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Variations in coagulation properties of cheese milk from three Danish dairy breeds as determined by a new free oscillation rheometry-based method

Pernille Dorthea Frederiksen · Marianne Hammershøj · Mette Bakman · Per Nyegaard Andersen · Jens Bech Andersen · Karsten Bruun Qvist · Lotte Bach Larsen

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Abstract The paper describes a study on the variation of coagulation properties in individual cow's milk measured by a novel free oscillation rheometry-based method. The work is part of a study on the occurrence and underlying causes of poorly and even non-coagulating (NC) milk in Denmark, and based on that, the paper reports on the frequency of non- and poorly coagulating milk in a herd of the three main Danish dairy breeds, Jersey, Danish red (RDM), and Danish Holstein-Friesian (SDM). The cows were milked by automatic milking. The rennet coagulation time, the coagulum firmness by storage modulus G' , and the curd firming rate of fresh skimmed milk were determined. More than 20% of the milk samples exhibited poor coagulation properties by the criteria set. Of these, the 17% resulted in a weak coagulum after addition of rennet, while another 3% were due to NC milk that did not aggregate to form a curd within the 40 min of measurement. Milk from Jersey cows exhibited superior coagulation properties when compared to milk from SDM and RDM cows. The dependence of curd firming rate on protein concentration was investigated, and found to depend on milk protein concentration to the power of three, emphasizing the importance of protein interactions, and not just the protein concentration itself. The paper gives a basis for the subsequent selection of NC milk samples with the

P. D. Frederiksen · M. Hammershøj · L. B. Larsen (✉)

Department of Food Science, Faculty of Agricultural Sciences, Aarhus University, PO Box 50, 8830 Tjele, Denmark

e-mail: lottebach.larsen@agrsci.dk

M. Bakman · P. N. Andersen

Arla Foods amba, Innovation Centre Brabrand, Brabrand, Denmark

J. B. Andersen

Department of Animal Health and Bioscience, Faculty of Agricultural Sciences, Aarhus University, Tjele, Denmark

K. B. Qvist

Cultures & Enzymes Division, Innovation, Chr. Hansen, Hørsholm, Denmark

aim to look in more detail for the underlying reasons for the phenomenon of NC milk.

摘要 - 新型振荡流变法测定源于丹麦三个品种奶牛的干酪用乳的凝乳特性

大量文献报道过丹麦的泽西种、丹麦红 (RDM) 种和丹麦荷尔斯坦因-弗里斯兰 (SDM) 种奶牛所产乳的凝乳特性非常差, 甚至不能够凝乳, 本研究是针对大量的关于这方面的文献报道, 采用新型的自由振荡流变法测定了不同品种干酪用乳凝固特性的变化。以自动挤奶机挤出的牛奶为测定样品, 测定了样品的凝乳酶凝乳时间以及基于储存模量 G' 和新鲜脱脂乳的凝块硬化速率所确定的凝块硬度。根据凝固标准, 20%牛奶样品的凝乳特性差, 17%样品加入凝乳酶后能形成较软的凝块, 而3%的样品由于是非凝固的牛奶, 所以在40 min的测定时间内不能够形成凝块。源于泽西奶牛的牛乳的凝固性优于其他两个品种牛乳的凝固性。研究了凝块硬化速率与蛋白质浓度之间的关系, 研究发现 3 种牛乳的凝块硬化速率与蛋白质浓度相关, 这种相关性不仅仅与蛋白质浓度的变化有关, 而是由于蛋白质之间的相互作用。本项研究仅仅是对这类非凝固牛奶的凝乳特性进行了探讨, 为今后进一步研究非凝固牛乳现象产生的原因奠定了基础。

Keywords Milk coagulation · Curd firming rate (CFR) · Rennet coagulation time (RCT) · Coagulum strength · Breed variation

