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

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Abstract

The aim of this study was to investigate the effects of milk protein genetic polymorphisms on the rennet and acid coagulation properties of milk after protein standardization. Skim milk samples were adjusted to a protein concentration of 6.07 ± 0.06 % by ultrafiltration (UF) before evaluating rennet coagulation by Formagraph and the acid coagulation properties by both Formagraph and low strain amplitude oscillatory test. Only the β-lactoglobulin (LG) genotypes influenced the rennet-clotting time before standardization 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 (CN) and β-LG genotypes. The results showed that the κ-CN genotypes significantly influenced the acid coagulation properties of both skim milk and retentate. However, 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 standardization 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}-CN C variant and the B variants of κ-CN, β-CN and 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 to 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 to 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, the aim of the current study was to investigate the effects of milk protein polymorphisms on the

49 rennet and acid coagulation properties of milk at equal protein content. In the present study, the
50 total protein content of individual milk samples was standardized by the use of laboratory-scale
51 ultrafiltration (UF) process in order to determine if milk protein genetic polymorphisms would
52 still influence milk coagulation.

53

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 by using pH meter (PHM61; Radiometer, Copenhagen., Denmark).

2.3 Milk UF-concentration

Immediately after cream separation, UF-concentration was performed of the skim milk by using a Labscale™ TFF system (Millipore, Oslo, Norway), with a Pellicon ® XL Cassette with a Biomax membrane 500 kDa (Cat number: PBX500C50; Millipore, Oslo, Norway), 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, Hillerød, Denmark), 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., Cork, Ireland), transferred to polystyrene cuvettes (DTS0012, Malvern Instruments GmbH, Herrenberg, Germany) and heated at 26 °C for 5 to 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, Høsholm, 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) 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, Padova, Italy) as described by Ketto, Schüller, Rukke, Johansen, and Skeie (2015). In brief, milk samples were heat treated at 95 °C for 5 minutes 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 minutes at 32 °C. Strain sweep (0.05 – 100%,

145 strain and 10 radsec^{-1}, frequency) was carried out to determine strain value within the linear
146 visco-elastic region (LVR). A constant strain from strain sweep, below the upper limit of LVR
147 (0.1%) was used when monitoring the acid coagulation process at 10 radsec^{-1}. Gelation time
148 (GT) from low strain amplitude oscillatory test was defined as the time from acidification to the
149 time when the elastic modulus (G') was ≥ 1 Pa, while on the Formagraph, GT was defined as the
150 time-interval between acid addition and the time when the width of the bifurcate increased to 1.2
151 mm. The GFR (gel-firming rate) was defined as the maximum slope of G' *vs*. time (Pa/min) and
152 G *vs.* time (mm/min) curves for the low strain amplitude oscillatory test and Formagraph,
153 respectively. Final gel firmness (G60) was recorded at 60 min in Pa (by the low strain amplitude
154 oscillatory test) and mm (by the Formagraph). Each sample was analysed once in the low strain
155 amplitude oscillatory test and three times in the Formagraph.

156 *2.9 Statistical analysis*

157 Statistical analysis was performed using a mixed procedure in SAS (SAS, 2015) to study the
158 effect of casein genotypes (α_{s1}-CN, κ-CN) and β-LG on the rennet and acid coagulation
159 properties of the skim milk and retentate after UF-concentration. The following statistical model
160 was used:

161 $$Y = X\beta + Zu + \text{residual}$$

162 Where:

163 Y = vector for the response variable (e.g., rennet or acid coagulation properties of the skim milk
164 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,
165 α_{s1}-CN, α_{s1}-CN-8P, α_{s1}-CN-9P, κ-CN and β-CN in the skim milk and retentate).

166 β = unknown vector for the fixed effects (α_{s1}-CN, κ-CN, β-LG genotypes).

167 u = vector for the random variables (Cow: 1, 2, 3, 4…and 18).

168 X and Z = known design matrices for fixed and random effects, respectively.

169

170 Statistical analyses were repeated with the milk protein and mineral concentration included in the
171 statistical model as the covariates in Xβ to test if the observed significant effects of milk protein
172 genotypes were confounded with milk protein composition (α-LA, β-LG, α_{s2}-CN (α_{s2}-CN-10P,
173 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
174 minerals (Ca, Mg and P).

175

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 to 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

198 pronounced before UF-concentration, the contents of αs2-CN-12P and β-LG were significantly
199 influenced by κ-CN and β-LG genetic polymorphisms respectively (Supplementary Table S1).

