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Casein retention in curd and loss of casein into whey at chymosin-induced coagulation of milk

Elin Hallén[1,*], Anne Lundén[2], Toomas Allmere[1] and Anders Andrén[1]

[1] Department of Food Science, Swedish University of Agricultural Sciences, Box 7051, 750 07 Uppsala, Sweden
[2] Department of Animal Breeding & Genetics, Swedish University of Agricultural Sciences, Box 7023, 750 07 Uppsala, Sweden

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Impact of milk protein composition on casein (CN) retention in curd during the milk coagulation process was studied using a model cheese making system. Individual milk samples from 110 cows in mid lactation of the Swedish Red and Swedish Holstein breeds with known genotypes of β-casein, κ-casein and β-lactoglobulin were defatted, coagulated with chymosin, subjected to syneresis and subsequent pressing simulated by centrifugation. The results indicated that κ-casein concentration of milk plays an important role in the curd formation process and initial syneresis (whey after cutting), whereas an increased CN ratio was associated with less casein in whey after simulated pressing. Increased κ-casein concentration of milk also characterized the milk samples with no measurable loss of casein in whey, compared with milk samples with casein lost in whey, both after cutting and after simulated pressing. Concentrations of α_{s1}-casein, β-casein, and total casein in milk were positively associated with fresh curd yield, which showed a strong correlation with amount of casein retained in curd. No effect of protein genotype on fresh curd yield or casein in whey was found. The β-lactoglobulin BB genotype was associated with increased casein retention in curd, most likely due to the association of this genotype with CN ratio.

Keywords: Milk coagulation, milk protein composition, whey composition, fresh curd yield, casein loss, *κ*-casein, *β*-lactoglobulin.

Cheese yield is largely determined by the concentrations of protein, particularly casein, and fat in milk (Lawrence, 1993). An important indicator of milk suitable for cheese production would therefore be casein number (weight percentage of casein to total protein). It is, however, possible that rather than regarding caseins as a homogenous group, some of the caseins may play a more significant role for the cheese yield, i.e. there may be room for improvement of the casein composition. Rather than analysing concentration of the various milk proteins, many studies have been looking for associations between polymorphisms in the milk protein coding genes and milk coagulation, thereby addressing the impact of structural variation of allelic milk protein variants. Studies have shown that selection for genetic variants of milk proteins could be an option to change the protein composition of milk (Ikonen et al. 1997; Lodes et al. 1997; Bobe et al. 1999; Hallén et al. 2008), thereby possibly obtaining improved processing properties resulting in a higher dairy product yield and quality (Rahali & Ménard, 1991; Boland & Hill, 2001; Ikonen et al. 1999).

Several of the studies on the detailed protein composition and cheese making potential of milk have focused on rheological properties such as curd firmness (Storry et al. 1983; van den Berg et al. 1992; Ikonen et al. 1997; Jõudu et al. 2008), whereas fewer have also related the protein composition to actual cheese yield (Ikonen et al. 1999; Auldist et al. 2004; Wedholm et al. 2006). Curd firmness at cutting has been positively associated with cheese yield (Bynum & Olson, 1982; Riddell-Lawrence & Hicks, 1989), but in practice gel firmness may have minor consequences for cheese yield as long as the coagulation process is relatively consistent (Lucey & Kelly, 1994). Hurtaud et al. (1995) showed that actual cheese yield (Camembert) was more accurately predicted by laboratory scale cheese yield than through coagulation measures obtained by Formagraph. Being the main constituents of cheese, measures of caseins lost to the whey may be more relevant for actual cheese yield than rheological properties.

The present work studied how the milk protein composition and the genetic polymorphism of milk proteins

*For correspondence; e-mail: elin.hallen@lmv.slu.se

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were associated with the retention of casein in curd at chymosin-induced coagulation after syneresis and simulated pressing of the curd.