关键词 乳的凝固 · 凝块硬化速率(CFR) · 凝乳酶凝乳时间 (RCT) · 凝块强度 · 品种间变化

1 Introduction

In Denmark at present, approximately 50% of the non-fat milk production is used for cheese making (Danish Dairy Board statistics 2008). During the last 30 years, the milk and protein yields from Danish dairy cows, of the three major breeds, have increased considerably (more than 75% increase in kilogram milk yield per cow; Statistics Denmark, Danish Cattle Federation), both due to specific breeding schemes and improved farm management. The coagulation properties of the milk are crucial in relation to cheese manufacture and cheese yield and are influenced by a range of different factors having implications for milk protein content and composition, including lactation stage, breed, genetic variants of the milk proteins, and composite genotypes, season, and feeding (Ekstrand et al. 1981; Hallen et al. 2007; Ng-Kwai-Hang et al. 1982). The rennet coagulation properties of milk are also highly affected by factors such as the total content of the caseins, content of α_{s1} -, α_{s2} - β - and κ -casein, their relative proportion, and furthermore by the content of the ions calcium, phosphorus, sodium, and potassium (Van Hooydonk et al. 1986; Zoon et al. 1988a; Zoon et al. 1988c). The general health status of the dairy cow also plays an important role in the quality of the milk. Milk with a high somatic cell count (SSC; $>500,000$ cells mL^{-1}) reduces the cheese yield, due to a higher proteolytic activity, a lower concentration of fat and casein, and a higher content of whey proteins, especially serum albumin and immunoglobulins (Auldist et al. 1996; Barbano et al. 1991). The rennetability and curd properties are further influenced by a large range of conditions such as pH (Hynes et al. 1999), rennet concentration (Zoon et al. 1988c), coagulation temperature (Bohlin et al. 1984), enzyme origin (Esteves et al. 2002), and heat treatment of the milk (Lucey 1995).

An increasing focus has recently been addressed to the problem relating to the occurrence of poor and non-coagulating (NC) milk (Ikonen et al. 1999; Joudu et al. 2007; Okigbo et al. 1985a; Wedholm et al. 2006). The underlying reasons are related both to genetic and environmental factors and are prevalent also in healthy cows in mid-lactation (Ikonen et al. 2004). Although there exists no literature that directly states that milk yield has a negative influence on the milk quality in relation to cheese production, it is known, for Danish Holstein cattle (SDM) and Danish Red (RDM), that the genetic association between production and health is antagonistic (Hansen et al. 2002; Norberg et al. 2009). Thus, we find it possible that the breeding schemes involving breeding for increased milk yield in these breeds also have consequences for the overall milk quality.

The lactodynamograph (Cassandro et al. 2008) and the Formagraph (Foss Electric, Denmark) are examples of instruments of an empirical nature giving information on coagulation time, gel strength, and gelation rate (Ipsen et al. 1997). The Formagraph had the advantage of being able to measure multiple samples simultaneously, but the disadvantage that output was available only in analog form, as a chart output. Among fundamental rheological methods, viscometry has been used as a sensitive means for determination of the onset of gelation. Controlled strain or stress oscillation measurements have been used to follow development of the gel modulus, while more recently, free oscillation rheometry has been introduced for the same purpose (Bohlin et al. 1984; Dejmek 1987; Hansen et al. 2009; McMahon and Brown 1982). The ReoRox4 instrument (Medirox AB, Nyköping, Sweden), used in the present screening study, is a free oscillation rheometer, allowing measurement of multiple samples simultaneously and giving digital output of modulus vs. time and various parameters derived thereof. Its use for characterisation of milk coagulation properties is new in the literature.

As differences in coagulation properties reflect the phenotypic differences between cows, i.e., the sum of genotypic, environmental, and random variation, a better understanding of the current intra- and inter-breed differences at individual cow's level is a step in the further characterisation of the underlying causes of poorly- and non-coagulating milk on the road for management decisions and breeding schemes at both cow and herd levels to improve the quality of cheese milk.

The present investigation was carried out as part of a larger study aiming for identifying underlying reasons for the occurrence of poor- and non-coagulating milk among Danish dairy cows and had as its aim to investigate the variation in coagulation properties at individual cow's level in a herd representing the three major dairy breeds in Denmark using the new free oscillation-based rheometry method, ReoRox. The results of this screening are presented here.

