International Dairy Journal 88 (2019) 18–24

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Effect of milk protein genetic polymorphisms on rennet and acid coagulation properties after standardisation of protein content

Isaya Appelesy Ketto [a, *], Ahmed Abdelghani [a], Anne-Grethe Johansen [a, b], Jorun Øyaas [c], Siv B. Skeie [a]

[a] Faculty of Chemistry, Biotechnology and Food Science (KBM), Norwegian University of Life Sciences (NMBU), 5003, N-1432 Ås, Norway
[b] TINE SA R&D, 7 Kalbakken, 0901 Oslo, Norway
[c] TINE Meieriet Tunga, Filterfermentor, 2490, Suppen 7005, Trondheim, Norway

ARTICLE INFO

Article history:
Received 4 June 2018
Received in revised form
11 August 2018
Accepted 11 August 2018
Available online 26 August 2018

ABSTRACT

The effects of milk protein genetic polymorphisms on the rennet and acid coagulation properties of milk after protein standardisation were investigated. Skim milk samples were adjusted to a protein concentration of 6.07 ± 0.06% by ultrafiltration (UF) before evaluating rennet coagulation and acid coagulation properties. Only the β-lactoglobulin (β-LG) genotypes influenced the rennet-clotting time before standardisation for the total protein concentration by UF; however, this effect was confounded with the β-LG concentration. After UF-concentration, a similar protein concentration between the samples was achieved in the retentate, then the rennet clotting time and rennet curd firmness at 30 min were significantly influenced by both the κ-casein (κ-CN) and β-LG genotypes. κ-CN genotypes significantly influenced the acid coagulation properties of both skim milk and retentate. Variations in the concentration of milk proteins (mostly α_{S2}-CN-12P) explained most of the differences in the rennet and acid coagulation properties of milk after protein standardisation by UF.

1. Introduction

The influence of milk protein genetic polymorphisms on milk composition and its coagulation properties is well documented in the literature. Improved rennet coagulation properties, such as a shorter rennet clotting time and higher curd firmness 30 min after rennet addition, have been shown for the α_{S1}-casein (α_{S1}-CN) C variant and the B variants of κ-CN, β-CN and β-lactoglobulin (β-LG) (Hallén, Allmere, Näslund, Andrén, & Lundén, 2007; Jõudu et al., 2007; Ketto et al., 2017). Ketto et al. (2017) reported a shorter gelation time and higher gel firmness at 60 min with κ-CN AA compared with the AB and BB genotypes after acidification of milk using glucono-δ-lactone. However, these studies on the effects of milk protein genetic polymorphisms on the rennet and acid coagulation properties of milk have been based on milk samples differing in protein concentration. For example, Ketto et al. (2017) investigated the effects of milk protein polymorphism on milk coagulation properties in milk varying in protein content from 2.59 to 3.96%. In fact, the B variants for both κ-CN and β-LG are associated with a higher concentration of total protein, κ-CN and fat concentration in addition to smaller casein micelle size (Bonfatti, Di Martino, Cecchinato, Vicario, & Carnier, 2010; Ikonen, Ojala, & Ruottinen, 1999), and these factors have been reported to influence the milk coagulation properties. In addition, the α_{S1}-CN BC genotype was associated with a higher milk protein percentage compared with the BB genotype, which was associated with a higher milk yield (Aleandri, Buttazzoni, Schneider, Caroli, & Davoli, 1990; Ng-Kwai-Hang, Hayes, Moxley, & Monardes, 1984).

Despite many reports on the effect of milk protein genetic polymorphisms on rennet coagulation properties, there is a lack of knowledge about the effects of milk protein genetic polymorphisms on the rennet and acid coagulation properties of milk at similar protein concentrations. Hence, in the current study the effects of milk protein polymorphisms on the rennet and acid coagulation properties of milk at equal protein content was investigated. In the present study, the total protein content of individual milk samples was standardised using a laboratory-scale ultrafiltration (UF) process to determine if milk protein genetic polymorphisms would still influence milk coagulation.

* Corresponding author. Tel.: +47 67232597.
E-mail addresses: isaya.ketto@nmbu.no, isayaketto@ymail.com (I.A. Ketto).

https://doi.org/10.1016/j.idairyj.2018.08.008
0958-6946/

2. Materials and methods

2.1. Blood samples and genotyping

Blood sampling, DNA sequencing and genotyping were performed as previously described by Ketto et al. (2017). In brief, the Norwegian Sequencing Centre, Oslo, Norway, performed DNA sequencing using a Hiseq 2500 platform (according to the manufacturer's protocol). After DNA sequencing, all reads were aligned to the bovine reference genome UMD 3.1 using BWA-mem version 0.7.10. Variant calling was performed using Freebayes version 1.0.2 (Garrison & Marth, 2012). Nine non-anonymous missense single nucleotide polymorphism (SNPs) were identified. Cows were genotyped for the identified SNPs using the MassArray genotyping platform (Agena Biosciences, San Diego, CA, USA).

2.2. Milk samples

Individual milk samples were collected from eighteen (18) Norwegian Red (NR) cows with similar genotype for β-CN (A^2A^2) and different genotypes of α_{S1}-CN, κ-CN and β-LG i.e., BB or BC, AA or BB and AB or BB respectively (Table 1). These cows belonged to the Centre for Animal Research (SHF) of the Norwegian University of Life Sciences (NMBU). The cows were excluded from the milking robot in the evening 10 h before milking, and the cows were milked individually in the morning in a separate milking parlour as described by Ketto et al. (2017). Immediately after milking, the milk samples were transported to the Faculty of Chemistry Biotechnology and Food Science (KBM) for milk processing and laboratory analyses. Milk treatments and analyses were made on the individual milk samples with the stated genetic composition. At the dairy pilot plant, milk samples were pre-heated to 55 °C before cream separation. Cream separation was done by using a 10-L batch electrical cream separator (Janschitz GmbH., Althofen, Austria). After cream separation, skim milk was analysed for fat, protein, lactose, and casein using a MilkoScan FT1 (Foss Electric A/S, Hillerød, Denmark). Milk pH was measured at 20 °C using a PHM61 pH meter (Radiometer, Copenhagen., Denmark).

