH	2.84	14.90**	6.49*	6.60	0.01

[1]WP = whey protein; CY_{t1} = weight of curd after 1 h as a percentage of weight of the milk processed; CY_{t48} = weight of curd after 48 h as a percentage of weight of the milk processed; CY_{t168} = weight of curd after 168 h as a percentage of weight of the milk processed; $REC_{PROTEIN}$ = protein of the curd as a percentage of the protein of the milk processed; REC_{FAT} = fat of the curd as a percentage of the fat of the milk processed.

[2]σ^2_{trial} = proportion of total variance accounted for by trial effect.

[3]RSD = residual standard deviation.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

These results partially agree with those of Vigolo et al. (2022c), who reported that the proportion between β-CN variants A1 and A2 in bulk milk in terms of amount did not affect the concentration of α_{S1}-CN. On the contrary, Heck et al. (2009) observed a relationship between the presence of the β-CN A1 variant, with greater concentrations of α_{S1}-CN and lower concentrations of α_{S2}-CN compared with β-CN A2. Finally, κ-CN was the fraction most affected by the different β-CN pools ($P < 0.001$) with a decrease of up to 2% (18.77 vs. 16.75% for pool 1 and pool 5, respectively; Table 4). Interestingly, the percentage of κ-CN gradually but significantly decreased when 25% of the pool or more constituted A2 milk (i.e., pool 2). This negative association between the relative concentration of κ-CN and the β-CN A2 allele is largely supported by literature (Heck et al., 2009; Bonfatti et al., 2011; Visker et al., 2011; Vigolo et al., 2022c). This association proves to be very enlightening in the quest to untangle the complex mechanisms that lead different β-CN genotypes to have different effects on milk processing and will be further discussed in the following section.

Effects of β-CN Variants on Cheese-Making Traits

All the effects included in the model were significant in explaining the variability of CY_{CURD} (Table 3). In particular, with the highest *F*-values, the effect of

Table 4. Least squares means and SE of detailed milk protein composition for 5 milk pools containing different mixes of β-CN A1 and A2 milks

Trait	β-CN pool[1] 1 100% A1	2 75% A1, 25% A2	3 50% A1, 50% A2	4 25% A1, 75% A2	5 100% A2	*P*-value
Protein composition (mg/mL of milk)						
Total CN	34.36 (0.74)^{ab}	34.87 (0.75)^{a}	32.88 (0.65)^{ab}	32.12 (0.70)^{ab}	31.18 (0.76)^{b}	0.0332
κ-CN	7.32 (0.18)^{ab}	7.37 (0.17)^{a}	6.90 (0.15)^{ab}	6.73 (0.16)^{ab}	6.53 (0.18)^{b}	0.0284
α_{S2}-CN	4.18 (0.07)^{ab}	4.20 (0.07)^{a}	3.93 (0.06)^{bc}	3.80 (0.07)^{cd}	3.61 (0.08)^{d}	0.0003
α_{S1}-CN	10.06 (0.23)^{a}	10.32 (0.23)^{a}	9.83 (0.20)^{a}	9.69 (0.21)^{a}	9.63 (0.23)^{a}	0.2687
β-CN	12.89 (0.29)^{ab}	13.04 (0.28)^{a}	12.23 (0.25)^{ab}	11.88 (0.27)^{ab}	11.62 (0.29)^{b}	0.0216
Total WP[2]	5.78 (0.18)^{b}	6.08 (0.17)^{ab}	6.13 (0.16)^{ab}	6.35 (0.17)^{ab}	6.54 (0.19)^{a}	0.0478
α-LA	1.46 (0.03)^{a}	1.50 (0.03)^{a}	1.47 (0.03)^{a}	1.49 (0.03)^{a}	1.52 (0.03)^{a}	0.4288
β-LG	4.32 (0.16)^{b}	4.58 (0.15)^{ab}	4.66 (0.14)^{ab}	4.86 (0.14)^{a}	5.03 (0.16)^{a}	0.0235
Protein composition (% of total protein in milk)						
Total CN	85.22 (0.27)^{a}	85.06 (0.28)^{a}	84.26 (0.24)^{ab}	83.75 (0.26)^{b}	83.12 (0.27)^{b}	0.0007
κ-CN	18.77 (0.43)^{a}	18.23 (0.42)^{b}	17.73 (0.41)^{c}	17.21 (0.42)^{cd}	16.75 (0.44)^{d}	0.0003
α_{S2}-CN	10.38 (0.07)^{a}	10.27 (0.07)^{a}	10.10 (0.06)^{ab}	9.91 (0.06)^{b}	9.59 (0.07)^{c}	<0.0001
α_{S1}-CN	24.93 (0.13)^{b}	25.1 (0.14)^{ab}	25.18 (0.12)^{ab}	25.27 (0.13)^{ab}	25.66 (0.13)^{a}	0.0333
β-CN	31.97 (0.15)^{a}	31.75 (0.16)^{a}	31.34 (0.14)^{ab}	31.00 (0.15)^{b}	30.97 (0.15)^{b}	0.0025
Total WP	14.78 (0.27)^{b}	14.94 (0.28)^{b}	15.74 (0.24)^{ab}	16.25 (0.26)^{a}	16.88 (0.27)^{a}	0.0007
α-LA	3.68 (0.04)^{b}	3.67 (0.04)^{b}	3.78 (0.03)^{ab}	3.84 (0.03)^{ab}	3.91 (0.04)^{a}	0.0083
β-LG	11.12 (0.23)^{c}	11.28 (0.24)^{c}	11.97 (0.21)^{b}	12.41 (0.22)^{ab}	12.89 (0.23)^{a}	0.0008