2 Materials and methods

2.1 Animals

Fifty-eight Danish Holstein-Friesian cows (SDM), 53 Danish red and white (RDM), and 40 Danish Jersey cows (JER) from the resident herd at the Danish Cattle Research Center (KFC, Tjele, Denmark) were used in the screening trial. The cows

were housed in a loose housing system in three groups, and each group was milked by an automatic milking system (AMS; Voluntary Milking System, DeLaval, Tumba, Sweden). The cows were fed ad libitum of a total mixed ratio and supplemented with concentrate according to standard practice. All cows received the same lactation diet for ad libitum intake throughout the experimental period post calving. A daily feed refusal of 5% or more was the aim for ad libitum fed. The lactation diet was formulated to fulfill the requirements of the Danish norms (Strudsholm F et al. 1999). A free cow traffic system was practiced in all three groups. During the first 4 weeks of lactation, cows were fetched for milking if >7 h had passed since their last milking. This was done twice daily; in the morning and in the afternoon. Likewise, cows in weeks 4–7 of lactation were fetched for milking if >14 h had passed since their last milking. To prevent overmilking, a minimum of 6 h after the preceding milking event had to pass before any cow was allowed another access to the AMS. Information regarding daily milk yields (kilograms), the milk yield (kilograms) in the actual milking, parity (1–4), lactation stage (weeks, days), and time since last milking (hours) was registered for each milk sample collected.

2.2 Milk samples

A total of 151 individual milk samples from the three breeds were collected from whole morning milking from August to October 2007. All cows were sampled once during this period. From each cow, a sample of milk (250 mL) was manually collected from the total milk yield of one milking event. The milk samples were immediately refrigerated at 5 °C for 1–4 h without preservatives before preparation for rheological analyses. Each milk sample was analyzed for concentration of milk fat, protein, casein, lactose, and urea by Milkoscan FT 6000 (Foss Electric, Hillerød, Denmark) and for somatic cell count using Fossomatic 5000 (Foss Electric) at Eurofins Laboratory (Holstebro, Denmark). Thirteen of the 151 milk samples had somatic cell counts above $500 \times 10^3 \text{ mL}^{-1}$, and these were excluded from the data analysis.

2.3 Dynamic rheological analysis

Measurements of the coagulation properties (rennet coagulation time (RCT), curd firming rate (CFR), and coagulum strength) were performed by continuous measurement during renneting using a ReoRox4 rheometer (Medirox, Nyköping, Sweden). Ten milliliters of milk was skimmed by centrifugation at $200 \times g$ for 20 min at 4 °C and adjusted to pH 6.5 with 10% (v/v) lactic acid. The milk sample was then pre-incubated for 30 min in a water bath at 33 °C before addition of $0.038 \text{ IMCU mL}^{-1}$ milk of ChyMax Ultra (Christian Hansen Laboratories A/S, Denmark). Simultaneous with the addition of the coagulant, an empty disposable polypropylene bob-cup with a diameter of 12 mm was placed in the ReoRox, thereby defining $t=0$. Immediately thereafter, 1 mL of sample was transferred to the bob-cup, and rheological measurements were performed at 33 °C for 40 min.

The free oscillation in ReoRox4 is initiated by a forced turn of 2° around the rotational axis, corresponding to a strain of 0.07, with 2.5-s intervals. After a brief holding time, the cup is released and allowed to oscillate freely along the vertical axis with a fixed oscillation frequency (f) of 10 Hz (angular frequency of $\omega=63 \text{ s}^{-1}$).

During the development of the milk coagulum, the frequency and the amplitude of oscillations of the sample cup are affected. The damping of the oscillations and frequency are measured by a photo-optic detector and presented as a function of time by the software GHI ReoRox4 viewer (v2.11k, Medirox). The software allows the calculation of the development of the storage modulus G' , the loss modulus G'' , and the complex modulus $G^*=(G'^2+G''^2)^{0.5}$, as well as the phase angle θ , where the phase difference $\tan \theta=G''/G'$.

The rennet coagulation time (RCT), the maximum coagulum strength by storage modulus G' (G'_{\max}), and curd firming rate, CFR, in the linear region of the coagulation curve were selected for description of the coagulation properties of the individual milk samples (Fig. 1). The RCT was defined as the time where $\theta=45^\circ$. The CFR, $[\Delta G'/\Delta t]_{\text{lin}}$, was calculated from consecutive points of the linear part of the gelation profile whereas the G'_{\max} value equals the highest value of the storage modulus G' obtained within the 40 min of measurement (Fig. 1). While the ReoRox4 instrument was developed with blood coagulation in mind and reports moduli in units of Pascal (Pa), we found the reported moduli, when measuring milk coagulation, to be considerably higher than what has been previously obtained using traditional controlled stress or strain rheometers. For this reason, it was decided to report moduli in “ReoRox Units” rather than in Pa. Each milk sample was measured in duplicates, and the mean coefficient of variation within the estimated traits, RCT, CFR, and G'_{\max} , were 5.0%, 7%, and 11.9%, respectively.