Table 1
Number of cows on each genotype of caseins and β-LG.

Protein	Genotype	Number of cows
α_{S1}-CN	BB	12
	BC	6
β-CN	A^2A^2	All cows (18)
κ-CN	AA	7
	BB	11
β-LG	AB	7
	BB	11

2.3. Milk UF-concentration

Immediately after cream separation, UF-concentration was performed on the skim milk using a Labscale™ TFF system (Millipore, Oslo, Norway), with a Pellicon ® XL Cassette and a Biomax membrane 500 kDa (Cat number: PBX500C50; Millipore), corresponding to a pore size of 0.02 μm. The skim milk samples (55 °C) were mixed gently to ensure homogeneity within the sample before UF-concentration. After mixing, the sample was poured into a 500-mL measuring cylinder and placed in a temperature-controlled water bath at 55 °C. Before the UF-concentration process, the system was flushed using another batch of the milk to be concentrated to ensure that the system was free from reagents used during cleaning. UF-concentration of the skim milk sample was performed at 50 °C at a pressure varying between 2 and 3 bar.

The retentate was analysed for casein concentration using a MilkoScan FT1 (Foss Electric A/S), and UF-concentration proceeded until the casein concentration of the retentate was ~4.5%. After UF-concentration, retentate and permeate were collected for further analyses, i.e., total protein concentration, mineral concentration (Ca, Mg and P), milk protein composition and acid and rennet coagulation properties. The samples for milk protein and mineral composition were frozen at −18 °C before analysis. Between samples, 0.1 N NaOH was used to clean the Labscale™ TFF system for about 30 min, followed by distilled water.

2.4. Total protein and milk minerals (Ca, Mg and P)

The total protein concentration of the skim milk, retentate and permeate was determined by the Kjeldahl method as described by IDF (2001). The concentrations of Ca, Mg and P in the skim milk, retentate and permeate were analysed by an 8800 Triple Quadrupole ICP-MS (Agilent Technologies, Tokyo, Japan), with WRM®-BD150 and CRM 063R (Institute of Reference Materials and Measurements, Geel, Belgium) used as reference materials for mineral quantification (Jørgensen et al., 2015).

2.5. Casein micelle size

The average diameter of the casein micelles in skim milk and retentate was determined by photon correlation spectroscopy (PCS) using a Zetasizer 3000HS (Malvern Instruments Ltd., Malvern, UK) as previously described by Devold, Brovold, Langsrud, and Vegarud (2000). Samples were diluted by using simulated milk ultrafiltrate (SMUF), prepared according to Jenness and Koops (1962). Before dilution, the SMUF was filtered through a 0.22-μm filter (Milex® GP, Millipore Ltd., Cork, Ireland). After dilution, the samples were filtered through 0.8-μm filters (Milex® GP, Millipore Ltd), transferred to polystyrene cuvettes (DTS0012, Malvern Instruments GmbH, Herrenberg, Germany) and heated at 26 °C for 5–10 min before measurement. During measurement, the light was scattered at a 90° angle at a constant temperature of 25 °C. Three measurements (each of 10 scans) were made for each sample, the average was used.

2.6. Milk protein composition

Milk protein composition was analysed in the frozen milk samples by capillary electrophoresis (CE) by using an Agilent G1600AX equipped with Agilent ChemStation software (Agilent Technologies, Germany) as described previously (Jørgensen et al., 2016; Ketto et al., 2017). Relative concentrations of α-LA, β-LG, α_{S1}-CN, α_{S2}-CN, κ-CN, and β-CN were calculated according to Heck et al. (2008). Because all samples were β-CN A^2, β-CN appeared as one single peak; hence, the relative concentrations of all minor peaks between the major κ-CN peak and β-CN A^2 were summed-up with the relative concentration of major κ-CN (i.e., κ-CN-1P) to estimate the total κ-CN. The relative concentration (%) of each protein identified by CE in each sample was calculated on the basis of the total protein concentration of each sample as analysed by Kjeldahl as described by Jørgensen et al. (2016).

2.7. Rennet coagulation properties

Rennet coagulation properties of the skim milk and retentate were analysed by Formagraph (LAT; Foss-Italia SpA, Padova, Italy) as described previously (Inglingstad et al., 2014; Ketto et al., 2017). In brief, samples (10 mL) were tempered at 63 °C for 30 min, cooled

to 32 °C, and then incubated at 32 °C for 30 min before addition of 200 μL of rennet (CHY-MAX; Chr. Hansen A/S, Hørsholm, Denmark), which was prepared by dilution (1:50) with acetate buffer (pH 5.6). The following parameters were obtained from the Formagraph: rennet-clotting time (RCT, min), a maximum slope of the coagulation curve (curd-firming rate (CFR, mm min^{-1})) and the width of the curves at 30 min (curd firmness at 30 min ($a_{30,}$ mm)). All measurements were made in triplicate.

2.8. Acid coagulation properties

Acid coagulation properties of the skim milk and retentate after UF-concentration were analysed simultaneously by using low strain amplitude oscillatory test by using a Physica MCR301 rheometer (Anton Paar GmbH, Graz, Austria) with a bob-cup measurement system and a Formagraph (LAT; Foss-Italia SpA) as described by Ketto, Schüller, Rukke, Johansen, and Skeie (2015). In brief, milk samples were heat treated at 95 °C for 5 min before cooling to 32 °C in ice water. For both methods, milk samples were acidified with 3% of glucono-δ-lactone (GDL) and then mixed simultaneously for 15 s before the acid coagulation trials. Acid coagulation was monitored for 60 min at 32 °C. Strain sweep (0.05–100%, strain and 10 rad s^{-1}, frequency) was carried out to determine strain value within the linear viscoelastic region (LVR).