$^{a-d}$Means with different letters within a row differ significantly ($P < 0.05$).

[1]A1 and A2 milk was extracted from cows that were A1A1 or A2A2 for the β-CN genotype, respectively.

[2]WP = whey protein.

protein content accounted for the largest proportion of variation, followed by β-CN pools and fat. These were expected results, as protein and fat play a primary role in the coagulation and syneresis processes that characterize cheese-making. Furthermore, to mimic the most common cheese-making practices in Italy, which use a range of fat-to-protein ratios [e.g., for Parmigiano Reggiano protected designation of origin (PDO) cheese], the milk used for the present cheese-making trials was not standardized. Thus, the correction for protein and fat contents in the statistical model was necessary to evaluate the actual effects of β-CN A1 and A2 relative contents. Moreover, β-CN pools significantly contributed to the variation of whey composition and REC parameters but did not affect cheese composition. The total variance explained by the random effect of cheese-making session on cheese-making traits ranged from 6.60 to 61.24% for cheese pH and whey lactose, respectively.

Values of CY_{t1} significantly worsened ($P < 0.05$) with the addition of 50% or more A2 milk (pools 3, 4, and 5; Table 5). Accordingly, REC traits that are commercially used as indices of cheese-making efficiency reflected a significantly ($P < 0.01$) less efficient process moving from pool 2 to pool 5, with $REC_{PROTEIN}$ and REC_{FAT} decreasing by up to 3% and 5.5%, respectively. Whey composition, representing the unrecovered milk nutrients, confirmed this result, as significantly lower protein and fat losses in whey from 100% A1 milk ($P < 0.01$) were observed (Table 5). These results mirror the well-documented adverse effects of the A2 variant on milk coagulation properties, which are ascribed mainly to lower hydrophobicity, lower Ca and P contents, and greater micelle size (Ketto et al., 2017; Bisutti et al., 2022; Fernández-Rico et al., 2022; Vigolo et al., 2022c). Indeed, the difference in the primary structure of β-CN A1 and A2 results in differing behaviors in various environments, thereby affecting the technical and functional properties of milk. In particular, the Pro^{67} unique to β-CN A2 (found within its P,Q-rich region) promotes the formation of secondary polyproline II helical structures, a conformation that results in larger CN micelle size (Daniloski et al., 2022). The β-CN A1 and A2 variants therefore experience great differences in protein packing and organization inside the casein micelles. In addition, the lower concentration of κ-CN associated with β-CN A2, as earlier discussed, affects the variability of the micelle size, which decreases inversely with the κ-CN content (Hewa Nadugala et al., 2022). Although it is difficult to separate the direct effect exerted by β-CN primary structure and κ-CN contents, they both lead to greater average micelle size of A2 milk, which is unfavorable for milk processing (Glantz et al., 2010). Even if differences in casein micelle size might also be influenced by factors other than β-CN and κ-CN