To compare the ReoRox rheometer with a conventional rheometer, two milk samples, prepared for analysis as described above, were measured in parallel by the

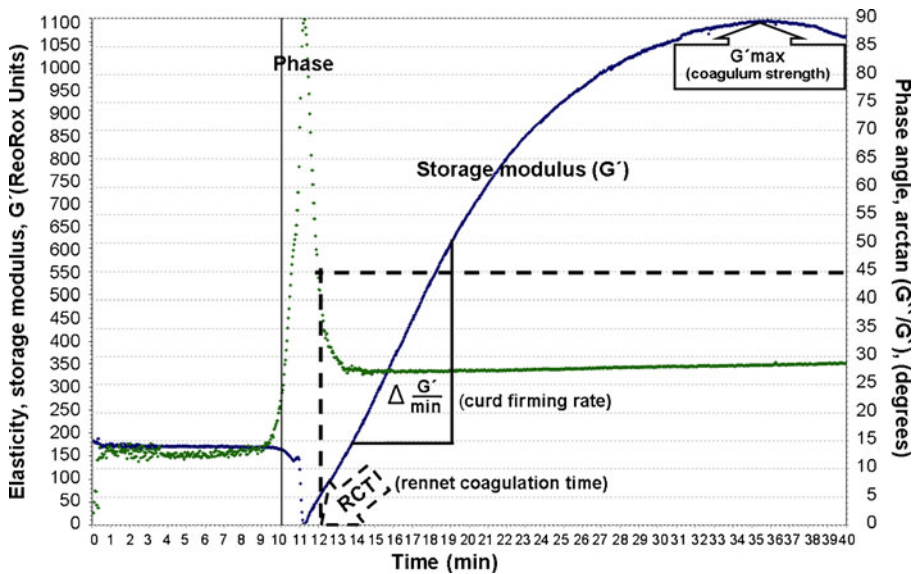


Fig. 1 The ReoRox output exemplifying parameters of coagulation properties. The rennet coagulation time, RCT, was defined as the time where the phase angle $\theta=45^\circ$. The curd firming rate was calculated from three consecutive points of the linear part of the gelation profile ($[\Delta G'/\Delta t]_{\text{lin}}$). The maximum coagulum strength value, G'_{\max} , was defined by the highest value of the storage modulus G' obtained within the 40 min of measurement

ReoRox4 and a TA 2000 rheometer (oscillation stress (0.03 Pa), 1 Hz, 40–60 min; TA Instruments, New Castle, Delaware (USA)).

2.4 Statistical analysis

Analysis of variance The GLM procedure from SAS (version 9.1.3, SAS Institute Inc., Cary, NC) was used to calculate the least squares means (LS-means) and standard errors (SE) and for testing of significant differences in the coagulation traits, RCT, G'_{\max} , CFR, and adjusted CFR (Fig. 2), as well as differences in the analyzed traits presented in Table 1, between the three breeds. A model was applied for studying the effect of breed and lactation state on the traits listed in Table 1, and days of lactation were included as a covariate in the statistical model to adjust the main effects. The two-way interactions between lactation day and breed and parity and breed were included in the model, but were found not to be significant. Significant differences between LS-means were evaluated based on F values.

Regression analyses Simple linear regression analyses (SAS) was used to detect and model the dependence of G'_{\max} on RCT and CFR; and the dependence of CFR on RCT for each of the three breeds by providing the linear least squares fit to the data (Fig. 3).

Expecting the curd firming rate (CFR) to depend on a power higher than one on the protein concentration (Garnot et al. 1982), Excel's Solver component was used to fit a model of the type $\text{CFR} = \alpha * \text{protein}\%^\beta$ across all three breeds by minimizing the error sum of squares.

