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Factors influencing chymosin-induced gelation of milk from individual dairy cows: Major effects of casein micelle size and calcium

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ABSTRACT

Optimisation of cheese yield is crucial for cheese production; a previous study showed large variations in chymosin-induced coagulation in milk from the second most common Swedish dairy breed, Swedish Red. In the present study, the effect of gross composition, protein composition, total and ionic calcium content, phosphorous content and casein micelle size on chymosin-induced gelation was determined in milk from 98 Swedish Red cows. The study showed that protein content and total calcium content, ionic calcium concentration and casein micelle size were the most important factors explaining the variation of gelation properties in this sample set. Non-coagulating milk was suggested to have lower ionic and total calcium content as well as lower relative concentrations of β-lactoglobulin than coagulating milk. The lower total calcium content in non-coagulating milk poses a problem as the difference was, theoretically, four times larger than the amount of calcium that is normally added in cheese processing.

1. Introduction

In 2011, 103,000 metric tons of cheese was produced in Sweden. As much as 87% of this cheese was sold in Sweden whereas the rest was exported (IDF, 2013). A previous study by Gustavsson et al. (2014a) showed that there were large variation in cheese milk quality in milk from 400 individuals of the Swedish Red (SR) breed and that as many as 18% of the cows in the study produced non-coagulating (NC) milk. This is alarming as SR is the second most common breed in Sweden at a frequency of 41% (Swedish Dairy Association, 2012). The presence of NC milk has been known for a few decades which has been reported by, e.g., Johnston and MacLachlan (1977), Okigbo, Richardson, Brown, and Ernstrom (1985) and van Hooydonk, Hagedoorn, and Boerrigter (1986). The occurrence of NC milk in individual cows varies in different breeds, e.g., Simmental (6.3%; Bonfatti, Cecchinato, Gallo, Blasco, & Carnier, 2011), Estonian Holstein (Vallas et al., 2010), Holstein Friesian and Brown Swiss (9.7% and 3.5%, respectively; Cecchinato et al., 2011) and Finnish Ayrshire and Finnish Holstein (8.6 and 1.3%, respectively; Tyrisevä, Vahlsten, Ruottinen, & Ojala, 2004).

In cheese production ~100 kg of milk is needed to produce about 8–16 kg of cheese (Walstra, Wouters, & Geurts, 2006). Optimisation of cheese yield is therefore of great importance within cheese production. The initial stage of cheese production is milk gelation (Lucey, 2002), which is initiated by the addition of rennet that contains the proteolytic enzyme chymosin. Chymosin hydrolyses the milk protein κ-casein (κ-CN), which removes the steric repulsion and reduces the net negative charge on the casein micelles. This makes the casein micelles susceptible to aggregation and a gel is formed (Horne & Banks, 2004; Lucey, 2009; Walstra et al., 2006). Rennet-induced gelation is known to be affected by gross composition of milk, detailed protein composition, pH and salt concentrations (Fox & Cogan, 2004; Walstra et al., 2006). As all these compositional factors vary with season, stage of lactation and parity of the cow, these are also contributing factors (Walstra et al., 2006).

It has previously been suggested that the size of the casein micelles could have an effect on rennet-induced gelation, where

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smaller casein micelles have been shown to give stronger gels and shorter gelation times (Ford & Grandison, 1986; Glantz et al., 2010; Horne & Banks, 2004; Niki et al., 1994). As previously reviewed (Bittante, Penasa, & Cecchinato, 2012; Caroli, Chessa, & Erhardt, 2009; Jakob & Puhan, 1992), the genetic protein variant κ-CN B has been reported to be associated with good gelation properties, and it has been suggested that this effect is partly due to that milk with κ-CN B has small casein micelles (Glantz, Lindmark-Månsson, Stålhammar, & Paulsson, 2011b; Walsh et al., 1998).

Calcium is present in milk either in the colloidal calcium phosphate (CCP) in the casein micelles (micellar calcium) or present in the milk serum (soluble calcium). Of the soluble calcium, some is present in complex with other ions and whey proteins or as free ions (ionic calcium; McMahon & Oommen, 2013; Walstra et al., 2006). Calcium content, and especially the concentration of ionic calcium, is crucial for the formation of rennet gels, as the paracasein micelles will not aggregate if the concentration of ionic calcium is too low (Lucey, 2009). Glantz et al. (2011b) found that the concentration of ionic calcium was correlated with gelation properties where a higher concentration gave shorter gelation times (t_g) and higher strength and yield stress of the gels. However, Glantz et al. (2011b) found no effect of total calcium contents. Malacarne et al. (2014) reported that well coagulating milk had higher contents of total and micellar calcium and phosphorus. It was also shown that well coagulating milk had higher contents of soluble phosphorous but that there was no difference in content of soluble calcium. This agreed, to a large extent, with the results of Jensen et al. (2012). In addition, Jensen et al. (2012) found that relative concentrations of the individual milk proteins could affect rennet-induced gelation, where the relative concentration of κ-CN was higher and the relative concentration of α_{S2}-casein (α_{S2}-CN) was lower in well coagulating milk compared with poor coagulating milk. Bonfatti, Di Martino, Cecchinato, Degano, and Carnier (2010) found that increased relative and absolute concentrations of κ-CN was associated with shorter t_g and higher gel strengths whereas relative and absolute concentrations of β-casein (β-CN) only affected gel strength.

