Interactions in Heated Skim Milk between Genetic Variants of β-Lactoglobulin and κ-Casein

Toomas Allmere,*,† Anders Andrén,† Anne Lundén,‡ and Lennart Björck†

Department of Food Science and Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Box 7051, S-750 07 Uppsala, Sweden

Bovine skim milk samples with different phenotypes of κ-casein (AB and BB; κ-cn) and β-lactoglobulin (AA, AB, and BB; β-lg) were heat-treated at 90 °C for different times (1−10 min). The residual native whey proteins in the ultracentrifugal supernatants were determined by fast performance liquid chromatography using a MonoQ HR5/5 column at pH 6.2, and the loss of native β-lg was recorded. The rate of heat-induced loss of native β-lg, expressed as the inverted half-life ($1/t_{1/2}$) of the reaction, was calculated. The reaction did not follow true first- or second-order kinetics, varying between the different genetic combinations. The highest $1/t_{1/2}$ values were found in milk from cows homozygous for κ-cn B or β-lg B. The same was true after adjustment for differences in casein number. Both the β-lg and κ-cn genetic variants were found to significantly influence the heat-induced aggregation reaction; with β-lg having the greatest effect. Statistical analysis showed that the two loci for β-lg and κ-cn accounted for more than half of the phenotypic variance in the experimental groups.

Keywords: *β-Lactoglobulin; κ-casein; casein micelles; heat treatment; genetic polymorphism*

INTRODUCTION

A correlation has been demonstrated between bovine milk protein phenotypes and milk composition and yield (McLean et al., 1984; Ng-Kwai-Hang et al., 1984, 1986, 1987; Kroeker et al., 1985a,b; Aleandri et al., 1990; van den Berg et al., 1992). The effects of genetic polymorphism on cheese-making properties of milk have also been studied thoroughly, and favorable effects of κ-casein (κ-cn) B have been demonstrated (Schaar, 1984; Schaar et al., 1985; Marziali and Ng-Kwai-Hang, 1986; Jakob, 1993). However, little information is available about any effects of genetic milk protein variants on the quality of other milk products. During the manufacture of liquid fermented milk products, milk is heated to achieve an aggregation of whey proteins with casein micelles. This results in increased water holding capacity and an improved consistency of the milk gel, which is directly related to the organoleptic properties of the final product. The aggregation is assumed to have a dual mechanism. It is suggested to be partially due to the formation of intermolecular disulfide bonds between β-lactoglobulin (β-lg) and κ-cn (Pearse et al., 1987; Mottar et al., 1989) and partially due to the hydrophobic bonds involved, especially in the initiating phase of the reaction (Smits and Brouwershaven, 1980; Haque and Kinsella, 1987; Parnell-Clunies et al., 1988). Doi et al. (1983) suggested that both disulfide−thiol interchange and hydrophobic interactions are involved in the κ-cn/β-lg complex formation. The exact mechanism of the aggregation between β-lg and casein micelles is, however, not yet fully understood.

The aggregation of whey proteins with casein micelles during heat treatment is influenced by the different genetic variants of the milk proteins. McKenzie et al. (1971) showed that κ-cn reacts more rapidly with β-lg B than with β-lg A in cacodylate buffer solutions at pH 6.6 and 74 °C. These findings were later supported by Hillier and Lyster (1979), Dannenberg and Kessler (1988a,b), and Parnell-Clunies (1988) during heat treatment of milk.

However, relatively few data are available regarding the heat interactions between defined genetic variants of κ-cn and β-lg in milk. In a previous publication (Allmere et al., 1997), it was shown that in skim milk containing κ-cn A, the concentration of native β-lg B in whey decreased more rapidly during heating at 90 °C than that of β-lg A. In this paper, additional data are presented on the heat-induced interactions between κ-cn and β-lg in skim milk regarding the other six possible combinations of genetic variants A and B of κ-cn and β-lg, respectively.