A constant strain from strain sweep, below the upper limit of LVR (0.1%) was used when monitoring the acid coagulation process at 10 rad s^{-1}. Gelation time (GT) from low strain amplitude oscillatory test was defined as the time from acidification to the time when the elastic modulus (G') was ≥1 Pa, while on the Formagraph, GT was defined as the time-interval between acid addition and the time when the width of the bifurcate increased to 1.2 mm. The GFR (gel-firming rate) was defined as the maximum slope of G' versus time (Pa min^{-1}) and G versus time (mm min^{-1}) curves for the low strain amplitude oscillatory test and Formagraph, respectively. Final gel firmness (G60) was recorded at 60 min in Pa (by the low strain amplitude oscillatory test) and mm (by the Formagraph). Each sample was analysed once in the low strain amplitude oscillatory test and three times in the Formagraph.

2.9. Statistical analysis

Statistical analysis was performed using a mixed procedure in SAS (SAS, 2015) to study the effect of casein genotypes (α_{S1}-CN, κ-CN) and β-LG on the rennet and acid coagulation properties of the skim milk and retentate after UF-concentration. The following statistical model was used:

$$Y = X\beta + Zu + \text{residual}$$

where: Y = vector for the response variable (e.g., rennet or acid coagulation properties of the skim milk and retentate or the content of α-LA, β-LG, α_{S2}-CN, α_{S2}-CN-10P, α_{S2}-CN-11P, and α_{S2}-CN-12P, α_{S1}-CN, α_{S1}-CN-8P, α_{S1}-CN-9P, κ-CN and β-CN in the skim milk and retentate); β = unknown vector for the fixed effects (α_{S1}-CN, κ-CN, β-LG genotypes); u = vector for the random variables (Cow: 1, 2, 3, 4…and 18); X and Z = known design matrices for fixed and random effects, respectively.

Statistical analyses were repeated with the milk protein and mineral concentration included in the statistical model as the covariates in $X\beta$ to test if the observed significant effects of milk protein genotypes were confounded with milk protein composition (α-LA, β-LG, α_{S2}-CN (α_{S2}-CN-10P, α_{S2}-CN-11P, and α_{S2}-CN-12P), α_{S1}-CN (α_{S1}-CN-8P, α_{S1}-CN-9P), κ-CN and β-CN) and milk minerals (Ca, Mg and P).

3. Results

3.1. Overall milk composition and pH

There were no differences in pH between the skim milk and retentate after UF-concentration (data not shown). The retentate obtained from the UF-concentration had, as expected, an increased protein (concentration factor, CF ≈ 1.7), casein, calcium and phosphorus concentration ($P < 0.05$; Table 2), and the variation (SD) in protein concentration between the samples was reduced by UF-concentration. The protein content in the skim milk ranged from 2.82 to 3.58%, while in the retentate the protein content ranged from 5.95 to 6.06%. All caseins were retained in the retentate; however, low concentrations of α_{S1}-CN (α_{S1}-CN-8P) and β-CN A^2 were detected in the permeate. The major whey proteins (β-LG and α-LA) were retained in the retentate, but they were present at a higher concentration in the permeate compared with the detected caseins (α_{S1}-CN and β-CN A^2).

Although lactose and fat concentrations did not vary significantly between the skim milk and retentate, the concentration of lactose was slightly reduced in most of the retentate samples, while the fat concentration was slightly increased in all retentate samples (Table 2). Furthermore, the casein micelles had a similar size in the skim milk and retentate (168 ± 11 and 167 ± 13 nm, respectively). Table 3 shows the content of milk proteins by each genotype of α_{S1}-CN, κ-CN and β-LG after UF-concentration of milk. Significant influence ($P < 0.05$) of κ-CN genetic polymorphism were observed on the content of α_{S2}-CN-12P, of α_{S1}-CN and of κ-CN genetic polymorphisms on the content of β-CN and of β-LG genetic polymorphism on the content of β-LG, while the content of α-LA, total α_{S2}-CN, α_{S2}-CN-10P, α_{S2}-CN-11P, total α_{S1}-CN, α_{S1}-CN-8P, α_{S1}-CN-9P, κ-CN were not influenced by milk protein genetic polymorphisms studied. The effects of milk protein genetic polymorphism on the contents of proteins were less pronounced before UF-concentration, the contents of α_{S2}-CN-12P and β-LG were significantly influenced by κ-CN and β-LG genetic polymorphisms respectively (Supplementary material, Table S1).

3.2. Rennet coagulation properties

The α_{S1}-CN genotypes did not influence the rennet coagulation properties of skim milk or the retentate, whereas the κ-CN

Table 2
Overall milk composition between the skim milk and retentate after UF-concentration.[a]

Parameter	Fractions of milk		
	Skim milk (before UF)	Retentate (after UF)	Permeate (after UF)
Milk composition (%)			
Total protein	3.58 ± 0.50	6.06 ± 0.06	0.12 ± 0.05
Casein	2.79 ± 0.29	4.48 ± 0.10	NA
Fat	0.11 ± 0.07	0.17 ± 0.11	NA
Lactose	4.73 ± 0.21	4.69 ± 0.21	NA
Milk minerals (g kg^{-1})			
Calcium, Ca	1.29 ± 0.17	1.96 ± 0.17	0.31 ± 0.05
Magnesium, Mg	0.12 ± 0.01	0.15 ± 0.02	0.08 ± 0.01
Phosphorus, P	0.97 ± 0.07	1.42 ± 0.09	0.35 ± 0.07
Protein composition (%)			
α_{S1}-CN	1.24 ± 0.17	2.13 ± 0.09	0.01 ± 0.01
α_{S2}-CN	0.31 ± 0.09	0.57 ± 0.09	ND
β-CN	1.15 ± 0.17	1.85 ± 0.15	0.01 ± 0.01
κ-CN	0.30 ± 0.07	0.50 ± 0.12	ND
α-LA	0.12 ± 0.02	0.20 ± 0.03	0.04 ± 0.02
β-LG	0.27 ± 0.18	0.44 ± 0.08	0.04 ± 0.02

[a] Values are the means ± standard deviation: ND, not detected; NA, not analysed.