Table 5. Least squares means and SE of cheese yield (CY), curd nutrient recoveries (REC) traits, whey composition, and curd chemical composition for 5 milk pools containing different mixes of β-CN A1 and A2 milks

	β-CN pool[2]					
Trait[1]	1 100% A1	2 75% A1, 25% A2	3 50% A1, 50% A2	4 25% A1, 75% A2	5 100% A2	*P*-value
CY_{CURD} (%)						
CY_{t1}	20.71 (0.41)^{a}	20.16 (0.39)^{a}	17.93 (0.35)^{b}	18.14 (0.38)^{b}	18.39 (0.42)^{b}	<0.0001
CY_{t48}	18.44 (0.39)^{a}	18.23 (0.39)^{ab}	16.82 (0.36)^{bc}	16.44 (0.37)^{c}	16.55 (0.40)^{bc}	0.0072
CY_{t168}	16.41 (0.35)^{a}	16.58 (0.34)^{a}	15.37 (0.30)^{a}	15.34 (0.32)^{a}	15.09 (0.35)^{a}	0.0276
REC (%)						
REC_{PROT}	81.76 (0.44)^{a}	80.43 (0.40)^{ab}	80.03 (0.35)^{b}	79.85 (0.38)^{b}	79.04 (0.42)^{b}	0.0020
REC_{FAT}	89.28 (0.79)^{a}	87.66 (0.91)^{ab}	85.03 (0.70)^{b}	84.33 (0.75)^{b}	85.19 (0.79)^{b}	0.0028
Whey composition (%)						
Protein	0.62 (0.01)^{b}	0.66 (0.01)^{ab}	0.67 (0.01)^{a}	0.68 (0.01)^{a}	0.71 (0.01)^{a}	0.0030
Fat	0.38 (0.03)^{b}	0.45 (0.03)^{ab}	0.54 (0.03)^{a}	0.57 (0.03)^{a}	0.54 (0.03)^{a}	0.0032
Lactose	5.07 (0.02)^{a}	5.05 (0.02)^{a}	4.97 (0.02)^{b}	4.89 (0.02)^{c}	4.90 (0.02)^{c}	<.0001
Cheese composition (%)						
Protein	16.46 (0.35)^{a}	16.42 (0.35)^{a}	17.09 (0.30)^{a}	17.23 (0.33)^{a}	16.86 (0.35)^{a}	0.3530
Fat	19.42 (0.34)^{a}	19.81 (0.34)^{a}	20.02 (0.30)^{a}	20.56 (0.32)^{a}	20.59 (0.35)^{a}	0.1861
Dry matter	40.59 (0.51)^{a}	40.83 (0.52)^{a}	41.59 (0.45)^{a}	42.06 (0.49)^{a}	41.99 (0.56)^{a}	0.2906
Moisture	59.55 (0.50)^{a}	59.37 (0.51)^{a}	58.49 (0.44)^{a}	57.95 (0.51)^{b}	58.28 (0.51)^{a}	0.2079
Salt	0.91 (0.07)^{a}	0.92 (0.07)^{a}	0.88 (0.07)^{a}	0.96 (0.06)^{a}	0.89 (0.07)^{a}	0.7471
pH	5.34 (0.01)^{a}	5.31 (0.01)^{ab}	5.29 (0.01)^{b}	5.29 (0.01)^{b}	5.30 (0.01)^{ab}	0.0567

$^{a–c}$Means with different superscript letters within a row differ significantly ($P < 0.05$).

[1]CY_{t1} = weight of curd after 1 h as a percentage of weight of the milk processed; CY_{t48} = weight of curd after 48 h as a percentage of weight of the milk processed; CY_{t168} = weight of curd after 168 h as a percentage of weight of the milk processed; $REC_{PROTEIN}$ = protein of the curd as a percentage of the protein of the milk processed; REC_{FAT} = fat of the curd as a percentage of the fat of the milk processed.