MATERIALS AND METHODS

Milk Samples. Skim milk from individual cows, that is, Swedish Red and White breed ($n = 41$) and Swedish Friesian breed ($n = 15$), was collected from the Experimental Dairy Farm of the Swedish University of Agricultural Sciences. To get a reasonable number of individuals with the κ-cn BB/β-lg BB combination, additional milk samples were collected from cows of Swedish Polled breed ($n = 9$) at other farms. This was due to rare occurrence of this phenotypic combination in the other two breeds.

The milk protein phenotypes were determined by isoelectric focusing within the pH interval 4.0−6.5 by Phastgel electrophoresis equipment (Pharmacia, Uppsala, Sweden) according to the method of Bovenhuis and Verstege (1989).

The phenotypes for α-lactalbumin (α-la) and α_{s2}-casein (α_{s2}-cn) were AA in all cows, whereas α_{s1}-casein (α_{s1}-cn) was represented by the phenotypes BB, BC, and CC and β-casein (β-cn) by the phenotypes A1A1, A1A2, A1A3, A1A5, A1B, A2A2, A2A5, and A2B.

* Author to whom correspondence should be addressed (email: toomas.allmere@lmv.slu.se).

† Department of Food Science.

‡ Department of Animal Breeding and Genetics.

S0021-8561(98)00290-8 CCC: $15.00
Published on Web 07/08/1998

Table 1. Number of Cows with the Different Combinations of the Genetic Variants of κ-cn and β-lg Used in the Experiments

	β-lg		
κ-cn	AA	AB	BB
AB	12	13	10
BB	9	9	12

Two samples from each cow were analyzed representing different lactation numbers and different stages of lactation. Only milk with a somatic cell count of <10 000 somatic cells/mL was used in the investigation.

Total protein, casein, and whey protein concentrations were determined for all of the skim milk samples by a Milkoscan 93 MIR instrument (A/S N. Foss Electric, Hillerød, Denmark). The whey protein concentration was determined after casein had been precipitated from skim milk by rennet. Casein number was calculated as the ratio between casein (casein = total protein − whey protein) and the total protein concentration.

Heat Treatment. Milk samples from the phenotypes AB and BB of κ-cn and AA, AB, and BB of β-lg were arranged into the six possible combinations (Table 1). The samples were treated and analyzed as described earlier by Allmere et al. (1997). Briefly, skim milk (5 mL) was heated rapidly to 90 °C in a shaken thermoblock and held at this temperature for between 0 and 10 min, at intervals of 1 min. Samples were cooled to 22 °C, and the nonaggregated whey proteins were separated from casein micelles by ultracentrifugation at 100000*g* and thereafter analyzed in duplicate by fast protein liquid chromatography (FPLC) (Pharmacia).

Calculation of Reaction Rates. The mean values of the concentration of native β-lg after different heating times for each genetic combination of κ-cn/β-lg were determined from the chromatograms and used for the kinetic calculations. For each of the genetic combinations, the apparent reaction order for the loss of native β-lg during heating was determined by plotting the mean logarithmic value of the β-lg concentrations (c_t) at each time versus the reaction rates (v_t). The slope of the line obtained from the plot, using the least-squares method for best fitting, was set equal to the reaction order. The half-life ($t_{1/2}$) of the reaction, that is, the loss of native β-lg, was derived from the plot c_t/c_0 versus time. The rate of the heat-induced loss of β-lg for each genetic combination of κ-cn/β-lg is expressed as the inverted half-life ($1/t_{1/2}$) of the reaction.