Table 3
Effect of milk protein genotypes on the content of milk proteins of the retentate (after UF-concentration).[a]

Genotypes		Content of milk proteins, %										
		α_{S1}-CN	α_{S1}-CN-8P	α_{S1}-CN-9P	α_{S2}-CN	α_{S2}-CN-10P	α_{S2}-CN-11P	α_{S2}-CN-12P	β-CN	κ-CN	α-LA	β-LG
α_{S1}-CN	BB	1.48 ± 0.27	1.38 ± 0.07	0.44 ± 0.04	0.57 ± 0.01	0.05 ± 0.02	0.02 ± 0.002	0.20 ± 0.01	1.92 ± 0.02	0.46 ± 0.03	0.20 ± 0.01	0.46 ± 0.01
	BC	1.22 ± 0.51	1.30 ± 0.13	0.59 ± 0.07	0.51 ± 0.02	0.07 ± 0.02	0.02 ± 0.002	0.20 ± 0.02	1.77 ± 0.05	0.56 ± 0.03	0.18 ± 0.01	0.49 ± 0.03
	p-value	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
κ-CN	AA	1.11 ± 0.42	1.37 ± 0.11	0.50 ± 0.06	0.49 ± 0.03	0.06 ± 0.02	0.02 ± 0.002	0.17 ± 0.02	1.92 ± 0.04	0.53 ± 0.04	0.19 ± 0.01	0.46 ± 0.02
	BB	1.57 ± 0.33	1.32 ± 0.08	0.53 ± 0.05	0.57 ± 0.02	0.09 ± 0.01	0.02 ± 0.002	0.23 ± 0.01	1.77 ± 0.03	0.49 ± 0.03	0.18 ± 0.01	0.49 ± 0.02
	p-value	NS	NS	NS	NS	NS	NS	***	**	NS	NS	NS
β-LG	AB	1.78 ± 0.42	1.36 ± 0.11	0.53 ± 0.06	0.54 ± 0.04	0.08 ± 0.01	0.02 ± 0.002	0.20 ± 0.02	1.13 ± 0.07	0.48 ± 0.04	0.19 ± 0.01	0.54 ± 0.02
	BB	0.91 ± 0.33	1.33 ± 0.08	0.50 ± 0.04	0.54 ± 0.04	0.07 ± 0.01	0.02 ± 0.002	0.20 ± 0.01	1.14 ± 0.06	0.54 ± 0.03	0.19 ± 0.01	0.41 ± 0.02
	p-value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**

[a] Values are the least square means ± standard error: NS, non-significant; *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Table 4
Effect of milk protein genotypes on the rennet coagulation properties of the skim milk and retentate (before and after UF-concentration, respectively).[a]

Protein	Variant	Skim milk (before UF)			Retentate (after UF)		
		RCT	CFR	a_{30}	RCT	CFR	a_{30}
α_{S1}-CN	BB	18.3 ± 1.0	1.9 ± 0.2	19.1 ± 2.1	16.5 ± 0.6	6.3 ± 0.6	35.6 ± 2.0
	BC	18.9 ± 1.5	2.0 ± 0.4	17.3 ± 2.4	18.5 ± 1.0	5.7 ± 0.9	35.6 ± 3.0
	p-value	NS	NS	NS	NS	NS	NS
κ-CN	AA	17.0 ± 1.2	1.9 ± 0.3	19.3 ± 2.9	15.9 ± 0.9	6.8 ± 0.8	39.8 ± 2.7
	BB	20.2 ± 1.1	2.0 ± 0.3	17.0 ± 2.4	19.1 ± 0.7	5.2 ± 0.6	31.4 ± 2.2
	p-value	NS	NS	NS	*	NS	*
β-LG	AB	16.1 ± 1.4	2.1 ± 0.4	21.0 ± 3.1	16.1 ± 0.9	6.8 ± 0.8	37.4 ± 2.9
	BB	21.0 ± 1.0	1.8 ± 0.3	15.4 ± 2.3	20.0 ± 0.7	5.1 ± 0.6	33.9 ± 2.1
	p-value	*	NS	NS	*	NS	NS

[a] Values are the least square means ± standard error: NS, non-significant, *$P < 0.05$. Rennet coagulation properties of milk fractions as measured by Formagraph [RCT, rennet-clotting time (min); CFR, curd-firming rate (mm min^{-1}); a_{30}, curd firmness at 30 min (mm)].

genotypes significantly influenced the coagulation of the retentate but not of the skim milk (Table 4). Favoured rennet coagulation properties of the retentate (low RCT and high a_{30}; $P < 0.05$) were linked with κ-CN AA compared with the BB genotype. For the β-LG genotypes, however, the RCT of both skim milk and retentate were influenced, and a shorter RCT was observed with the AB compared with the BB genotype ($P < 0.05$). In the retentate, the effect of the κ-CN genotypes on RCT was confounded with the concentration of total α_{S2}-CN and the individual concentration of α_{S2}-CN-10P, 11P and 12P, β-LG and α-LA (Fig. 1). In skim milk, the effect of the β-LG genotypes ($P < 0.05$) on RCT was confounded with its concentration of β-LG (Fig. 2).

3.3. Acid coagulation properties

Only the κ-CN genotypes influenced ($P < 0.05$) the acid coagulation properties of skim milk and retentate (Table 5). In both skim milk and retentate, κ-CN AA was correlated with improved acid coagulation properties (i.e., shorter gelation time (GT), higher gel-firming rate (GFR) and higher gel firmness at 60 min) compared