Materials and Methods

Milk samples

Individual morning milk samples were collected during five non-consecutive months between November 2003 and March 2005 from 110 cows in the experimental dairy herd of the Swedish University of Agricultural Sciences (Jälla, Uppsala, Sweden). Seventy cows were of the Swedish Red breed (SRB) and 40 of the Swedish Holstein breed (SLB). Of the SRB cows, 37 belonged to a selection line for high milk fat percentage (SRB H) and 33 to a selection line for low milk fat percentage (SRB L), but with equivalent total milk energy production in both lines. One milk sample from each cow was analysed. To reduce effects of lactation stage or mastitis, the sample was collected when the cow was in lactation week 10–35 and an upper limit was set for somatic cell count (SCC $\leqslant$250,000). Samples were cooled directly after milking and kept at 4 °C. Fat, protein and lactose concentration was analysed by mid-infrared spectroscopy (MilkoScan FT120, A/S Foss Electric, Hillerød, Denmark) and SCC by flow cytometry (Fossomatic 5200, A/S Foss Electric). Protein content and composition was analysed by reversed phase HPLC (RP-HPLC). Information regarding morning milk yield, parity number, lactation week and time of sampling was collected for each sample.

Typing for protein variants

Typing for variants of the β-CN (A^1, A^2, A^3, B) and κ-CN (A, B, E) genes was carried out by Pyrosequencing™ (Biotage AB, Uppsala, Sweden) as previously described by Hallén et al. (2007).

Milk sample and chymosin preparation

On the day of sample collection, fresh milk samples were pre-warmed (30 °C, 30 min) and defatted by centrifugation (2465 ***g***, 3 °C, 25 min) (Centrifuge 5810R, Eppendorf AG, Hamburg, Germany). Samples were then kept refrigerated. Chromatographically pure chymosin (Andrén et al. 1980), 174,000 International Milk Clotting Units/g, was used to prepare a working solution of 1·5 mg chymosin/ml in a 0·1 M-phosphate buffer (pH 5·7).

Curd and whey preparation

Milk coagulation was performed the day after sample collection, using a procedure similar to Hurtaud et al. (1995) and Othmane et al. (2002). Defatted milk samples (10 ml) were incubated in test tubes in a shaking water bath (30 °C, 30 min). Chymosin solution (25 µl) was added to each sample, after which they were vortexed and incubated for another 30 min. The coagulum was vertically cut in four equally sized sections, using a four-edged knife specifically made to fit the tubes. After another 30 min incubation the tubes were removed from the water bath and a 300 µl sample of the expelled whey was withdrawn by pipette (Whey1). Pressing of the curd was simulated by centrifugation at room temperature (1258 ***g***, 15 min) (Centrifuge 5810R, Eppendorf AG). Expelled whey was decanted by a standardized protocol and measured by weighing (Whey2). Fresh curd yield (Yf) was calculated as the weight difference between the initial milk sample and the expelled whey, and expressed as grams of curd per 100 g milk. The repeatability was 0·8 for the Whey2 measurement, calculated according to International Standardization Organization guidelines (ISO, 2005) at an initial trial conducted on 30 samples analysed in triplicate. Samples of defatted milk, Whey1 and Whey2 were stored at −80 °C pending analysis of protein composition by RP-HPLC.

HPLC analysis

Skim milk and whey samples were analysed for milk protein composition by RP-HPLC. The method, including equipment, reagents and buffers, was as described by Hallén et al. (2008). Concentration of 'major proteins' was calculated as the sum of concentrations of the individual proteins (α_{s1}-CN, α_{s2}-CN, β-CN, κ-CN, β-lg and α-lactalbumin; α-la). Casein (CN) ratio was calculated as the sum of individual concentrations of the analysed caseins (α_{s1}-CN, α_{s2}-CN, β-CN and κ-CN) divided by concentration of 'major proteins'. Casein concentrations in Whey1 and Whey2 (CNwhey1 and CNwhey2) were calculated as the sum of individual concentrations of the analysed caseins in the respective whey fraction. Casein retention in curd (retCN) was calculated as the weight difference between total amount of casein in the initial milk sample and total amount of casein in Whey2 expelled from this milk sample.

Statistical analysis

Effects of milk protein composition of the original milk on CNwhey1, CNwhey2 and Yf were analysed using the Mixed procedure of the statistical software SAS (SAS Institute Inc, Cary, USA). The time of sampling parameter was entered as a random effect. Fixed effects of parity, lactation week, breed, β-lg genotype, β-/κ-CN genotype, milk yield, SCC, initial concentration of fat and lactose were not significant and dropped from the subsequent analyses. Models were also run where the individual protein concentrations were exchanged with concentration of major proteins, total casein, and CN ratio, respectively.