Statistical Calculations. The effects of different factors on the $1/t_{1/2}$ were statistically analyzed using the General Linear Model (GLM) procedure of SAS (1985). The following fixed effects were considered in the analysis: the genetic variants of α_{s1}-cn (only polymorphic in the Swedish Friesian breed), β-cn, κ-cn, and β-lg; the concentration of total protein, casein, whey protein, β-lg, α-la, lactose, and milk fat; casein number; somatic cell count; milk volume per milking; lactation week and number; breed; sire; sampling occasion. A test of the overall significance of the different factors was performed as an ordinary *F* test. Since none of the environmental effects, or α_{s1}-cn and β-cn, were found to significantly influence the $t_{1/2}$ value, the final model included only the genetic variants of κ-cn and β-lg. In an attempt to illustrate the relative contribution of κ-cn or β-lg to the variance of the $1/t_{1/2}$ values, this contribution was estimated as the relative reduction in mean square error (MSE) after κ-cn or β-lg was individually added to the model, according to

$$(MSE_{RM} - MSE_{FM})/MSE_{RM}$$

where MSE_{RM} represents the reduced model, without either κ-cn or β-lg phenotypes, and MSE_{FM} represents the full model including both phenotypes.

RESULTS

There was little variation in pH among the samples, all being within the range of pH 6.61−6.70 prior to heating. The average total protein concentration ranged from 3.03 to 3.73% (Table 2), the highest values in milk from cows with phenotype κ-cn BB/β-lg BB. The latter is most likely an effect of the large proportion of Swedish Polled cows in this phenotypic combination, a breed known to have a protein content in milk above the average of Swedish dairy breeds. The average casein concentrations of the groups were approximately the same, that is, between 2.78 and 2.81%, resulting in a low casein number for the milk with κ-cn BB/β-lg BB.

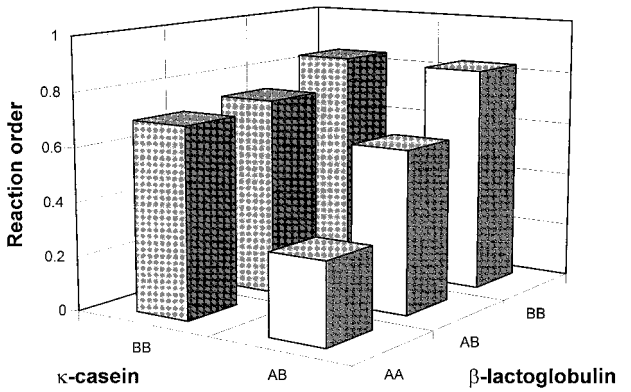

Figure 1. Reaction orders of the loss of native β-lg in skim milk containing different combinations of genetic variants of κ-cn and β-lg during heating at 90 °C.

The apparent reaction orders of the heat-induced loss of native β-lg ranged from 0.30 to 0.85 for the different milk protein combinations (Figure 1). Milk from cows homozygous for either κ-cn B or β-lg B had similar and the highest values, whereas the values of the reaction order for the κ-cn AB/β-lg AA milk were lowest. The reaction order for the combination κ-cn AB/β-lg AB was intermediate. Within κ-cn AB, the reaction order increased with the number of β-lg B alleles. The same trend was also seen within κ-cn BB, although less pronounced.

The heat-induced loss of β-lg for each genetic combination of κ-cn/β-lg, expressed as the inverted half-life ($1/t_{1/2}$) of the reaction, is shown in Table 2. To elucidate any influence of casein/whey protein ratio, the $1/t_{1/2}$ values were adjusted for casein number (Table 2). The indexed values of $1/t_{1/2}$ showed that milk containing κ-cn AB in combination with any of the three β-lg variants had similar $1/t_{1/2}$ values. In milk with κ-cn BB, on the other hand, the $1/t_{1/2}$ value increased with the number of B alleles of β-lg. After adjustment for the casein number, the indexed $1/t_{1/2}$ values were similar to the values without adjustment.

The *F* test showed two significant effects on $1/t_{1/2}$, that is, genetic variants of β-lg and κ-cn. The contributing factors test showed that the genotypes of β-lg and κ-cn explained 30.5 and 19.7% of variance, respectively. In the statistical analyses, the results from the combination κ-cn BB/β-lg BB were excluded, since 9 of 12 cows in this group were of the Swedish Polled breed, which was not represented in any of the other phenotypic combinations.