3 Results

3.1 Variations in milk composition between breeds

Means and measures of the analyzed traits for the milk samples ($n=138$) are listed by breed in Table 1. The SDM and RDM breeds only differ significantly from each other in four of the 18 variables listed in the Table—lactation day, urea content, RCT, and the daily milk yield. On the contrary, Jersey cows are significantly different from both SDM and RDM in 13 of the listed traits. As expected, the Jersey cows have a significantly lower milk yield per day ($P<0.001$) compared with SDM and RDM, but deliver milks with a significantly higher content of protein, casein, and fat ($P<0.001$). By calculating the daily output of casein and protein, however, the JER cows have significantly lower values than both SDM and RDM ($P<0.001$). The proportion of the protein being casein was significantly lower in milk from Jersey cows ($P<0.001$). The breed, stage of lactation, and lactation day of the cows all influence the parameters describing the daily casein output and the content of fat, protein, and casein. The lactation number also influences the milk yield, the somatic cell count as well as the pH. The breed influences the RCT, the casein number, and the lactation day, whereas, the pH, somatic cell count, and time since last milking do not differ between breeds in the investigated herd. The parameter, lactation day,

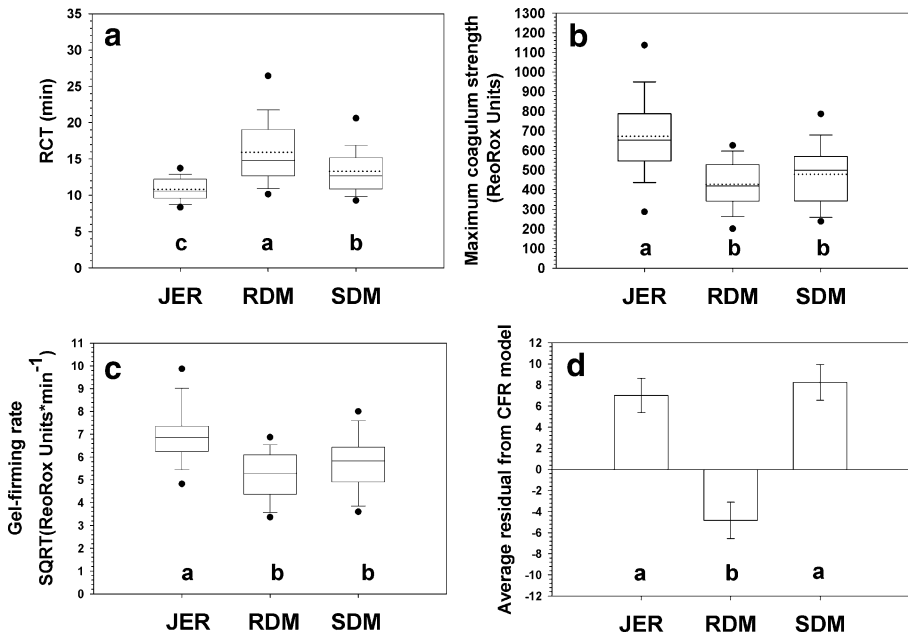


Fig. 2 Comparison of renneting properties of skimmed milk samples from individual cows between breeds: Danish Holstein-Friesian (SDM), Jersey (JER), and Danish red and white (RDM). A, B and C; by analysis of variance the calculated least squared means of the three coagulation traits, rennet coagulation time (RCT), maximum coagulum strength (G'_{max}), and curd firming rate (CFR), respectively. Different letters indicate significant differences (min $P < 0.01$). The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, the punctuated line indicates the means and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) indicate the 90th and 10th percentiles and the 5th and 95th percentiles are shown as filled circles. D; plot of the average residuals representing the difference between the observed CFR and the predicted CFR from the model $CFR = \alpha * \text{protein} \%^{\beta}$, using values of α and β of 0.737 and 3.06, respectively

shows no significant association with the milk yield, the casein number, the RCT, or the pH of the milk.