The following statistical model was used:

$$y_{ijklmnop} = \mu + b_1\alpha_{s1}CN_i + b_2\alpha_{s2}CN_j + b_3\beta CN_k + b_4\kappa CN_l + b_5\beta lg_m + b_6\alpha la_n + sampl_o + \varepsilon_{ijklmnop}$$

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Table 1. Mean composition (with standard deviations) of morning milk samples and of the whey after cutting (Whey1) and after simulated pressing (Whey2), respectively, along with proportion of protein retained in the curd, at coagulating individual defatted milk samples with chymosin

	Milk (g/l) (n=110)		Whey1 (g/l) (n=106)¶		Whey2 (g/l) (n=106)¶		Retained in curd (%) (n=106)¶	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Milk yield, kg	20·1	4·6						
Protein	32·9	3·3						
Fat	34·9	6·9						
Lactose	48·9	2·0						
ln SCC	3·60	0·87						
Major proteins†	32·54	3·75	7·97	5·24	8·48	4·00	72·8	11·8
Casein‡	26·21	3·19	2·05	5·01	2·30	3·23	93·3	9·2
Whey protein§	6·32	1·59	5·91	1·34	6·18	1·49	26·7	10·5
α_{s1}-casein	8·66	1·12	0·71	1·86	0·78	1·20	93·3	10·3
α_{s2}-casein	1·42	0·62	0·06	0·20	0·03	0·11	97·7	8·6
β-casein	12·62	1·72	0·89	2·30	1·22	1·66	92·7	9·8
κ-casein	3·51	1·15	0·40††	0·71	0·27††	0·36	93·6	7·7
β-lactoglobulin	5·43	1·46	5·09	1·30	5·22	1·44	26·7	11·4
α-lactalbumin	0·99	0·24	0·82	0·17	0·96	0·18	25·7	14·7

† α_{s1}-CN+α_{s2}-CN+β-CN+κ-CN+β-LG+α-LA
‡ α_{s1}-CN+α_{s2}-CN+β-CN+κ-CN
§ β-LG+α-LA
¶ Non-coagulating samples excluded
††para-κ-casein

where:

$y_{ijklmnop}$=CNwhey1 or CNwhey2 or Yf for cow *ijklmnop*
μ is the general mean
b_1, b_2, …b_6=regression coefficients of $y_{ijklmnop}$ on the respective protein concentration in milk
$\alpha_{s1}CN_i$, $\alpha_{s2}CN_j$, βCN_k, κCN_l, βlg_m, αla_n=individual protein concentration *i/j/k/l/m/n* in milk of cow *ijklmnop*
$sampl_o$=random effect of time of sampling *o* (*o*=1, 2,…5)
$\varepsilon_{ijklmnop}$=random residual effect

The above model was also used in the analysis of CNwhey1 and CNwhey2 as categorical traits (no measurable casein in whey=0, casein in whey=1) using the GLIMMIX procedure of SAS (SAS Institute Inc, Cary).

Effects of genetic polymorphism of milk proteins on protein composition of milk were analysed using the general linear model (GLM) procedure (SAS Institute Inc, Cary). Due to the close genetic linkage between the casein loci (Ferretti et al. 1990; Threadgill & Womack, 1990), aggregate β-/κ-CN genotype was entered as a fixed effect in the model. Genotypes comprising less than three cows were dropped from the subsequent analysis (see Table 3). The group variable specified cow breed and selection line and consisted of three classes: SRB H, SRB L and SLB. Parity was grouped into four classes; first, second, third, and fourth or higher parity. Fixed effects of milk yield, SCC, fat and lactose concentration were not significant and dropped from the subsequent analyses.

$$y_{ijklmno} = \mu + \beta/\kappa CN_i + \beta\, lg_j + group_k + parity_1 + sampl_m + b_1\, lactwk_n + \varepsilon_{ijklmno}$$

where:

$y_{ijklmno}$=milk protein variable for cow *ijklmno*
$\beta/\kappa CN_i$=fixed effect of β-/κ-CN genotype *i* (*i*=1, 2, …10; see Table 3)
βlg_j=fixed effect of β-lg genotype *j* (*j*=AA, AB, BB)
$group_k$=fixed effect of group *k* (*k*=SRB H, SRB L, SLB)
$parity_l$=fixed effect of parity *l* (*l*=1, 2, …4)
$sampl_m$=fixed effect of time of sampling *m* (*m*=1, 2, …5)
b_1=regression coefficient of $y_{ijklmno}$ on lactation week
$lactwk_n$=lactation week *n* of cow *ijklmno*