The reaction patterns of the heat-induced loss of native β-lg in the ultracentrifugal supernatants were found to vary among the different combinations of genetic variants (Figure 2). In the combination κ-cn BB/β-lg AA, the initial phase of the reaction was longer, resulting in a low value of $1/t_{1/2}$, although the loss of native β-lg was more rapid once the reaction had been initiated. The combinations κ-cn AB/β-lg AA and κ-cn

Table 2. Average Total Protein and Casein Number of Unheated Milk with Different κ-cn/β-lg Phenotypes[a]

κ-cn/β-lg	BB/AA	BB/AB	BB/BB	AB/AA	AB/AB	AB/BB
total protein concn, % (w/w)	3.18	3.19	3.73	3.24	3.03	3.43
casein number	0.78	0.78	0.75	0.76	0.82	0.81
$t_{1/2}$, s	340	305	270	295	280	280
$1/t_{1/2}$, s-1 × 10−3	2.94	3.28	3.70	3.39	3.57	3.57
$1/t_{1/2}$, indexed	1.00	1.11	1.26	1.15	1.21	1.21
$(1/t_{1/2})$/casein number	3.77	4.20	4.94	4.46	4.36	4.41
$(1/t_{1/2})$/casein number, indexed	1.00	1.11	1.31	1.18	1.16	1.17

[a] Inverted half-lives, $1/t_{1/2}$, of heat-induced loss of native β-lg in supernatants of skim milk ultracentrifuged (100000*g*) after heating at 90 °C are presented.