3.2 Variation in milk coagulation properties between breeds

The coagulation properties of the individual milk samples were assessed by comparing the three coagulation traits: the rennet coagulation time (RCT), the curd firming rate (CFR), and the coagulum strength (G'_{max}) from the rheological analysis (Table 1 and Fig. 2). The strength of the coagulum was, besides the ReoRox measurements, visually evaluated at the end of analysis time, and it was observed that a coagulum of a final strength below 350 ReoRox units was characterized by a loose or weak aggregate. In a separate study where the coagulation process was followed by an experienced dairy technologist in parallel with the ReoRox4 analyses, we found that, at the time of cutting, the samples all had coagulum-strength values above 220 ReoRox units (data not shown). According to these observations, we found that of the 138 milk samples, a total number of 4, corresponding to 3%, did not aggregate to form an adequate curd within the 40 min of measurement (two

Table 1 Concentrations and comparisons of analyzed traits regarding production, composition, and coagulation properties in milk from cows of Danish Holstein-Friesian (SDM), Jersey (JER), and Danish red (RDM)

	SDM			JER			RDM			Influence/effect of		
	Least squares means±standard error	LSM±SE (n=53)	Number of individuals (n)	Least squares means±standard error	LSM±SE (n=38)	Number of individuals (n)	Least squares means±standard error	LSM±SE (n=47)	Number of individuals (n)	Breed	Lactation day	Lactation number
Daily milk yield	kg	31.64±1.15 a		21.21±1.2 c			28.72±1.16 b		***	***	n.s.	
Milk yield	kg	14.50±0.68 a		9.21±0.71 b			14.21±0.68 a		***	n.s.	***	
Lactation day	–	204.07±13.39 a		134.66±15.81 b			153.15±14.21 b		**	–	n.s.	
Time since last milking	h	11.53±0.86		10.96±0.9			12.77±0.86		n.s.	**	n.s.	
Protein%	g·100 g ⁻¹	3.47±0.053 b		3.81±0.056 a			3.52±0.052 b		***	***	**	
Fat%	g·100 g ⁻¹	3.88±0.13 b		5.06±0.14 a			3.84±0.13 b		***	***	*	
Casein%	g·100 g ⁻¹	2.78±0.05 b		2.98±0.06 a			2.79±0.05 b		**	***	*	
Milk protein output ((protein%×10)×daily milk yield)	g	1,002.53±49.39 a		741.59±51.49 b			974.83±49.57 a		***	***	n.s.	
Casein output ((casein%×10)×daily milk yield)	g·day ⁻¹	868.31±34.31 a		635.49±35.46 b			795.32±33.09 a		***	***	**	
Casein number (casein%/protein%)	–	0.80±0.004 a		0.78±0.004 b			0.80±0.004 a		***	n.s.	n.s.	
Urea	mmol·L ⁻¹	4.42±0.13 b		4.36±0.13b			5.10±0.13 a		***	*	n.s.	
SCC (somatic cell count)	(log·mL ⁻¹)	1.81±0.06		1.66±0.06			1.79±0.06		n.s.	*	**	
Lactose	g·100 g ⁻¹	4.73±0.06 a		4.11±0.06 b			4.77±0.06 a		***	**	n.s.	
RCT (rennet coagulation time)	min	13.1±0.62b		10.73±0.65 c			15.77±0.63 a		***	n.s.	n.s.	
Coagulum strength	ReoRox units	495.31±27.07 b		705.71±28.22 a			463.10±27.16 b		***	**	n.s.	
Curd firming rate (CFR)	ReoRox units·min ⁻¹	34.33±2.31 b		53.46±2.41 a			31.63±2.32 b		***	***	n.s.	
pH	–	6.68±0.009		6.70±0.01			6.68±0.009		n.s.	n.s.	**	

n.s. not significant ($P>0.05$)

The different lowercase letters indicate significant differences in rows (min $P<0.05$)

