Genetic and **isoform variability** of major milk proteins in relation to **milk coagulation**

Hanne B. Jensen, Nina A. Poulsen, and Lotte B. Larsen
University of Aarhus, Department of Food Science, Denmark,

Tel: +4587157502
Hanne.bakjensen@agrsci.dk

Introduction

Milk coagulation is essential for cheese manufacturing and cheese yield. There is great variation in coagulation ability between individual cows and breeds. Some cows produce milk that is better at making cheese than others (**Picture 1**), The occurrence of poorly- and non-coagulating milk is becoming a recognized problem in many studied dairy cow populations.

Among others, it this recognized that milk coagulation is partly hereditary (~40%),

Picture 1. Variability of coagulation properties. Milk samples from Holstein-Friesian cows assessed 1 hr after addition of rennet; One samples shows poor/non-coagulation characteristics (to the left), while the other samples show good characteristics (to the right) as it has formed a gel-structure.

Objective

To uncover the genetic and isoform variation of the major milk proteins in milk samples with different ability to coagulate.

Experimental setup

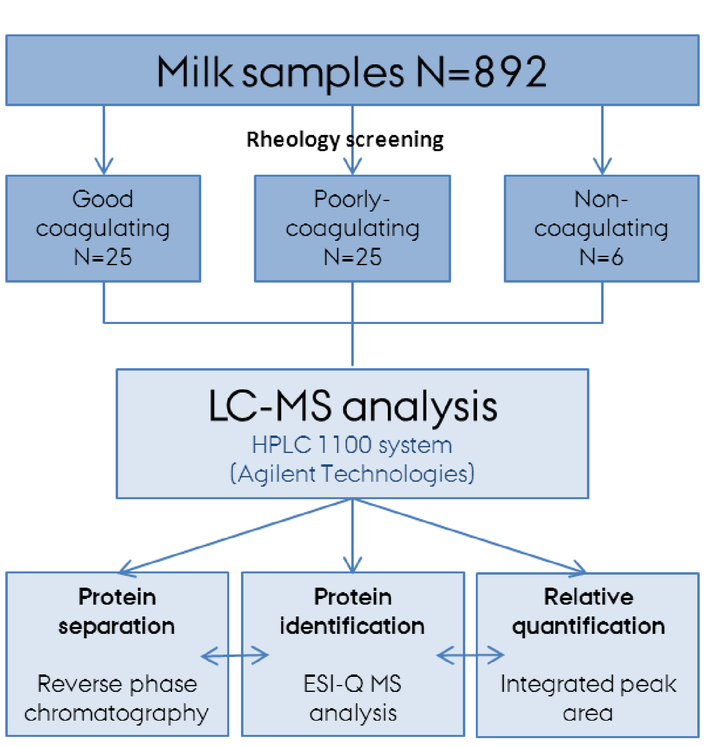

Figure 1. Outline of experimental setup. Liquid chromatography-mass spectrometry (LC-MS) was used to analyze milk samples from Danish Holstein-Friesian cows. Environmental factors possible influencing milk coagulation was minimized as: All cows were in mid-lactation (week 19 to 32), parity 1 to 3, housed in loose-housing systems, fed according to standard practice, milked twice a day, and somatic cell count in milk was < 500x10³ cell/mL.

Results and conclusions

- The major milk proteins (caseins, α_{S1}-CN, α_{S2}-CN, β-CN, κ-CN; whey proteins, α-lactalbumin (α-LA), β-lactoglobulin (β-LG)), their genetic variants (etc., A, B, C) and their isoform variants (varying in level of phosphorylation (P) and glycosylation (G)) were separated with high resolution in relation to coagulation groups using reverse phase chromatography (**Figure 2**), followed by MS identification.