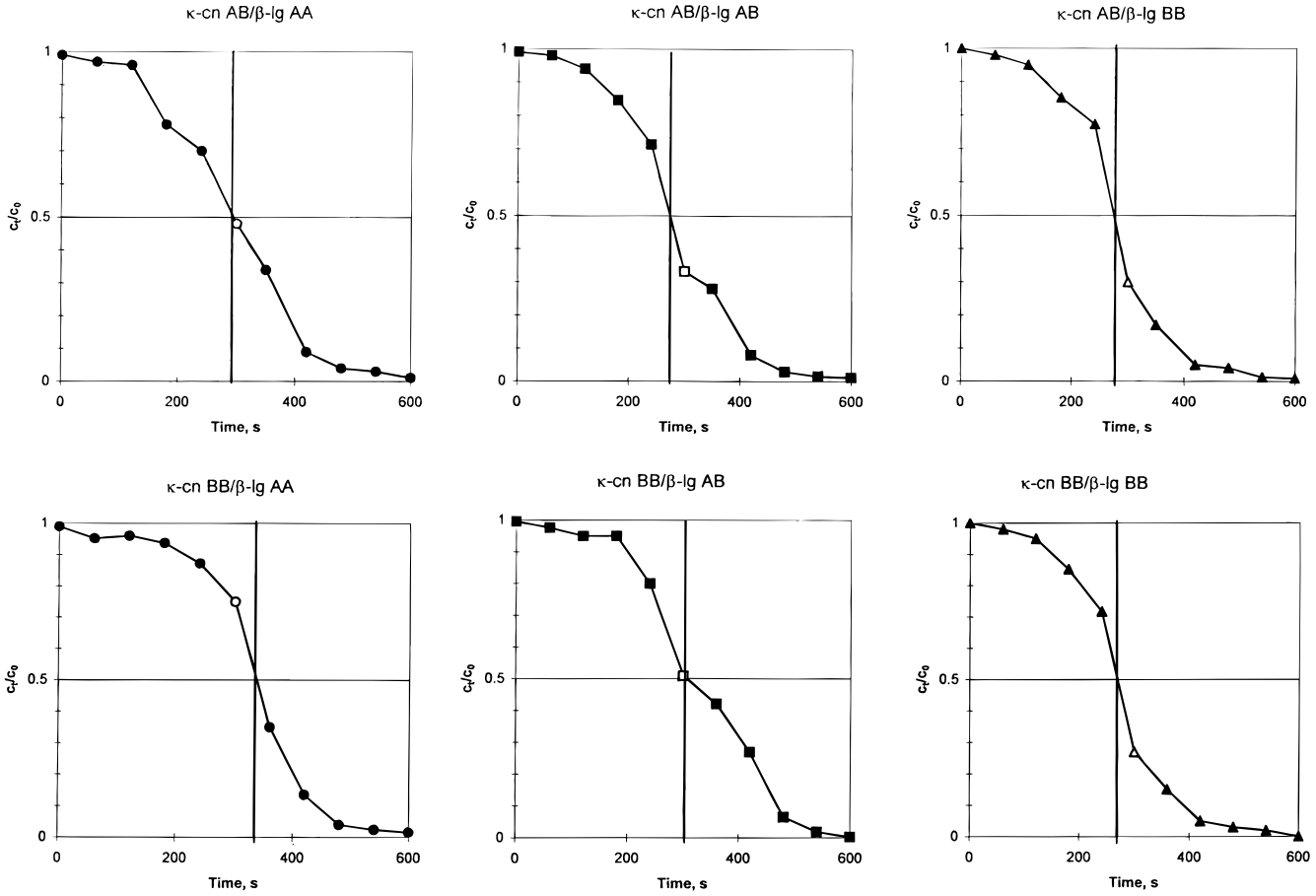

Figure 2. Loss of native β-lg (c_t/c_0) versus time (s) in skim milk containing different combinations of genetic variants of κ-cn and β-lg during heating at 90 °C. The amount of native β-lg in skim milk at 300 s is indicated with an open symbol (○).

BB/β-lg AB had both, compared to the other combinations, a shorter initial or final phase and a less rapid decrease of native β-lg.

DISCUSSION

The pH is believed to be one of the major factors influencing heat-induced reactions between milk proteins. Smits and Brouwershaven (1980) demonstrated that the proportion of β-lg that interacts with casein micelles decreases with increasing pH in the interval between 6.8 and 7.3. The small variation in pH of the milk samples in this investigation could thus be considered as insignificant and cannot be considered to be any major cause of the observed differences.

McKenzie et al. (1972) have demonstrated that the interaction between β-lg and κ-cn does not follow true first- or second-order kinetics. Later, Dalgleish (1990) proposed that the reaction follows pseudo-first-order kinetics. The broad range in the reaction orders found in this investigation (0.30−0.84) indicates that the heat-induced loss of native β-lg in skim milk does not follow a specific reaction order but is influenced by the genetic variants of both β-lg and κ-cn. The reaction orders were found to be similar in milk from cows either homozygous for β-lg B or homozygous for κ-cn B, whereas it increased with the number of β-lg B alleles in milk from cows heterozygous for κ-cn (AB). This is in analogy with our previous results for milk homozygous for κ-cn A (Allmere et al., 1997).

The reaction order of about 0.3 for β-lg AA and 0.8 for β-lg BB in milk containing κ-cn AB and reaction orders of 0.7−0.8 for the different genetic variants of β-lg in milk with κ-cn BB (Figure 1) are not in agreement with the reaction orders previously reported by Dannenberg and Kessler (1988a). They found only a small difference in reaction orders for the denaturation of β-lg A and β-lg B in skim milk, that is, 1.4 and 1.5, respectively. They, however, did not use milk samples

with known genetic variants of κ-cn, and this is a likely explanation for the discrepancy in results.

The rate of decrease of native β-lg in the supernatant was found to be most rapid in milk from cows with β-lg BB (Figure 2). This is in agreement with previously published results demonstrating that β-lg B is the most heat-sensitive genetic variant (Hillier and Lyster, 1979; Dannenberg and Kessler, 1988a; Parnell-Clunies et al., 1988).