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

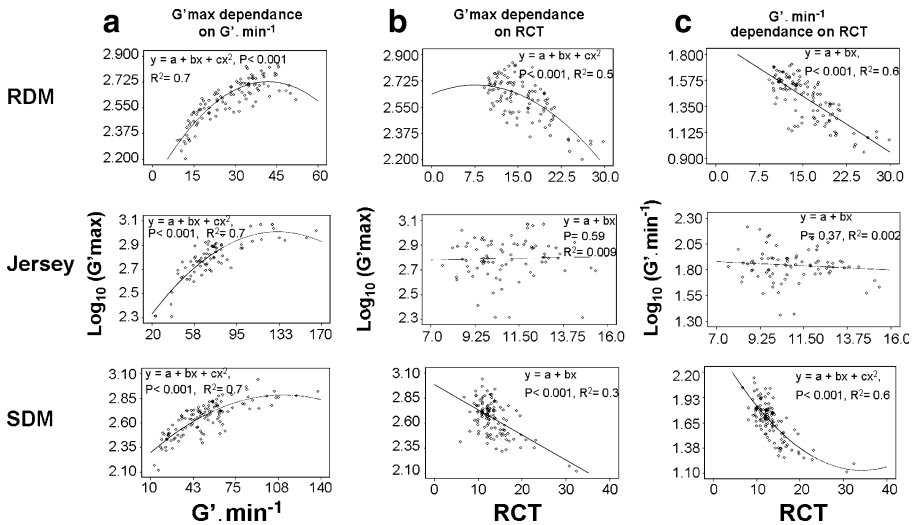


Fig. 3 Regression analysis exemplifying the relationship between the three coagulation parameters; rennet coagulation time (RCT), maximum coagulum strength (G'_{\max}), and curd firming rate (CFR), by breed. The line or curve passing through the data points indicates the best fit of displayed models

samples from RDM, one from Jersey, and one from SDM). The G'_{\max} values of these four samples were all below 200 ReoRox units. Furthermore, 24 samples (17%) formed a weak coagulum, with final coagulum-strength values below 350 ReoRox units (10 samples from RDM, 1 from Jersey, and 13 from SDM).

The differences in the coagulation properties, i.e., RCT, CFR, and the coagulum strength of milk samples, from each of the three breeds are depicted in Fig. 2, and by analysis of variance, the calculated LS-means of the three coagulation traits between the breeds were compared (Table 1 and Fig. 2). The RCT differs significantly between all breeds, whereas the milk from RDM and SDM has comparable coagulation properties with respect to CFR and coagulum strength. In contrast, the coagulation time of SDM milk is shorter than in milk from RDM cows. The milk samples from Jersey cows have significantly better coagulation properties than milk from cows of either the other two breeds, i.e., the coagulation time is shorter, and the resulting curd is both firmer and formed with a higher rate.

As aggregation is a bimolecular process, we expected CFR to depend on a power higher than one of protein concentration. The data therefore fitted the model describing the relation between CFR and protein concentration ($\text{CFR} = \alpha \cdot \text{protein}\%^\beta$). Using this model, α was determined to be 0.737 and a β value of 3.06, with an R^2 of 0.470. This shows that CFR depends on a power of three of the protein concentration of the milk used for coagulation. The residuals, i.e., the difference between the observed CFR and the predicted CFR from the model, represent the part of the CFR that is not accounted for by protein content. From the average residuals for each of the three breeds and their corresponding standard deviations (Fig. 2d), it can be seen that Jersey and SDM cows systematically gave higher than expected CFR based on their protein content, while RDM gave lower CFR values. This shows that in Jersey and SDM, other factors than protein concentration itself are of significance for CFR.

We investigated the correspondence of measurements carried out on the ReoRox apparatus with rheological measurements from a conventional rheometer. We found the estimate of RCT to be linearly positively correlated with the gelation time measured by the rheometer. Also, the estimates of the coagulum strength values, ReoRox units versus Pascal units, were seen to be positively associated, although not in a simple linear manner (data not shown). In order to implement the ReoRox instrument for rheological analyses of milk coagulation, further research is needed to establish its exact correlation to conventional rheometers.

3.3 Correlation between coagulation parameters

The CFR and the coagulum strength (G'_{\max}) was found to correlate positively in milk from all three breeds ($r^2=0.7$, $P<0.01$; Fig. 3a). Furthermore, a negative correlation was observed between RCT and the gel strength (Fig. 3b) and between RCT and the curd firming rate (Fig. 3c) in milk from SDM and RDM cows ($r^2=0.6$, $P<0.01$), while no significant correlation was obtained for the Jersey cows between these traits. Hence, the milk samples from RDM and SDM cows having a short coagulation time also yield a firmer coagulum, with a higher rate of formation than milk samples with a longer coagulation time.