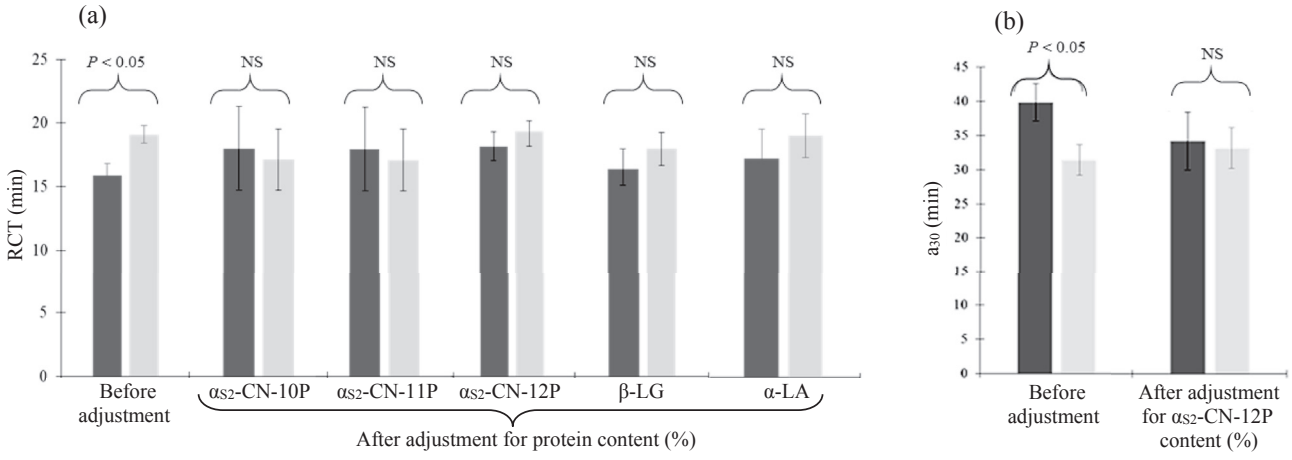

Fig. 1. Effect of κ-CN genotypes (■, κ-CN AA; ■, κ-CN BB) on (a) the rennet clotting time (RCT) of the retentate before and after adjustment for α_{S2}-CN-10P, α_{S2}-CN-11P, α_{S2}-CN-12P, β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) contents and (b) curd firmness (a_{30}) before and after being adjusted for α_{S2}-CN-12P content in the statistical model (NS, non-significant).

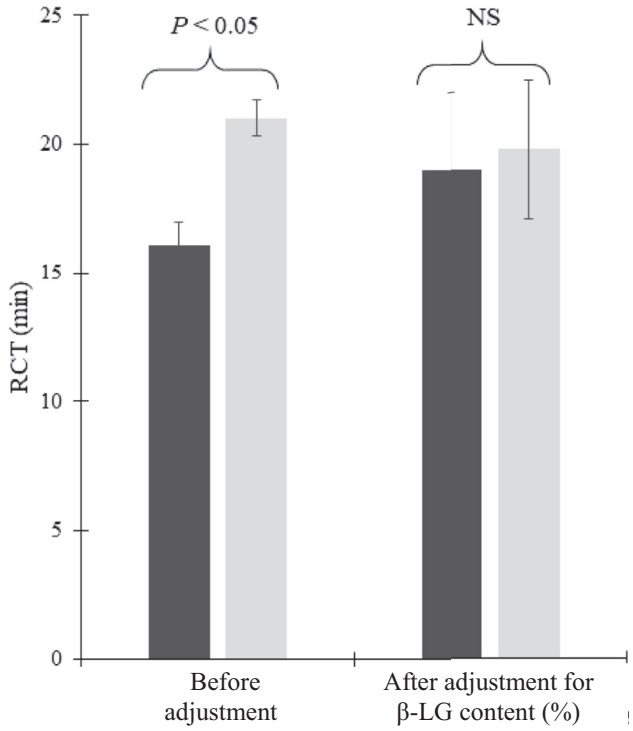

Fig. 2. Effect of β-LG genotypes (■, β-LG AB; ■, β-LG BB) on the rennet clotting time (RCT) of the skim milk before and after adjustment for the β-LG content in the statistical model.

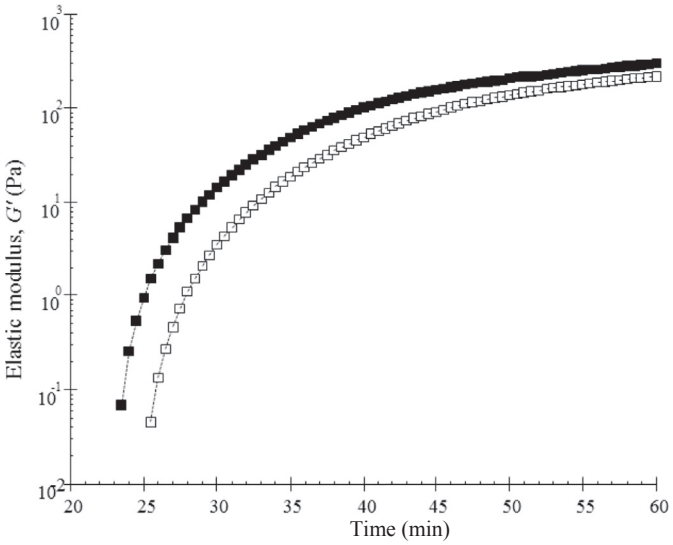

Fig. 3. Acid coagulation pattern obtained from Physica MCR 301 between two samples with different κ-CN genotypes (i.e.: □, AA; ■, BB) and similar genotypes for α_{S1}-CN, β-CN and β-LG (i.e., BC, A^2A^2 and BB, respectively).

with the BB genotype. The acid coagulation results obtained by the low strain amplitude oscillatory test in Fig. 3 corresponded with the results obtained by the Formagraph. In both methods, κ-CN AA was correlated with improved acid coagulation properties of milk. The effects of the κ-CN genotypes on the GT of skim milk were, however, confounded by the inclusion of the concentration of α_{S2}-CN-12P in the statistical model (Fig. 4a). Likewise, in the retentate, the effect of the κ-CN genotype on G60 was confounded by the α_{S2}-CN-12P concentration (Fig. 4b). The concentration of α_{S2}-CN-12P in the skim milk and retentate was significantly ($P < 0.05$) lower in κ-CN AA compared with BB (Fig. 5).

4. Discussion

A membrane with a cut-off as used in the current study (500 kDa–0.02 μm), will allow some of the whey proteins and individual caseins, not associated with the casein micelle, to pass through the membrane (Jørgensen et al., 2016). This may alter the total protein to casein ratio between skimmed milk and retentate as shown in the current study. The fact that the whey protein to casein ratio will influence the firmness of the acid gel network and that an increased casein content will increase the buffer capacity of the milk (Jørgensen et al., 2015), in addition to the large range in total protein content between the different skim milk samples, makes it difficult to compare the coagulation properties of skimmed milk with retentate in this study. The focus of the present study is therefore to determine if milk protein genetic polymorphisms would still influence milk coagulation at a standardised protein concentration.