Results and Discussion

Means and measures of variation for gross composition of milk and for detailed protein composition of milk, Whey1, and Whey2 are given in Table 1. About one third of the Whey1 and Whey2 samples, respectively, contained no measurable amounts of casein and the majority contained <2 g/l, whereas 6% of the Whey1 samples and 3% of the Whey2 samples contained >10 g/l (Fig. 1). Although mean levels of casein were similar in Whey1 and Whey2 (Table 1), a higher proportion of the Whey1 samples contained less than 2 g casein/l compared with Whey2 (90% and 60%, respectively). The casein lost in whey at cheese making has been reported to be around 1 g/kg milk (Lucey & Kelly, 1994). In this trial mean casein loss in Whey2 was 1·8 g/kg milk (SD 2·5, range 0–13·9 g/kg). Of the 110 milk samples, four (3·6%) did not aggregate to form a curd within the set time of the coagulation experiment (>1 h).

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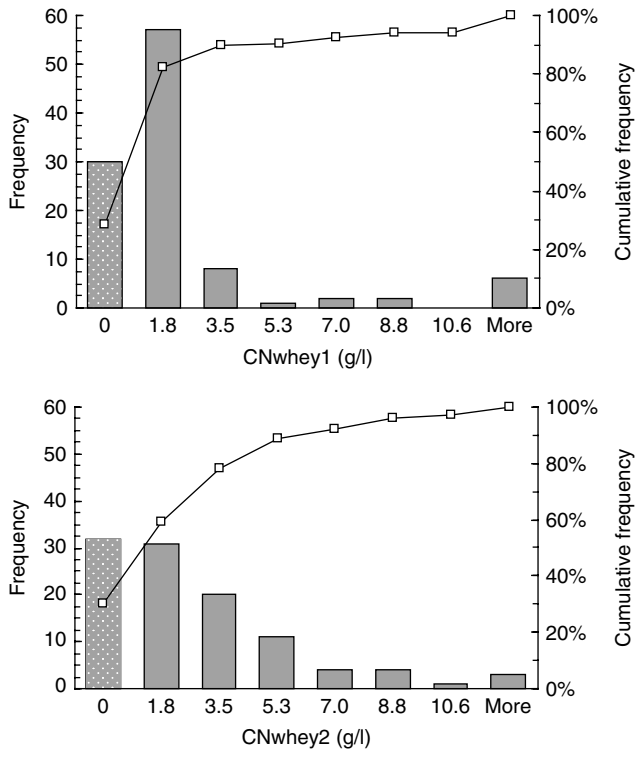

Fig. 1. Distribution of values for casein content of whey after cutting (CNwhey1) and after simulated pressing (CNwhey2), respectively, at chymosin-induced coagulation of individual defatted milk samples (n=106).

These non-coagulating samples were not included in further calculations.

Mean fresh curd yield was 23·9 g/100 g milk (SD 4·3, range 14·5–34·1 g/100 g). Similar results were reported by Othmane et al. (2002), and Hurtaud et al. (1995), who also analysed small volumes of milk (26·5% in 10 ml, and 30·6% in 30 ml, respectively). Average protein content of whey was 0·85% (8·48 g/l), equivalent to 27·3% of the protein originally present in the milk (Table 1). These percentages correspond well to the 1% and 30·7%, respectively, reported by Ng-Kwai-Hang et al. (1989). About 27% of the protein in whey consisted of casein, which can be considered to constitute yield losses. Of the casein and major proteins from the original milk, 93·3% and 72·8%, respectively, was retained in the curd. In the production of cheddar cheese, recoveries of casein ranging from 93 to 99% and of total protein of approximately 74% have been reported (Lucey & Kelly, 1994).