200 **3.2 Rennet coagulation properties**

201 The α_{s1}-CN genotypes did not influence the rennet coagulation properties of skim milk or the
202 retentate, whereas the κ-CN genotypes significantly influenced the coagulation of the retentate
203 but not of the skim milk (Table 4). Favoured rennet coagulation properties of the retentate (low
204 RCT and high a_{30}; $P < 0.05$) were linked with κ-CN AA compared to the BB genotype. For the
205 β-LG genotypes, however, the RCT of both skim milk and retentate were influenced, and a
206 shorter RCT was observed with the AB compared to the BB genotype ($P < 0.05$). In the
207 retentate, the effect of the κ-CN genotypes on RCT was confounded with the concentration of
208 total α_{s2}-CN and the individual concentration of α_{s2}-CN-10P, 11P and 12P, β-LG and α-LA (Fig
209 1). In skim milk, the effect of the β-LG genotypes ($P < 0.05$) on RCT was confounded with its
210 concentration of β-LG (Fig. 2).

211 **3.3 Acid coagulation properties**

212 Only the κ-CN genotypes influenced ($P < 0.05$) the acid coagulation properties of skim milk and
213 retentate (Table 5). In both skim milk and retentate, κ-CN AA was correlated with improved acid
214 coagulation properties (i.e., shorter gelation time (GT), higher gel-firming rate (GFR) and higher
215 gel firmness at 60 minutes) compared to the BB genotype. The acid coagulation results obtained
216 by the low strain amplitude oscillatory test in Fig. 3 corresponded with the results obtained by
217 the Formagraph. In both methods, κ-CN AA was correlated with improved acid coagulation
218 properties of milk. The effects of the κ-CN genotypes on the GT of skim milk were, however,
219 confounded by the inclusion of the concentration of α_{s2}-CN-12P in the statistical model (Fig. 4a).
220 Likewise, in the retentate, the effect of the κ-CN genotype on G60 was confounded by the α_{s2}-

221 CN-12P concentration (Fig. 4b). The concentration of α_{s2}-CN-12P in the skim milk and retentate
222 was significantly ($P < 0.05$) lower in κ-CN AA compared to BB (Fig. 5).
223

4 Discussion

A membrane with a molecular weight 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 standardized 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 to 4.26% in Swedish Red and Holstein cows, close to the protein range of 2.59 to 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 to 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 standardized 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

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

252 Marziali and Ng-Kwai-Hang (1986) studied the effects of milk protein genotypes (β-CN, κ-CN,
253 and β-LG) on the rennet coagulation properties of Holstein Friesian milk after adjusting the
254 protein and fat concentrations by using a statistical model. They found that neither β-CN nor κ-
255 CN genetic variants influenced the rennet coagulation properties of the milk; however, the A
256 variant of β-LG was associated with a shorter clotting time and higher curd firmness compared to
257 the B variant. This was in accordance with the current study, which reported a shorter rennet
258 clotting time of skim milk with β-LG AB compared to BB. The observed effects of κ-CN genetic
259 polymorphisms in the current study on the rennet coagulation properties of retentate (RCT and
260 a_{30}) were confounded with the concentration of α_{s2}-CN and its phosphorylation states (10P, 11P,
261 and 12P), α-LA and β-LG. Previous studies have reported poor rennet and coagulation properties
262 with an increase in the proportion of phosphorylated caseins (α_{s1}-CN-9P or α_{s2}-CN-12P) and the
263 amount of α-LA (Frederiksen et al., 2011; Jensen et al., 2012; Ketto et al., 2017; Poulsen, Jensen,
264 & Larsen, 2016).

265 The good agreement between the acid coagulation results from the low strain amplitude
266 oscillatory and Formagraph corresponds to a previous study (Ketto et al., 2015). The κ-CN AA
267 genotype improved the acid coagulation properties and is in agreement with the results of a
268 previous study on regular unadjusted milk from the same breed (Ketto et al., 2017). The content
269 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 showed 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 to 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 agrees 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 iso-electric 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.