Improved rennet coagulation properties were obtained in the retentate related to the A variant of both κ-CN and β-LG. This is inconsistent with previous reports performed on the milk at different total protein concentrations (Hallén et al., 2007; Jõudu et al., 2007; Ketto et al., 2017). The aforementioned studies were conducted on milk with different protein contents, i.e., Hallén et al. (2007) reported a protein range of 2.54–4.26% in Swedish Red and Holstein cows, close to the protein range of 2.59–3.96% reported by Ketto et al. (2017) in Norwegian Red cattle and Jõudu et al. (2007) who reported a protein range of 2.5–4.72% in Estonian Native cattle. These studies reported a favourable effect of the B variant of the two proteins (κ-CN and β-LG) on rennet coagulation, probably due to their effects on the total protein content. In the present study, the protein and casein contents in the retentate were

Table 5
Effect of milk protein genotypes on the acid coagulation properties of the skim milk and retentate (before and after UF-concentration, respectively).[a]

Protein	Variant	Skim milk (before UF)			Retentate (after UF)		
		GT	GFR	G60	GT	GFR	G60
α_{S1}-CN	BB	25.5 ± 1.0	2.1 ± 0.1	35.0 ± 2.1	35.6 ± 1.6	1.7 ± 0.1	28.0 ± 2.4
	BC	25.4 ± 1.5	2.0 ± 0.2	35.2 ± 3.3	36.9 ± 2.5	1.8 ± 0.2	30.9 ± 3.7
	p-value	NS	NS	NS	NS	NS	NS
κ-CN	AA	22.5 ± 1.4	2.4 ± 0.2	41.0 ± 3.0	32.5 ± 2.2	2.0 ± 0.2	34.3 ± 3.3
	BB	28.4 ± 1.1	1.7 ± 0.1	29.2 ± 2.4	40.0 ± 1.8	1.4 ± 0.2	24.6 ± 2.6
	p-value	**	**	**	*	*	*
β-LG	AB	25.5 ± 1.5	1.9 ± 0.2	31.5 ± 3.2	36.4 ± 2.3	1.7 ± 0.2	29.1 ± 3.5
	BB	25.4 ± 1.1	2.2 ± 0.1	38.6 ± 2.3	36.1 ± 1.7	1.8 ± 0.1	29.8 ± 2.6
	p-value	NS	NS	NS	NS	NS	NS

[a] Values are the Least square means ± standard error; NS, non-significant, *$P < 0.05$,**$P < 0.01$. Acid coagulation properties of milk fractions as measured by Formagraph [GT, gelation time (min); GFR, gel-firming rate (mm min^{-1}); G60, acid gel firmness at 60 min (mm)].

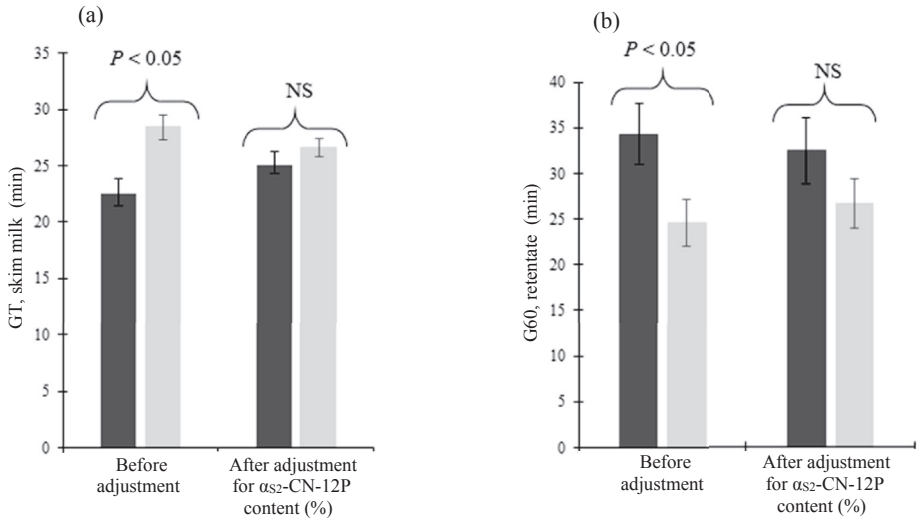

Fig. 4. Effect of κ-CN genotypes (■, κ-CN AA; ■, κ-CN BB) on (a) the gelation time (GT) of the skim milk and (b) the acid gel firmness at 60 min (G60) of the retentate before and after adjustment for the α_{S2}-CN-12P content (NS, non-significant).

standardised to 6.07 ± 0.06% and 4.48 ± 0.10% respectively; this could be the reason for the different findings between the current study and the previous studies.

The negative effect of β-LG BB on the rennet coagulation properties (i.e., rennet clotting time) could be linked to its positive correlation with β-LG content (Ketto et al., 2017), which is negatively correlated with the casein index (%) (Schopen et al., 2011). The significant effect of milk protein genetic polymorphism on the contents of milk proteins was less pronounced in the current study compared with other studies, for example Ketto et al. (2017), probably because of fewer number of cows used in the current study.