Influence of protein composition of milk on CNwhey1 and CNwhey2 is given in Table 2. A higher concentration of κ-CN in milk was associated with lower levels of casein in whey after cutting, CNwhey1, whereas concentrations of the other individual proteins, total casein, or major proteins showed no significant effect. Milk with high κ-CN content has been shown to contain smaller casein micelles compared with milk with low κ-CN content (Niki et al. 1994; Walsh et al. 1998). This allows for a more compact and uniform arrangement of the gel network, which may reduce losses in whey by an improved entrapping ability (Nuyts-Petit et al. 1997; Walsh et al. 1998). A faster coagulation reaction of samples with a high κ-CN concentration (van den Berg et al. 1992; Nuyts-Petit et al. 1997) and thus firmer curd at cutting might have reduced the losses of casein in Whey1, as previously suggested by Ng-Kwai-Hang et al. (1989). CNwhey2, i.e. losses after simulated pressing, was negatively associated with CN ratio and positively associated with levels of major proteins and α-la concentration in milk, whereas concentrations of individual caseins or total casein, respectively, were not significant (Table 2). Somewhat contrasting results were reported by Verdier-Metz et al. (2001), who found protein losses in whey to be lower in milk with high protein (and fat) content, although casein content was not analysed. Consequently, according to our results increasing the overall protein content of milk may not have the desired effect, whereas a high proportion of casein to total protein would decrease casein losses in whey. Why κ-CN concentration was found significant for casein loss in Whey1 as described above, but not in Whey2, might have been due to additional factors affecting casein loss as the pressing force was introduced. Samples with CNwhey1 above 2 g/l were found to have CNwhey2 values lower than this (data not shown), suggesting that casein aggregates in the whey were caught in the coagulum during simulated pressing. Further, 50% of the samples with CNwhey1 below 2 g/l exhibited increased CNwhey2 values compared with CNwhey1 (data not shown), indicating weak gels losing casein during simulated pressing. Further analyses would have been necessary to explain these occurrences.

Of the protein components in milk, only concentrations of α_{s1}-CN and β-CN were positively associated with Yf (Table 2). This may have been due to the lower accuracy of the HPLC method when analysing small protein fractions, resulting in comparatively large standard errors of the estimates for α_{s2}-CN and κ-CN. Marziali & Ng-Kwai-Hang (1986) found α_s-CN (α_{s1}-CN+α_{s2}-CN) and β-CN concentrations to have a positive effect on actual cheese yield, whereas Wedholm et al. (2006) in addition found a positive effect of κ-CN concentration. It has also been reported that whereas total concentration of casein in milk is positively related to cheese yield, variation in casein composition is not (Christian et al. 1999). This is supported by the present results, where total casein and major proteins concentrations were positively associated with Yf (Table 2), whereas CN ratio and relative proportions of the different caseins showed no association (data not shown).

Aggregate β-/κ-CN genotype was not found to have effect on Yf, CNwhey1 or CNwhey2. However, β-/κ-CN genotype was associated with concentration of κ-CN in milk (Table 3) (see Hallén et al. 2008). The BB genotype of β-lg was associated with increased retCN compared with

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Table 2. Regression coefficients (±standard error) of casein concentration in whey after cutting (CNwhey1) and after simulated pressing (CNwhey2), and of fresh curd yield (Yf) at chymosin-induced coagulation of defatted milk on milk protein composition

	CNwhey1 (g/l)			CNwhey2 (g/l)			Yf (g/100 g milk)		
Parameter	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
Major proteins†, g/l	0·18	±0·14	n.s.¶	0·24	±0·08	**	0·60	±0·10	***
Casein‡, g/l	0·19	±0·16	n.s.	0·16	±0·09	n.s.	0·78	±0·11	***
Casein ratio§	−4·07	±12·63	n.s.	−16·59	±7·01	*	15·8	±10·1	n.s.
α_{s1}-casein, g/l	0·90	±0·68	n.s.	0·12	±0·36	n.s.	1·29	±0·45	**
α_{s2}-casein, g/l	−0·50	±0·82	n.s.	−0·53	±0·43	n.s.	0·73	±0·54	n.s.
β-casein, g/l	0·18	±0·42	n.s.	0·08	±0·22	n.s.	0·69	±0·28	*
κ-casein, g/l	−0·96	±0·46	*	0·27	±0·24	n.s.	0·27	±0·31	n.s.
β-lactoglobulin, g/l	0·26	±0·39	n.s.	0·37	±0·21	n.s.	−0·08	±0·26	n.s.
α-lactalbumin, g/l	0·92	±2·53	n.s.	3·51	±1·32	**	1·24	±1·67	n.s.