The observed differences in the reaction rates found in this investigation may be related to a number of factors, an important one being the differences in size of casein micelles. Devold et al. (1994) have shown that the sizes of the casein micelles in milk from cows homozygous for β-lg B are relatively smaller compared to those in milk containing κ-cn AA. The κ-cn BB phenotype has in addition been found to be associated with a lower β-lg concentration (Ng-Kwai Hang et al., 1986). The most rapid loss of native β-lg could thus be expected with the combination of κ-cn BB/β-lg BB, since this combination has presumably the lowest β-lg content and the smallest casein micelle size. Therefore, this combination has the largest total micelle surface and contains more available κ-cn per mass unit of β-lg. In this investigation, however, the casein number was low in milk containing κ-cn BB, indicating that the differences in reaction are affected by factors other than κ-cn availability and β-lg concentration.

An additional cause of the observed variability may be any differences in reactivity of thiol groups in β-lg, especially since one of the amino acid substitutions is located close to the free thiol group (McKenzie et al., 1972). On the other hand, Sawyer (1969) proposed that the thiol groups are more important for the initial aggregation of β-lg itself than for the subsequent κ-cn/β-lg interaction, and this probably has a great importance also for the reaction in total. Haque and Kinsella (1987) showed that in the initial phases of complex formation between β-lg and κ-cn hydrophobic forces predominate. Hartman (1967) showed that a sulfhydryl blocking reagent does not prevent the reaction between unfolded (denatured) β-lg and unheated (native) κ-cn, which supports the theory of the involvement of hydrophobic forces in the reaction. Therefore, heat-induced reactions between κ-cn and β-lg are probably related to hydrophobic reactions as well as both intra- and intermolecular reactions involving thiol groups. Consequently, the observed differences between the different genetic milk protein variants reported here most likely reflect differences of both hydrophobic interactions and reactions involving thiol groups of the genetic variants of κ-cn as well as β-lg.

Another possible factor for differences in the reaction rate ($1/t_{1/2}$) between the different genetic combinations is the heterogeneity of κ-cn. Doi et al. (1979) and Haque and Khalifa (1992) have shown that different fractions of κ-cn have different thermal properties, possibly including the susceptibility to aggregation with β-lg during heating. In addition, Doi et al. (1983) have shown a positive correlation between the amount of carbohydrate in κ-cn and the susceptibility of κ-cn to react with β-lg. Dalgleish (1986) and Robitaille et al. (1991) showed that the κ-cn in milk with κ-cn AB contained significantly higher concentrations of carbohydrate than that in milk with κ-cn AA. From these findings it could be assumed that the κ-cn BB samples in this investigation should be more glycosylated and consequently show a higher reaction rate than the κ-cn AB samples. However, the results in this study do not support that assumption (Table 2).

Noh et al. (1989) have also shown that the α_{s2}-cn with its thiol group could influence the heat-induced protein complex formation. However, it should be pointed out that in our experiments, the milk contained only α_{s2}-cn variant A, which eliminates any effect caused by the genetic polymorphism of α_{s2}-cn.

If the data from our previously published results (Allmere et al., 1997) are combined with results published in this investigation, a complete picture is obtained of the reactivity between all nine possible combinations of the phenotypes of κ-cn (AA, AB, and BB) and β-lg (AA, AB, and BB). It can then be concluded that the reaction rate in milk homozygous for κ-cn A or κ-cn B increased with the number of β-lg B alleles. The influence of β-lg genotype was highly significant ($P < 0.0001$), despite the exclusion of the high values of the combination κ-cn BB/β-lg BB in the statistical analysis due to the nonrandom distribution of this genotype combination over breed, herd, and date of analysis. The number of β-lg B alleles, however, did not influence the reaction rates in the milk from cows heterozygous for κ-cn (AB) to any notable degree.

In conclusion, the loss of native β-lg in skim milk during heating at 90 °C has been shown to be significantly influenced by the genotypes of both κ-cn and β-lg, together accounting for more than half of the phenotypic variation in the reaction rate. The double-homozygote κ-cn BB/β-lg BB was also found to have the highest reaction rate ($1/t_{1/2}$), that is, ∼1.4 times higher compared to the other double-homozygote (κ-cn AA/β-lg AA).