In the present study gross composition, detailed protein composition, total and ionic calcium contents, phosphorous content and casein micelle size were determined together with chymosin-induced gelation properties in 98 SR cows. The aim of the study was to elucidate the reasons for the variation in gelation properties in these milk samples and to try to explain the high frequency of NC milk in the present sample set.

2. Materials and methods

2.1. Sample collection and milk composition

Morning milk samples and blood samples were collected from 395 individual cows from 21 different farms located in the southern part of Sweden. The samples were collected in April to May, 2010 and in September 2010 to April 2011. Pedigree information was used to select cows that were as unrelated as possible. At the day of sampling milk samples were cooled and transported to Lund University, Sweden and Aarhus University, Denmark for further analyses. All fresh milk samples were analysed for fat, protein and casein contents using an infra-red technique (Milkoscan FT2, Foss Electric, Hillerød, Denmark). The somatic cell count was determined using flow cytometry (Combifoss 5000, Foss Electric) at a certified dairy analysis laboratory (Eurofins Steins Laboratory, Jönköping, Sweden). Also, pH was measured on all fresh milk samples. Milk samples were centrifuged at 4 °C and 2000 × g for 30 min, fat was removed from the milk and the skim milk samples were frozen and stored at −20 °C until further analyses. Frozen skim milk samples were thawed and relative concentrations of α-lactalbumin (α-LA), β-lactoglobulin (β-LG), α_{S2}-CN, α_{S1}-casein (α_{S1}-CN) 8P, α_{S1}-CN 9P, total α_{S1}-CN, κ-CN 1P and β-CN were determined using capillary zone electrophoresis as previously described (Åkerstedt, Wredle, Lam, & Johansson, 2012; Gustavsson et al., 2014b).

A subset of 98 milk samples were selected for further analyses and all these milk samples had somatic cell counts below 300,000 cells mL^{-1}. The subset was randomly selected to represent the genotype frequencies of the whole sample population described above. The cows in the subset were in days in milk (DIM) 56–377, whereof 4 cows were in early lactation (days 56–91), 31 cows in mid lactation (days 111–208), 53 cows in late lactation (days 210–321) and 10 cows in very late lactation (days 322–377). Furthermore, 31 of the cows were in their first parity, 42 in their second parity and 25 in their third parity. In the subset, total calcium and phosphorus contents were measured using inductively coupled plasma atomic emission spectroscopy ICP-AES (Nordic Committee on Food Analysis, 1998). The measurements were performed by a certified dairy analysis laboratory (Eurofins Environment Sweden, Lidköping, Sweden). Measurements of ionic calcium were performed at 32 °C using a calcium electrode (Orion 97–20 Ionplus Calcium Electrode; Thermo Electron Corp., Beverly, MA) as previously described (Glantz et al., 2011b). Before measurements, frozen skim milk samples were thawed overnight at 4 °C and then tempered in a water bath at 32 °C for 4 h.

2.2. Casein micelle size

A Mastersizer 2000 (Malvern Instruments, Malvern, UK) with a Hydro 2000 SM sampling unit was used to determine casein micelle size distributions using laser light scattering. Before measurements, frozen skim milk samples were thawed over night at 4 °C, tempered in a water bath at 32 °C for 4 h and then let to cool to room temperature. The sampling unit was filled with room tempered pure water which was prepared with a Milli-Q system (Millipore Corp., Bedford, MA, US), the sampler unit pump was set at 1500 rpm, milk was added until 8% obscuration was obtained and the measurement was started. This procedure was repeated twice for each sample. Using the software supplied with the instrument (Malvern application, v 5.60, Malvern Instruments) the average volume-weighted diameter, D[4,3], was determined in each milk sample. To avoid interference of remaining fat, only particles with diameters between 0.02 and 0.83 μm were included when estimating the volume-weighted diameter of the casein micelles.

2.3. Chymosin-induced gelation properties

Rheological measurements of chymosin-induced skim milk gels were performed on fresh milk samples at the week of sampling. Samples were prepared and measurements were performed as previously reported (Gustavsson et al., 2014a). The gel strength measured 40 min after addition of chymosin to each sample was defined as G'_{40}, and the time when the gel strength starts to increase continuously was defined as gelation time, t_g. The yield stress, σ_y, was defined as the shear stress when the viscosity reached 90% of the maximum recorded viscosity in the performed stress sweep. Skim milk samples which had not started to form a gel after 40 min, $t_g > 40$ min and $G'_{40} = 0$ Pa, were defined as NC milk.

2.4. Genotyping

Taqman SNP genotyping assays were used to genotype all cows for genetic variants of α_{S1}- (*CSN1S1*), β- (*CSN2*) and κ-CN (*CSN3*) and