† α_{s1}-CN+α_{s2}-CN+β-CN+κ-CN+β-LG+α-LA
‡ α_{s1}-CN+α_{s2}-CN+β-CN+κ-CN
§ (α_{s1}-CN+α_{s2}-CN+β-CN+κ-CN)/(α_{s1}-CN+α_{s2}-CN+β-CN+κ-CN+β-LG+α-LA)
¶ not significant
* $P<0·05$; ** $P<0·01$; ** $P<0·001$

Table 3. Least squares means (±standard error) for effect of β-lactoglobulin (β-LG) genotype on casein ratio and β-LG concentration, and for effect of aggregate β-/κ-casein (β-/κ-CN) genotype on κ-CN concentration in individual milk samples

Genotype	n†	Casein ratio‡	β-LG (g/l)
β-LG AA	17	$0·79^{a}$±0·007	$6·07^{a}$±0·24
β-LG AB	59	$0·79^{a}$±0·005	$5·70^{a}$±0·17
β-LG BB	34	$0·86^{b}$±0·005	$3·46^{b}$±0·17
			κ-CN (g/l)
β-/κ-CN A^1A^1/AA	6		$3·52^{a,b}$±0·35
β-/κ-CN A^1A^1/AB	4		$3·93^{a,c}$±0·44
β-/κ-CN A^1A^1/AE	4		$3·35^{a,b}$±0·42
β-/κ-CN A^1A^1/EE	1		—
β-/κ-CN A^1A^2/AA	26		$3·38^{a}$±0·18
β-/κ-CN A^1A^2/AB	13		$4·06^{a,c}$±0·26
β-/κ-CN A^1A^2/AE	13		$2·89^{b}$±0·26
β-/κ-CN A^1A^2/BE	1		—
β-/κ-CN A^1B/AB	1		—
β-/κ-CN A^2A^2/AA	20		$3·14^{a,b}$±0·22
β-/κ-CN A^2A^2/AB	12		$3·54^{a}$±0·26
β-/κ-CN A^2A^2/AE	1		—
β-/κ-CN A^2A^2/BB	3		$5·06^{c}$±0·49
β-/κ-CN A^2B/AA	1		—
β-/κ-CN A^2B/AB	4		$4·86^{c}$±0·43

† Number of cows
‡ (α_{s1}-CN+α_{s2}-CN+β-CN+κ-CN)/(α_{s1}-CN+α_{s2}-CN+β-CN+κ-CN+β-LG+α-LA)
[a, b, c] values within column with differing letters in superscript are statistically significant ($P<0·05$)

AB in the present study ($P<0·01$, data not shown). This effect was probably due to the association of β-lg BB with CN ratio (Table 3), since if CN ratio was added to the model, β-lg genotype did not remain significant. Higher cheese yield has been associated with β-lg B in several other studies (Marziali & Ng-Kwai-Hang, 1986; Wedholm et al. 2006) possibly through its impact on CN ratio (Rahali & Ménard, 1991; van den Berg et al. 1992; Boland & Hill, 2001).

Fresh curd yield (Yf) was dependent on amount of casein available for curd formation, reflecting the milk casein content (Table 2), whereas there was no association between casein content of milk and casein content of whey. The retCN parameter, representing amount of curd forming casein, was therefore a better predictor of Yf ($R^2=0·60$). An increased casein retention of 0·1 g/l milk resulted in 1 g/l higher fresh curd yield in this study. There was also a high correlation between retCN and casein content of milk ($R^2=0·51$). The measure of CNwhey2 (casein loss in whey after simulated pressing) showed a weak association with Yf, as milk with a high casein concentration and a large loss of casein in whey could still result in a large yield, whereas milk with a low casein concentration exhibiting only a small loss would also give a small yield.

Aiming to keep the losses of casein in whey to a minimum, milk samples with no (measurable) losses of casein in whey could be considered desirable. In an attempt to find characteristics in the protein composition which might distinguish 'casein loss in whey' from 'no/negligible casein loss in whey' CNwhey1 and CNwhey2 were analysed as binary traits. The results showed that a higher concentration of κ-CN in milk reduced the risk of casein loss in whey, both after cutting (Whey1) and after simulated pressing (Whey2) ($P<0·05$, data not shown), whereas the other protein fractions analysed showed no association with casein loss. The result for κ-CN is consistent with previously reported positive effects of κ-CN concentration on milk coagulation and cheese yield (e.g. Storry et al. 1983; Rahali & Ménard, 1991; van den Berg et al. 1992; Ikonen et al. 1997; Walsh et al. 1998; Wedholm et al. 2006) and supports the possibility to select on protein

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genotype for improving the coagulation and thereby cheese yield potential of milk.

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