# Effect of Protein Composition on the Cheese-Making Properties of Milk from Individual Dairy Cows

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#### **ABSTRACT**

The objective of this study was to evaluate the effect of variations in milk protein composition on milk clotting properties and cheese yield. Milk was collected from 134 dairy cows of Swedish Red and White, Swedish Holstein, and Danish Holstein-Friesian breed at 3 sampling occasions. Concentrations of  $\alpha_{S1}$ -,  $\beta$ -, and  $\kappa$ -casein (CN),  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin (LG) A and B were determined by reversed phase liquid chromatography. Cows of Swedish breeds were genotyped for genetic variants of  $\beta$ - and  $\kappa$ -CN. Model cheeses were produced from individual skimmed milk samples and the milk clotting properties were evaluated. More than 30% of the samples were poorly coagulating or noncoagulating, resulting in weak or no coagulum, respectively. Poorly and noncoagulating samples were associated with a low concentration of  $\kappa$ -CN and a low proportion of  $\kappa$ -CN in relation to total CN analyzed. Furthermore, the  $\kappa$ -CN concentration was higher in milk from cows with the AB genotype than the AA genotype of  $\kappa$ -CN. The concentrations of  $\alpha_{S1}$ -,  $\beta$ -, and  $\kappa$ -CN and of  $\beta$ -LG B were found to be significant for the cheese yield, expressed as grams of cheese per one hundred grams of milk. The ratio of CN to total protein analyzed and the  $\beta$ -LG B concentration positively affected cheese yield, expressed as grams of dry cheese solids per one hundred grams of milk protein, whereas  $\beta$ -LG A had a negative effect. Cheesemaking properties could be improved by selecting milk with high concentrations of  $\alpha_{S1}$ -,  $\beta$ -, and  $\kappa$ -CN, with high  $\kappa$ -CN in relation to total CN and milk that contains β-LG B.

**Key words:** cheese yield, milk protein composition, milk clotting properties, poorly coagulating milk

## INTRODUCTION

In many milk-producing countries, a large fraction of the milk produced is used for cheese making. In the

Scandinavian countries, about one-third of the total volume is used for this purpose. The quality and amount of cheese obtained, not only per volume of milk but also per gram of protein in cheese milk, is important for the economic outcome of the dairy industry. The milk clotting properties are important both with regard to quality and yield of cheese. It has been suggested that a firmer curd at cutting is positively correlated to yield of cheese (Aleandri et al., 1989; Martin et al., 1997). Johnson et al. (2001) showed that a firmer curd at cutting resulted in reduced-fat Cheddar cheeses with higher moisture content. However, the milk clotting properties are variable, and factors influencing these properties include the concentrations of total CN and calcium (Storry et al., 1983), pH (Najera et al., 2003), genetic polymorphism of milk proteins (Schaar et al., 1985; Mayer et al., 1997; Ikonen et al., 1999a), stage of lactation (Okigbo et al., 1985c; Ostersen et al., 1997), season (O'Brien et al., 1999), and feeding (Verdier-Metz et al., 1998). Differences in milk clotting properties between different breeds have been previously studied in the United Kingdom (Verdier-Metz et al., 1998), France (Macheboeuf et al., 1993), Italy (Chiofalo et al., 2000), Ireland (Auldist et al., 2002), and New Zealand (Auldist et al., 2004).

Genetic polymorphism of milk proteins has been associated with composition, production traits, and technological properties of milk. Concentration of  $\beta$ -LG is higher in milk with the AA genotype than with AB or BB (McLean et al., 1984; Ng-Kwai-Hang et al., 1987; Graml et al., 1989), which results in a lower CN number in AA milk (Lunden et al., 1997; Schaar, 1984; van den Berg et al., 1992). Several workers have reported significantly higher concentrations of  $\kappa$ -CN in milk with the B allele (McLean et al., 1984; van den Berg et al., 1992; Bobe et al., 1999). The B allele of  $\kappa$ -CN has also been associated with improved milk clotting properties (Schaar, 1984) and a higher cheese yield (Schaar et al., 1985), whereas the E allele has been related to unfavorable milk clotting properties (Ikonen et al., 1999a; Caroli et al., 2000). Many studies have been carried out on the effects of genetic polymorphism on

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milk clotting properties and cheese yield (Ng-Kwai-Hang, 1998), whereas studies evaluating the actual milk protein composition in relation to cheese-making properties of individual milk samples are less frequent (Auldist et al., 2004). The CN to total protein ratio has decreased in Swedish bulk milk during the last decades. making it less suitable for cheese making (Lindmark-Månsson et al., 2003). Deteriorating trends like this may also occur in other countries, and therefore, it is important to identify suitable quality markers for cheese milk. In Sweden, milk is currently graded according to concentrations of total protein and milk fat. It could be an economical advantage to the dairy industry if a specific marker could be used to identify milk suitable for cheese making; that is, good milk clotting properties and high cheese yield. The object of this study was thus to evaluate the effect of protein composition on milk clotting properties and cheese yield from milk of individual cows.