4 Discussion

It is well recognized that Jersey milk as compared to Friesian milk has a higher fat content, and that the milk possesses better cheese-making properties, mainly caused by the higher total protein content of the milk (Auldust et al. 2004). In addition to increasing the cheese yield, the higher protein content in particular will improve the coagulation properties of the milk (Zoon et al. 1988a; Zoon et al. 1988c), as confirmed here. The relationship between CFR and protein content in the milk samples was expressed as a polynomial equation across the breeds, $\text{CFR}=\alpha*\text{protein}\%^\beta$. According to this equation, we found the exponent β to be 3.06. This value is in good agreement with previously reported values of 2.4 and 2.6 for bulk milk, indicating a similar relationship (Garnot et al. 1982; Zoon et al. 1988b), and strengthens the bimolecular nature of the aggregation. The plot of the residuals, i.e., the difference between the observed and predicted CFR using this model, showed that in Jersey and SDM, other factors than the protein concentration itself contribute positively to the CFR, like, e.g., protein composition and/or specific genetic variants, while in RDM, other factors than protein concentration contributed negatively to CFR. Elucidation of these factors would require further studies on the detailed milk and milk protein composition.

The casein number in milk from Jersey cows was found to be significantly lower than for milk of SDM and RDM cows (Table 1). This result is in contrast with other reports that indicate a generally higher casein number for Jersey milk (Ng-Kwai-Hang et al. 1986; NgKwaiHang and Kim 1996; Sorensen et al. 2003). Even though the lactation stage is known to affect the casein number of cow's milk (Barbano et al. 1991; Blake et al. 1980), this was in the present analysis not found to be significant, perhaps because the relationship between casein number and lactation stage is complex and cannot be assumed to be the same for all breeds.

The dissimilar correlation patterns of coagulation parameters between the three breeds (Fig. 3) as well as previous observations on poorly coagulating milk samples with normal RCT, but low curd firming rates (Wedholm et al. 2006), support our choice to use the CFR as a discriminative parameter for coagulation properties of milk samples, instead of the RCT, which was otherwise used in a number of publications. For future evaluation of coagulation properties of milk from different breeds, the CFR should be used as the discriminator, since also the curd firmness, but not the RCT, has been shown to be positively associated to the total protein content in milk (Hansen et al. 2002; Okigbo et al. 1985c). Although it has been shown that coagulation properties of milk shows large lactational changes (Coulon et al. 1991; De Marchi et al. 2007; Okigbo et al. 1985c), several reports find the differences in RCT and in maximal curd firmness in relation to lactation stage to be nonsignificant whereas curd firming rates were found to be significantly different between lactation periods (Coulon et al. 1998; Summer et al. 2003). Whether the poorer coagulating property of milk from Danish Holstein cattle (SDM) is caused by the extensive breeding for a higher yield in this breed needs further investigations, but it has already been suggested that this dairy character should be given a negative rather than a positive weight in the breeding goal (Hansen et al. 2002).

More than 20% of the milk samples measured by ReoRox4 exhibited poor coagulation properties by the criteria set. The underlying reasons to this phenomenon are currently under investigation.

Of the 138 samples, 3% failed to form an adequate curd within the 40 min of measurement and would as such be considered as non-coagulating samples. The prevalence of non-coagulating (NC) milk from cows of Danish Dairy breeds is thus lower than reported for milk from Finnish Ayrshire cows (Tyriseva et al. 2003) and more similar to what has been reported for Estonian Dairy cows (Joudu et al. 2008). Recently, two candidate genes associated with NC milk were identified in Finnish Ayrshire Cows (Tyriseva et al. 2008). A genetic factor in the occurrence of NC milk does not exclude influence of environmental factors, like feeding, or other production factors and udder health. Based on logistic regression analyses, the highest risk of a Finnish Ayrshire cow producing NC milk was thus at peak lactation or in mid-lactation and, consequently, high milk yield, low fat, and protein content increased the risk of non-coagulation (Zoon et al. 1988a). Fortunately, it has been shown that addition of calcium can restore the coagulation of NC milk (Van Hooydonk et al. 1986), although not to the level of well-coagulating milk (Okigbo et al. 1985b).

5 Conclusion

The variation in coagulation properties of individual cow's milk in a herd of three Danish dairy breeds was measured by a novel free oscillation method that can be used for larger screening studies of coagulation properties. The paper reports on the frequency of non- and poorly coagulating milk in the herd. More than 20% of the samples were characterized by non- or poor coagulation properties, and of these, 3% were due to non-coagulating milk that did not aggregate to form a curd. The paper gives a basis for the subsequent selection of non-coagulating milk samples with the aim to look in more detail for the underlying reasons for the phenomenon of non-coagulating milk.

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