[2]A1 and A2 milk was extracted from cows that were A1A1 or A2A2 for the β-CN genotype, respectively.

concentrations (specifically their genetic variants and post-translational modifications, cow genetics, protein content, farming practices, and environmental factors; Bijl et al., 2014), in the present study the influence of external factors was minimized by sampling only one farm within a short timeframe and selecting animals for a balanced κ-CN and β-LG phenotype. Because casein micelle size seems to affect only the initial step of cheese-making, this hypothesis is consistent with data obtained in the present study: differences in CY among β-CN pools are appreciable only 48 h after draining, whereas no significant differences between experimental treatments after 7 d of aging (CY_{t168}) were observed. A paucity of studies is available for the evaluation of β-CN genetic polymorphisms on cheese-making traits. Bisutti et al. (2022) evaluated the effects of A1 and A2 variants of β-CN from individual milk using a laboratory cheese-making procedure (9 mL/sample) and did not report any significant effects on CY_{CURD} and REC traits. Similarly, no significant differences were observed for cheese chemical composition after 7 d of ripening. It is important to emphasize that in the present study the gel cut time and size were standardized for all trials to simulate the dairy plant routine, which performed most of the cheese-making operations based on a pre-established schedule. However, cutting at the optimum curd firmness rate (which could be different for each processing) may influence the loss of fat and fines in the whey and, consequently, the final cheese yield (Fox et al., 2017).

CONCLUSIONS

To the best of our knowledge, this is the first study that has attempted to evaluate the effects of β-CN A1/A2 genetic polymorphism on cheese-making traits in field conditions. We demonstrated that the percentage of β-CN variants in bulk milk used in milk processing plants affects the relative proportion of protein fraction, which, in turn, is directly linked to changes in dairy processing. Recoveries in curd and losses in whey were not constant but were clearly influenced by genetic factors; the β-CN A1 variant was the more efficient variant. Overall, proportions of ≥75% A1 milk had favorable effects on CY_{t1} and CY_{t48}. In contrast, β-CN mixes did not affect the final cheese composition. Because the frequency of the β-CN A2 allele in Italy is greater than 60%, it is reasonable to assume that most of the milk processed by the Italian dairy industry is represented by pools 3 and 4 of the present study (50–75% A2 milk, respectively). According to the results of the present study, this may have negative effects on both milk coagulation and cheese-making traits. Considering that the percentage of A2A2 cows is increasing, technical and economic implications should be carefully evaluated.

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REFERENCES

Banks, J. M. 2007. Cheese yield. Pages 100–114 in Cheese Problems Solved. P. L. H. McSweeney, ed. Woodhead Publishing Ltd.

Benedet, A., C. L. Manuelian, M. Penasa, M. Cassandro, F. Righi, M. Sternieri, P. Galimberti, A. V. Zambrini, and M. De Marchi. 2018. Factors associated with herd bulk milk composition and technological traits in the Italian dairy industry. J. Dairy Sci. 101:934–943. https://doi.org/10.3168/jds.2017-12717.

Bijl, E., R. de Vries, H. van Valenberg, T. Huppertz, and T. van Hooijdonk. 2014. Factors influencing casein micelle size in milk of individual cows: Genetic variants and glycosylation of κ-casein. Int. Dairy J. 34:135–141. https://doi.org/10.1016/j.idairyj.2013.08.001.

Bisutti, V., S. Pegolo, D. Giannuzzi, L. F. M. Mota, A. Vanzin, A. Toscano, E. Trevisi, P. Ajmone Marsan, M. Brasca, and A. Cecchinato. 2022. The β-casein (CSN2) A2 allelic variant alters milk protein profile and slightly worsens coagulation properties in Holstein cows. J. Dairy Sci. 105:3794–3809. https://doi.org/10.3168/jds.2021-21537.

Bland, J. H., A. S. Grandison, and C. C. Fagan. 2015. Effect of blending Jersey and Holstein-Friesian milk on Cheddar cheese processing, composition, and quality. J. Dairy Sci. 98:1–8. https://doi.org/10.3168/jds.2014-8433.