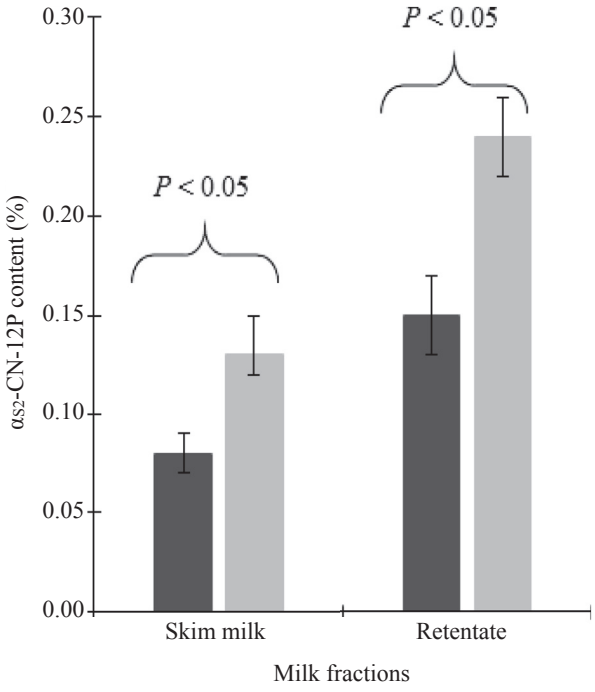

Fig. 5. Variation in α_{S2}-CN 12P content between the κ-CN genotypes (■, AA; ■, BB) in skim milk and retentate.

Marziali and Ng-Kwai-Hang (1986) studied the effects of milk protein genotypes (β-CN, κ-CN, and β-LG) on the rennet coagulation properties of Holstein Friesian milk after adjusting the protein and fat concentrations by using a statistical model. They found that neither β-CN nor κ-CN genetic variants influenced the rennet coagulation properties of the milk; however, the A variant of β-LG was associated with a shorter clotting time and higher curd firmness compared with the B variant. This is in accordance with the current study, which determined a shorter rennet clotting time of skim milk with β-LG AB compared with BB. The observed effects of κ-CN genetic polymorphisms in the current study on the rennet coagulation properties of retentate (RCT and a_{30}) were confounded with the concentration of α_{S2}-CN and its phosphorylation states (10P, 11P, and 12P), α-LA and β-LG. Previous studies have reported poor rennet and coagulation properties with an increase in the proportion of phosphorylated caseins (α_{S1}-CN-9P or α_{S2}-CN-12P) and the amount of α-LA (Frederiksen et al., 2011; Jensen et al., 2012; Ketto et al., 2017; Poulsen, Jensen, & Larsen, 2016).

The good agreement between the acid coagulation results from the low strain amplitude oscillatory and Formagraph corresponds to a previous study (Ketto et al., 2015). The κ-CN AA genotype improved the acid coagulation properties and is in agreement with the results of a previous study on regular unadjusted milk from the same breed (Ketto et al., 2017). The content of α_{S2}-CN-12P was negatively correlated with both rennet and acid coagulation properties of milk (Ketto et al., 2017). The findings from the current study show that the κ-CN BB genotype was positively correlated with a higher concentration of α_{S2}-CN-12P. This could be the reason for the poor acid coagulation properties with the κ-CN BB compared with the AA genotype. UF-concentration of skim milk increased the concentration of protein in the retentate including α_{S2}-CN-12P.

The negative correlation between a higher concentration of α_{S2}-CN-12P and milk acid coagulation could be linked to the higher buffering capacity of a high concentration of highly phosphorylated caseins. A study by Salaün, Mietton, and Gaucheron (2005) reported an increased buffering capacity in milk with higher concentrations of colloidal calcium phosphate and highly phosphorylated caseins. Studies by Mistry and Kosikowski (1985), Salvatore, Pirisi, and Corredig (2011) and Srilaorkul, Ozimek, Wolfe, and Dziuba (1989), provided some evidence on the increase in buffering capacity with

poor acidification/fermentation properties of milk after UF treatment. These findings agree with the current research that the increase in the concentration of α_{S2}-CN-12P (after UF-concentration) impaired the acid coagulation properties of milk. Post-translational modifications in α_S-CN and β-CN (i.e., phosphorylation) and κ-CN (mostly glycosylation) alter the properties of the casein micelles since both glycosylation (only κ-CN) and phosphorylation change the properties of caseins, for example the isoelectric point, molecular weight, hydrophobicity and net charge of the caseins (Huppertz, 2013; Huppertz, Fox, & Kelly, 2018). These modifications together with the increase in buffering capacity, would change the physicochemical properties of casein micelles, and the technological properties of the concentrated milk, especially after rennet and acid addition.

5. Conclusions

The findings from this research suggest that the effects of κ-CN genotypes on the rennet and acid coagulation properties of milk, when the protein concentration in milk is increased (CF 1.7) and made equal, could be explained by variations in the detailed milk protein composition (especially, α_{S2}-CN-12P). In addition to controlling the variations in total protein content, the variations in the detailed milk protein composition also need to be considered when studying the effects of milk protein genetic polymorphisms on the rennet and acid coagulation properties of milk.

Acknowledgements

The authors wish to acknowledge the Norwegian Research Council (Grant numbers: 234114 and 208674/F50) and TINE SA (Grant number: 52114115) for their financial support of this study and the infrastructure grant (Grant number: 208674) for financing the dairy pilot plant. We appreciate the contributions from May Helene Aalberg and Ola Tjåland regarding milk treatment and analyses of the total protein concentration by Kjeldahl and gross milk composition by MilkoScan FT1. We also thank the workers at the SHF for collecting the milk samples and Solfrid Lohne from the Faculty of Environmental Sciences and Nature Management for mineral analysis.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.idairyj.2018.08.008.

References

Aleandri, R., Buttazzoni, L. G., Schneider, J. C., Caroli, A., & Davoli, R. (1990). The effects of milk protein polymorphisms on milk components and cheese-producing ability. *Journal of Dairy Science, 73*, 241–255.

Bonfatti, V., Di Martino, G., Cecchinato, A., Vicario, D., & Carnier, P. (2010). Effects of β-κ-casein (CSN2-CSN3) haplotypes and β-lactoglobulin (BLG) genotypes on milk production traits and detailed protein composition of individual milk of Simmental cows. *Journal of Dairy Science, 93*, 3797–3808.

Devold, T. G., Brovold, M. J., Langsrud, T., & Vegarud, G. E. (2000). Size of native and heated casein micelles, content of protein and minerals in milk from Norwegian Red Cattle—effect of milk protein polymorphism and different feeding regimes. *International Dairy Journal, 10*, 313–323.