#### **MATERIALS AND METHODS**

## Milk Collection and Experimental Design

Evening whole milk samples (approximately 10 L) from 16 Swedish Red and White (SRB), 15 Swedish Holstein (SLB), and 14 Danish Holstein-Friesian (SDM) cows were collected in September 2003. Milk from SRB and SLB were collected at the experimental dairy herd at Jälla (Swedish University of Agricultural Sciences) and milk from SDM was collected at the Research Centre Foulum (Danish Institute of Agricultural Sciences). A second sampling from 22 SRB, 8 SLB, and 15 SDM took place in January 2004, and the last sampling from an additional 20 SRB, 9 SLB, and 15 SDM was carried out in May 2004. A total of 134 cows was thus included in the study and no cow was sampled twice. Feeding and management was carried out according to standard practices in the respective countries and the cows were held indoors on the 3 sampling occasions. All cows included in the study were healthy and milked twice a day. The cows were grouped into 4 classes according to stage of lactation: early (wk 6 to 15; n = 27), mid (wk 16 to 30; n = 69), late (wk 31 to 45; n = 30), and very late (wk 46 or later; n = 8). All breeds were represented within each stage of lactation class. The cows were also grouped into 4 classes according to lactation number: first lactation (1; n = 50), second lactation (2; n = 42), third lactation (3; n = 22), and fourth or more lactation (4; n = 20). All breeds were represented within each lactation number class.

#### Milk Composition

Samples of fresh milk were analyzed for concentration of milk fat, lactose, urea, and pH by a Milkoscan FT 6000 (A/S N., Foss Electric, Hillerød, Denmark) and for somatic cells using a Fossomatic 5000 (A/S N., Foss Electric) at Steins Laboratory (Holstebro, Denmark). Calcium content was determined using atom absorption spectrophotometry after drying to ash at 525°C for 6 h, followed by dissolving in acid and dilution in lanthanchloride solution. Concentrations of major milk proteins were determined using the reversed phase liquid chromatography (**RP-HPLC**) method modified by Wedholm et al. (2006) according to Bordin et al. (2001). No measurement was made for  $\alpha_{S2}$ -CN. Concentration of total protein analyzed was defined as the sum of the concentrations of  $\alpha_{S1}$ -CN,  $\beta$ -CN,  $\kappa$ -CN,  $\beta$ -LG A,  $\beta$ -LG B, and  $\alpha$ -LA. Concentration of total CN analyzed comprised the sum of the concentrations of  $\alpha_{S1}$ -CN,  $\beta$ -CN, and  $\kappa$ -CN, and concentration of total whey protein analyzed comprised the sum of the concentrations of  $\beta$ -LG A,  $\beta$ -LG B, and  $\alpha$ -LA. Proteolysis in individual pasteurized skimmed milk samples were determined as the level of free amino terminals using the fluorescamine method modified for milk samples (Larsen et al., 2004). Level of free amino terminals was expressed as leucine equivalents (in mM) according to a standard curve for leucine.

## Genotyping for $\kappa$ - and $\beta$ -CN Genetic Variants

Blood samples were collected from SRB and SLB and the DNA was genotyped for genetic variants of  $\beta$ - and  $\kappa$ -CN using pyrosequencing, as described by E. Hallén, T. Allmere, J. Näslund, A. Andrén, and A. Lundén, Departments of Food Science and Animal Breeding and Genetics, Swedish Univ. Agric. Sci., Uppsala, Sweden; personal communication).

#### Cheese Making

The model cheeses were produced from skimmed milk to reduce the number of variables influencing cheese yield. After 2 d of cold storage (4°C), individual milk samples were preheated to 40°C, defatted, and heated in a pilot plate heating apparatus (72°C for 15 s), as described by Allmere et al. (1998). Four liters of skimmed milk was inoculated with a commercial starter culture (0.1 g/L of Lactobacillus helveticus 174 and 0.1 g/L of Probat 404, Danisco, Sweden), and incubated at  $30^{\circ}\mathrm{C}$  for 30 min. This was followed by addition of chymosin (1.25 mL/L of Chy-Max Plus, 190 International Milk Clotting Units/mL, Christian Hansen A/S, Denmark) and gentle stirring. After 30 min at 30°C, the gel formed was cut into 2-cm cubes. To allow syneresis, the curd was incubated at 50°C for another 30 min during gentle stirring. The whey was removed and the curd was pressed (0.04 kg/cm<sup>2</sup>) for 20 h at room temper-

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