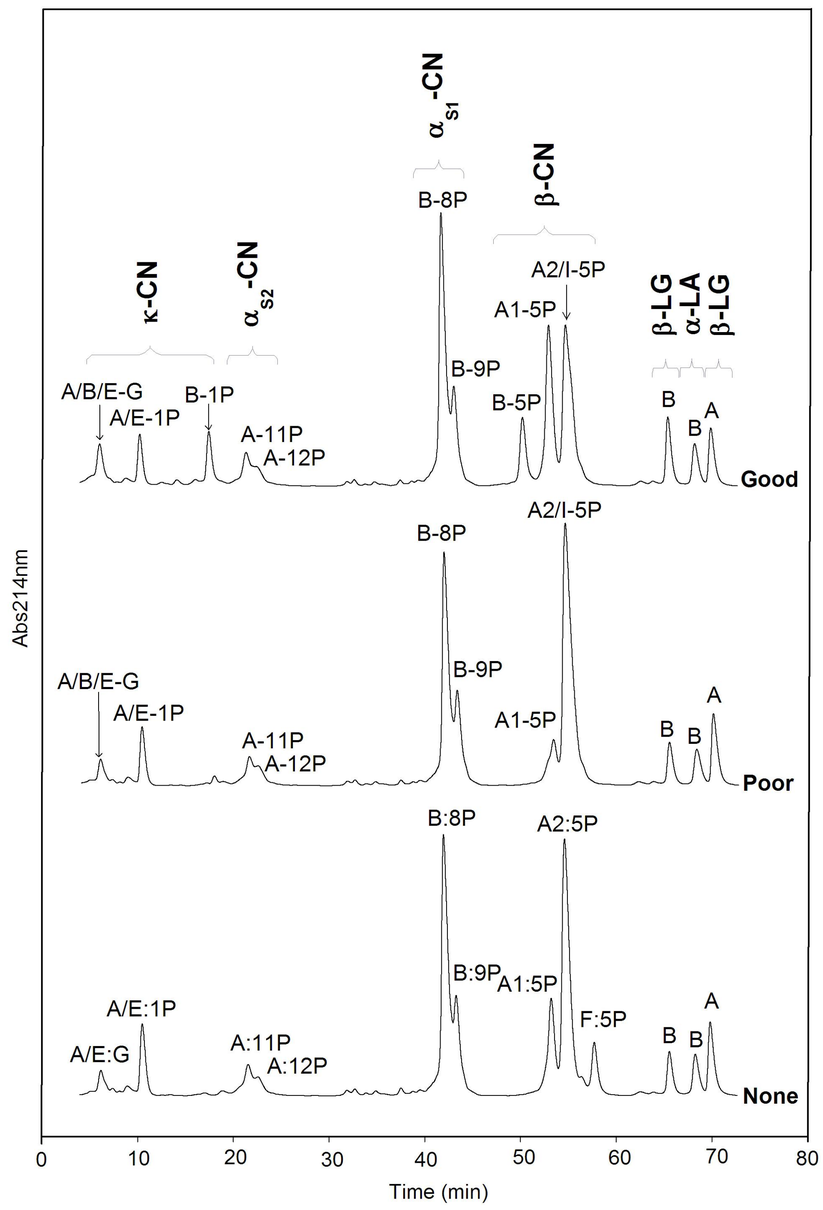

Figure 2. Comparative chromatograms of bovine milk samples in relation to coagulation groups (good, poor, none; pooled milk samples). All identified major proteins (vertical text) are marked, along with genetic variants and isoform (varying in phosphorylation, P, or glycosylation, G; horizontal text).

- A variety of genetic variants of the major milk proteins was identified, including α_{S1}-CN (variants B and C), α_{S2}-CN (A), β-CN (A^1, A^2, B, I, F), κ-CN (A, B and E), α-LA (B), and β-LG (A, B, and C).
- In both poorly and non-coagulating milk the most prevalent composite genotype of α_{S1}-, β- and κ-casein was BB-A^2A^2-AA, confirming a genetic contribution in relation to milk coagulation.
- Interestingly, relative quantification revealed that milk with impaired coagulation abilities had a significant lower fraction of the least phosphorylated α_{S1}-CN form (α_{S1}-CN 8P), along with a lower fraction of glycosylated κ-CN, compared with good coagulating milk.
- This study is one of the first to identify subtle variations in level of isoform modifications in relation to milk coagulation properties, which affect the physic-chemical properties of the proteins, the micelle stability, and thus have importance during the aggregation stage of coagulation.

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