ACKNOWLEDGMENT

We thank Magnus Lindersson, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, for phenotyping the cows.

LITERATURE CITED

Aleandri, R.; Buttazoni, L. G.; Schneider, J. C. The effects of milk protein polymorphisms on milk components and cheese producing ability. *J. Dairy Sci.* **1990**, *73*, 241−255.

Allmere, T.; Andrén, A.; Björck, L. Studies of the interactions between different genetic variants of β-lactoglobulin and κ-casein A during heating of skim milk. *J. Agric. Food Chem.* **1997**, *45*, 1564−1569.

Bovenhuis, H.; Verstege, A. J. M. Improved method for phenotyping milk protein variants by isoelectric focusing using PhastSystem. *Neth. Milk Dairy J.* **1989**, *43*, 447−451.

Dalgleish, D. G. Analysis by fast liquid protein chromatography of variants of κ-casein and their relevance to micellar structure and renneting. *J. Dairy Res.* **1986**, *53*, 43−51.

Dalgleish, D. G. Denaturation and aggregation of serum proteins and caseins in heated milk. *J. Agric. Food Chem.* **1990**, *38*, 1995−1999.

Dannenberg, F.; Kessler, H. G. Reaction kinetics of the denaturation of whey proteins. *J. Food Sci.* **1988a**, *53*, 258−263.

Dannenberg, F.; Kessler, H. G. Application of reaction kinetics to the denaturation of whey proteins in heated milk. *Milchwissenschaft* **1988b**, *43*, 1−37.

Devold, T. G.; Vegarud, G. E.; Langsrud, T. Milk protein polymorphism related to average micellar size and protein composition. *Proc. 24th Int. Dairy Congr. (Melbourne), Brief Commun.* **1994**, 58.

Doi, H.; Ibuki, F.; Kanamori, M. Heterogeneity of reduced bovine κ-casein. *J. Dairy Sci.* **1979**, *62*, 195−203.

Doi, H.; Ideno, S.; Ibuki, F.; Kanamori, M. Participation of the hydrophobic bond in complex formation between κ-casein and β-lactoglobulin. *Agric. Biol. Chem*. **1983**, *47*, 407−409.

Haque, Z.; Khalifa, M. Y. κ-casein heterogeneity and milk heating effects on susceptibility to chymosin action. *J. Food Sci*. **1992**, *57*, 49−54.

Haque, Z.; Kinsella, J. E. Heat-induced changes in the hydrophobicity of casein and β-lactoglobulin. *Agric. Biol. Chem*. **1987**, *51*, 2245−2247.

Hartman, G. H. Heat treatment of mixtures of β-lactogloulin and κ-casein. *Diss. Abstr*. **1967**, *28*, 474.

Hillier, R. M.; Lyster, R. L. J. Whey protein denaturation in heated milk and cheese whey. *J. Dairy Res*. **1979**, *46*, 95−102.

Jakob, E. Beziehungen zwischen dem genetischen Polymorphismus der Milchproteine und der Labfähigkeit von Milch. Ph.D. Dissertation, Swiss Federal Institute of Technology, ETH No. 10224, 1993.

Kroeker, E. M.; Ng-Kwai-Hang, K. F.; Hayes, J. F.; Moxley, J. E. Effects of environmental factors and milk protein polymorphism on composition of casein fraction in bovine milk. *J. Dairy Sci*. **1985a**, *68*, 1637−1641.

Kroeker, E. M.; Ng-Kwai-Hang, K. F.; Hayes, J. F.; Moxley, J. E. Effect of β-lactoglobulin variant and environmental factors on variation in the detailed composition of bovine milk serum proteins. *J. Dairy Sci*. **1985b**, *68*, 1752−1757.

Marziali, A. S.; Ng-Kwai-Hang, K. F. Relationships between milk protein polymorphisms and cheese yielding capacity. *J. Dairy Sci*. **1986**, *69*, 1193−1201.