Frederiksen, P. D., Andersen, K. K., Hammershoj, M., Poulsen, H. D., Sorensen, J., Bakman, M., et al. (2011). Composition and effect of blending of noncoagulating, poorly coagulating, and well-coagulating bovine milk from individual Danish Holstein cows. *Journal of Dairy Science, 94*, 4787–4799.

Garrison, E., & Marth, G. (2012). *Haplotype-based variant detection from short-read sequencing*. Preprint at https://arxiv.org/pdf/1207.3907.pdf.

Hallén, E., Allmere, T., Näslund, J., Andrén, A., & Lundén, A. (2007). Effect of genetic polymorphism of milk proteins on rheology of chymosin-induced milk gels. *International Dairy Journal, 17*, 791–799.

Heck, J. M. L., Olieman, C., Schennink, A., van Valenberg, H. J. F., Visker, M. H. P. W., Meuldijk, R. C. R., et al. (2008). Estimation of variation in concentration, phosphorylation and genetic polymorphism of milk proteins using capillary zone electrophoresis. *International Dairy Journal, 18*, 548–555.

Huppertz, T. (2013). Chemistry of the caseins. In P. L. H. McSweeney, & P. F. Fox (Eds.), *Advanced dairy chemistry. Vol. 1A. Proteins: Basic aspects* (4th edn., pp. 135–160). New York, USA: Springer Science+Business.

Huppertz, T., Fox, P. F., & Kelly, A. L. (2018). The caseins: Structure, stability, and functionality. In R. Y. Yada (Ed.), *Proteins in food processing* (2dn edn., pp. 49–92). Duxford, UK: Woodhead Publishing.

IDF. (2001). *Milk – Determination of nitrogen content – Part 4: Determination of non-protein-nitrogen content. IDF 20-4:2001*. Brussels, Belgium: International Dairy Federation.

Ikonen, T., Ojala, M., & Ruottinen, O. (1999). Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows. *Journal of Dairy Science, 82*, 1026–1033.

Inglingstad, R. A., Steinshamn, H., Dagnachew, B. S., Valenti, B., Criscione, A., Rukke, E. O., et al. (2014). Grazing season and forage type influence goat milk composition and rennet coagulation properties. *Journal of Dairy Science, 97*, 3800–3814.

Jenness, R., & Koops, J. (1962). Preparations and properties of a salt solution which stimulates milk ultrafiltrate. *Netherland Milk and Dairy Journal, 16*, 153–164.

Jensen, H. B., Poulsen, N. A., Andersen, K. K., Hammershøj, M., Poulsen, H. D., & Larsen, L. B. (2012). Distinct composition of bovine milk from Jersey and Holstein-Friesian cows with good, poor, or noncoagulation properties as reflected in protein genetic variants and isoforms. *Journal of Dairy Science, 95*, 6905–6917.

Jørgensen, C. E., Abrahamsen, R. K., Rukke, E.-O., Johansen, A.-G., Schüller, R. B., & Skeie, S. B. (2015). Improving the structure and rheology of high protein, low fat yoghurt with undenatured whey proteins. *International Dairy Journal, 47*, 6–18.

Jørgensen, C. E., Abrahamsen, R. K., Rukke, E.-O., Johansen, A.-G., Schüller, R. B., & Skeie, S. B. (2016). Optimization of protein fractionation by skim milk microfiltration: Choice of ceramic membrane pore size and filtration temperature. *Journal of Dairy Science, 99*, 6164–6179.

Jõudu, I., Henno, M., Varv, S., Kaart, T., Kart, O., & Kalamees, K. (2007). Milk protein genotypes and milk coagulation properties of Estonian native cattle. *Agricultural and Food Science, 16*, 222–231.

Ketto, I. A., Knutsen, T. M., Øyaas, J., Heringstad, B., Ådnøy, T., Devold, T. G., et al. (2017). Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. *International Dairy Journal, 70*, 55–64.

Ketto, I. A., Schüller, R. B., Rukke, E.-O., Johansen, A. G., & Skeie, S. B. (2015). Comparison between Formagraph and low-amplitude oscillation rheometery in monitoring coagulation properties of acid induced gels in bovine milk. *Annual Transactions The Nordic Rheology Society, 23*, 181–187.

Marziali, A. S., & Ng-Kwai-Hang, K. F. (1986). Effects of milk composition and genetic polymorphism on coagulation properties of milk. *Journal of Dairy Science, 69*, 1793–1798.

Mistry, V. V., & Kosikowski, F. V. (1985). Growth of lactic acid bacteria in highly concentrated ultrafiltered skim milk potentates. *Journal of Dairy Science, 68*, 2536–2543.

Ng-Kwai-Hang, K. F., Hayes, J. F., Moxley, J. E., & Monardes, H. G. (1984). Association of genetic variants of casein and milk serum proteins with milk, fat and protein production by dairy cattle. *Journal of Dairy Science, 67*, 835–840.

Poulsen, N. A., Jensen, H. B., & Larsen, L. B. (2016). Factors influencing degree of glycosylation and phosphorylation of caseins in individual cow milk samples. *Journal of Dairy Science, 99*, 3325–3333.

Salaün, F., Mietton, B., & Gaucheron, F. (2005). Buffering capacity of dairy products. *International Dairy Journal, 15*, 95–109.

Salvatore, E., Pirisi, A., & Corredig, M. (2011). Gelation properties of casein micelles during combined renneting and bacterial fermentation: Effect of concentration by ultrafiltration. *International Dairy Journal, 21*, 848–856.

SAS. (2015). *Statistical analysis system user guide*. Version 9.4. Cary, NC, USA: SAS Institute, Inc.

Schopen, G. C. B., Visker, M. H. P. W., Koks, P. D., Mullaart, E., van Arendonk, J. A. M., & Bovenhuis, H. (2011). Whole-genome association study for milk protein composition in dairy cattle. *Journal of Dairy Science, 94*, 3148–3158.

Srilaorkul, S., Ozimek, L., Wolfe, F., & Dziuba, J. (1989). The effect of ultrafiltration on physicochemical properties of retentate. *Canadian Institute of Food Science and Technology Journal, 22*, 56–62.