Bonfatti, V., A. Cecchinato, G. Di Martino, M. De Marchi, L. Gallo, and P. Carnier. 2011. Effect of κ-casein B relative content in bulk milk κ-casein on Montasio, Asiago, and Caciotta cheese yield using milk of similar protein composition. J. Dairy Sci. 94:602–613. https://doi.org/10.3168/jds.2010-3368.

Caroli, A., P. Bolla, E. Budelli, G. Barbieri, and P. Leone. 2000. Effect of κ-casein E allele on clotting aptitude of Italian Friesian milk. Zootec. Nutr. Anim. 3:127–130.

Cipolat-Gotet, C., A. Cecchinato, M. De Marchi, and G. Bittante. 2013. Factors affecting variation of different measures of cheese yield and milk nutrient recovery from an individual model cheese-manufacturing process. J. Dairy Sci. 96:7952–7965. https://doi.org/10.3168/jds.2012-6516.

Cipolat-Gotet, C., A. Cecchinato, M. Malacarne, G. Bittante, and A. Summer. 2018. Variations in milk protein fractions affect the efficiency of the cheese-making process. J. Dairy Sci. 101:8788–8804. https://doi.org/10.3168/jds.2018-14503.

CLAL. 2022. Italy: Dairy sector. Accessed Sep. 7, 2022. https://www.clal.it/en/?section=quadro_italia.

Daniloski, D., N. M. D. Cunha, N. A. McCarthy, T. F. O'Callaghan, S. McParland, and T. Vasiljevic. 2021. Health-related outcomes of genetic polymorphism of bovine β-casein variants: A systematic review of randomised controlled trials. Trends Food Sci. Technol. 111:233–248. https://doi.org/10.1016/j.tifs.2021.02.073.

Daniloski, D., N. A. McCarthy, T. Huppertz, and T. Vasiljevic. 2022. What is the impact of amino acid mutations in the primary structure of caseins on the composition and functionality of milk and dairy products? Curr. Res. Food Sci. 5:1701–1712. https://doi.org/10.1016/j.crfs.2022.09.026.

Fernández-Rico, S., A. C. Mondragón, A. López-Santamarina, A. Cardelle-Cobas, P. Regal, A. Lamas, I. S. Ibarra, A. Cepeda, and J. M. Miranda. 2022. A2 milk: New perspectives for food technology and human health. Foods 11:2387. https://doi.org/10.3390/foods11162387.

Fox, P. F., T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney. 2017. Fundamentals of Cheese Science. Springer US.

Glantz, M., T. G. Devold, G. E. Vegarud, H. Lindmark Månsson, H. Stålhammar, and M. Paulsson. 2010. Importance of casein micelle size and milk composition for milk gelation. J. Dairy Sci. 93:1444–1451. https://doi.org/10.3168/jds.2009-2856.

Heck, J. M. L., A. Schennink, H. J. F. van Valenberg, H. Bovenhuis, M. H. P. W. Visker, J. A. M. van Arendonk, and A. C. M. van Hooijdonk. 2009. Effects of milk protein variants on the protein composition of bovine milk. J. Dairy Sci. 92:1192–1202. https://doi.org/10.3168/jds.2008-1208.

Hewa Nadugala, B., C. N. Pagel, J. K. Raynes, C. S. Ranadheera, and A. Logan. 2022. The effect of casein genetic variants, glycosylation and phosphorylation on bovine milk protein structure, technological properties, nutrition and product manufacture. Int. Dairy J. 133:105440. https://doi.org/10.1016/j.idairyj.2022.105440.

Hohmann, L. G., C. Weimann, C. Scheper, G. Erhardt, and S. König. 2021. Genetic diversity and population structure in divergent German cattle selection lines on the basis of milk protein polymorphisms. Arch. Anim. Breed. 64:91–102. https://doi.org/10.5194/aab-64-91-2021.

Ketto, I. A., T. M. Knutsen, J. Øyaas, B. Heringstad, T. Ådnøy, T. G. Devold, and S. B. Skeie. 2017. Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. Int. Dairy J. 70:55–64. https://doi.org/10.1016/j.idairyj.2016.10.010.