McKenzie, H.; Norton, R. S.; Sawyer, W. H. Heat induced interaction of β-lactoglobulin and κ-casein. *J. Dairy Sci*. **1971**, *38*, 343−351.

McKenzie, H. A.; Ralston, G. B.; Shaw, D. C. Location of sulfhydryl and disulfide groups in bovine β-lactoglobulins and effects of urea. *Biochemistry* **1972**, *11*, 4539−4547.

McLean, D. M.; Graham, E. R. B.; Ponzoni, R. W.; McKenzie, H. A. Effects of milk protein genetic variants on milk yield and composition. *J. Dairy Res*. **1984**, *51*, 531−546.

Mottar, J.; Bassier, A.; Joniau, M.; Baert, J. Effect of heat-induced association of whey proteins and casein micelles on yoghurt texture. *J. Dairy Sci*. **1989**, *72*, 2247−2256.

Ng-Kwai-Hang, K. F.; Hayes, J. F.; Moxley, J. E.; Monardes, H. G. Variability of test-day milk production and composition and relation of somatic cell counts with yield and compositional changes of bovine milk. *J. Dairy Sci*. **1984**, *67*, 361−366.

Ng-Kwai-Hang, K. F.; Hayes, J. F.; Moxley, J. E.; Monardes, H. G. Relationships between milk protein polymorphisms and major milk constituents in Holstein-Friesian cows. *J. Dairy Sci*. **1986**, *69*, 22−26.

Ng-Kwai-Hang, K. F.; Hayes, J. F.; Moxley, J. E.; Monardes, H. G. Variation in milk protein concentrations associated with genetic polymorphism and environmental factors. *J. Dairy Sci*. **1987**, *70*, 563−570.

Noh, B.; Creamer, L.; Richardson, T. Thermally induced complex formation in an artificial milk system. *J. Agric. Food Chem*. **1989**, *37*, 1395−1400.

Parnell-Clunies, E. M.; Kakuda, Y.; Irvine, D. M. Heat-induced protein changes in milk processed by vat and continuous heating systems. *J. Dairy Sci*. **1988**, *71*, 1472−1483.

Pearse, M. J.; Linklater, P. M.; Hall, R. J.; MacKinlay, A. G. Effect of heat induced interaction between β-lactoglobulin and κ-casein on syneresis. *J. Dairy Res*. **1985**, *52*, 159−165.

Robitaille, G.; Ng-Kwai-Hang, K.-F.; Maonardes, H. G. Variation of *N*-acetylneuraminic acid content of bovine κ-casein. *J. Dairy Sci*. **1991**, *74*, 3314−3317.

Sawyer, W. H. Complex between β-lactoglobulin and κ-casein. A review. *J. Dairy. Sci*. **1969**, *52*, 1347−1355.

Schaar, J. Effects of κ-casein genetic variants and lactation number on the renneting properties of individual milks. *J. Dairy Res*. **1984**, *51*, 397−406.

Schaar, J.; Hansson, B.; Pettersson, H. E. Effects of genetic variants of κ-casein and β-lactoglobulin on cheesemaking. *J. Dairy Res*. **1985**, *52*, 429−437.

Smits, P.; van Brouwershaven, J. H. Heat-induced association of β-lactoglobulin and casein micelles. *J. Dairy Res*. **1980**, *47*, 313−325.

Van den Berg, G.; Escher, J. T. M.; de Koning, P. J.; Bovenhuis, H. Genetic polymorphism of κ-casein and β-lactoglobulin in relation to milk composition and processing properties. *Neth. Milk Dairy J*. **1992**, *46*, 145−168.

Received for review March 19, 1998. Revised manuscript received May 19, 1998. Accepted May 19, 1998. We gratefully acknowledge the Swedish University of Agricultural Sciences for a scholarship to Toomas Allmere and the Swedish Farmers' Foundation for Agricultural Research for providing research funds.

JF980290R