Lundén, A., M. Nilsson, and L. Janson. 1997. Marked effect of β-lactoglobulin polymorphism on the ratio of casein to total protein in milk. J. Dairy Sci. 80:2996–3005. https://doi.org/10.3168/jds.S0022-0302(97)76266-0.

Marziali, A. S., and K. F. Ng-Kwai-Hang. 1986. Effects of milk composition and genetic polymorphism on coagulation properties of milk. J. Dairy Sci. 69:1793–1798. https://doi.org/10.3168/jds.S0022-0302(86)80603-8.

Morris, C. A., S. M. Hickey, N. G. Cullen, C. G. Prosser, R. M. Anderson, and M. L. Tate. 2005. Associations between β-casein genotype and milk yield and composition in grazing dairy cows. N. Z. J. Agric. Res. 48:441–450. https://doi.org/10.1080/00288233.2005.9513678.

Niero, G., G. Visentin, S. Ton, M. De Marchi, M. Penasa, and M. Cassandro. 2016. Phenotypic characterisation of milk technological traits, protein fractions, and major mineral and fatty acid composition of Burlina cattle breed. Ital. J. Anim. Sci. 15:576–583. https://doi.org/10.1080/1828051X.2016.1250128.

Noni, I. D., R. J. FitzGerald, H. J. T. Korhonen, Y. L. Roux, C. T. Livesey, I. Thorsdottir, D. Tomé, and R. Witkamp. 2009. Scientific report of EFSA prepared by a DATEX working group on the potential health impact of β-casomorphins and related peptides. EFSA Sci. Rep. 231.

Sebastiani, C., C. Arcangeli, M. Torricelli, M. Ciullo, N. D'avino, G. Cinti, S. Fisichella, and M. Biagetti. 2022. Marker-assisted selection of dairy cows for β-casein gene A2 variant. Ital. J. Food Sci. 34:21–27. https://doi.org/10.15586/ijfs.v34i2.2178.

Stocco, G., C. Cipolat-Gotet, V. Gasparotto, A. Cecchinato, and G. Bittante. 2018. Breed of cow and herd productivity affect milk nutrient recovery in curd, and cheese yield, efficiency and daily production. Animal 12:434–444. https://doi.org/10.1017/S1751731117001471.

Sturaro, A., M. De Marchi, E. Zorzi, and M. Cassandro. 2015. Effect of microparticulated whey protein concentration and protein-to-fat ratio on Caciotta cheese yield and composition. Int. Dairy J. 48:46–52. https://doi.org/10.1016/j.idairyj.2015.02.003.

Vigolo, V., M. Franzoi, F. Cendron, G. Salvadore, M. Penasa, M. Cassandro, and M. De Marchi. 2022a. Characterization of the genetic polymorphism linked to the β-casein A1/A2 alleles using different molecular and biochemical methods. J. Dairy Sci. 105:8946–8955. https://doi.org/10.3168/jds.2022-22136.

Vigolo, V., G. Niero, M. Penasa, and M. De Marchi. 2022b. Effects of preservative, storage time, and temperature of analysis on detailed milk protein composition determined by reversed-phase high-performance liquid chromatography. J. Dairy Sci. 105:7917–7925. https://doi.org/10.3168/jds.2022-22069.

Vigolo, V., M. Franzoi, M. Penasa, and M. De Marchi. 2022c. β-Casein variants differently affect bulk milk mineral content, protein composition, and technological traits. Int. Dairy J. 124:105221. https://doi.org/10.1016/j.idairyj.2021.105221.

Visker, M. H. P. W., B. W. Dibbits, S. M. Kinders, H. J. F. van Valenberg, J. A. M. van Arendonk, and H. Bovenhuis. 2011. Association of bovine β-casein protein variant I with milk production and milk protein composition. Anim. Genet. 42:212–218. https://doi.org/10.1111/j.1365-2052.2010.02106.x.

Wedholm, A., L. B. Larsen, H. Lindmark-Månsson, A. H. Karlsson, and A. Andrén. 2006. Effect of protein composition on the cheese-making properties of milk from individual dairy cows. J. Dairy Sci. 89:3296–3305. https://doi.org/10.3168/jds.S0022-0302(06)72366-9.

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