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398

Fig. 1

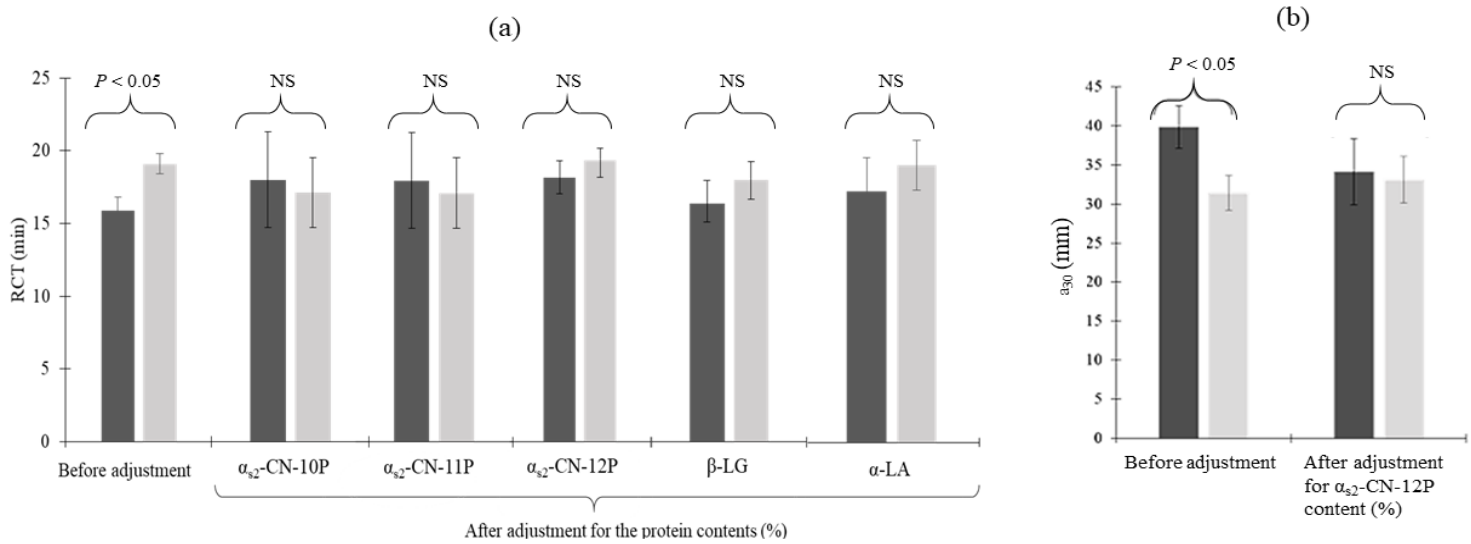
(a)
25
20
15
10
5
0
RCT (min)
P < 0.05
NS
NS
NS
NS
NS
Before adjustment
αs2-CN-10P
αs2-CN-11P
αs2-CN-12P
β-LG
α-LA
After adjustment for the protein contents (%)
(b)
45
40
35
30
25
20
15
10
5
0
a30 (mm)
P < 0.05
NS
Before adjustment
After adjustment for αs2-CN-12P content (%)

Fig. 2

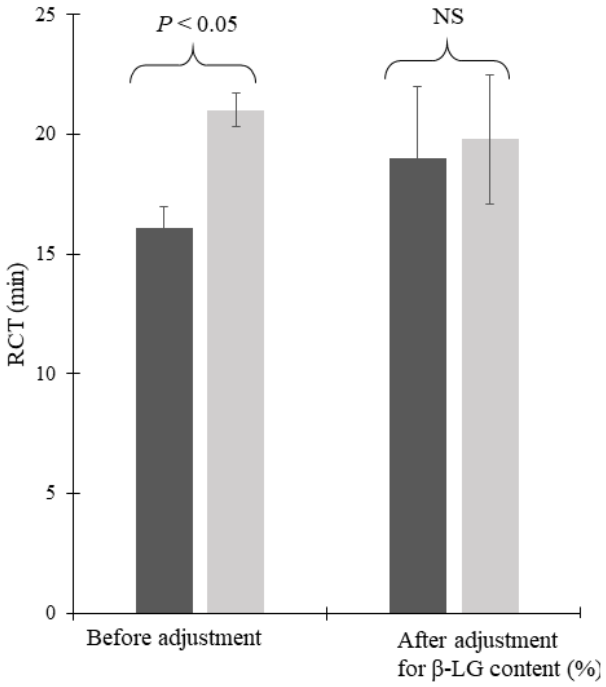
25
20
15
10
5
0
RCT (min)
P < 0.05
NS
Before adjustment
After adjustment
for β-LG content (%)

Fig. 3

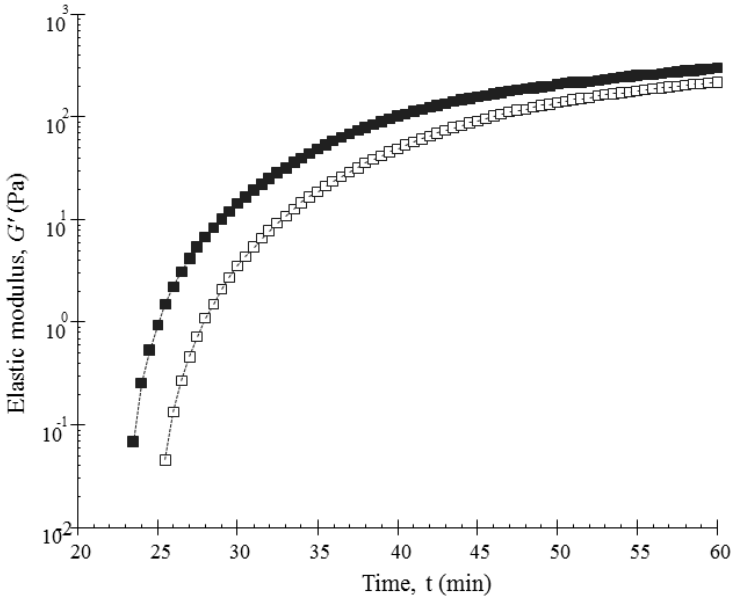
Elastic modulus, G' (Pa)
10^3
10^2
10^1
10^0
10^-1
10^-2
20
25
30
35
40
45
50
55
60
Time, t (min)

Fig. 4

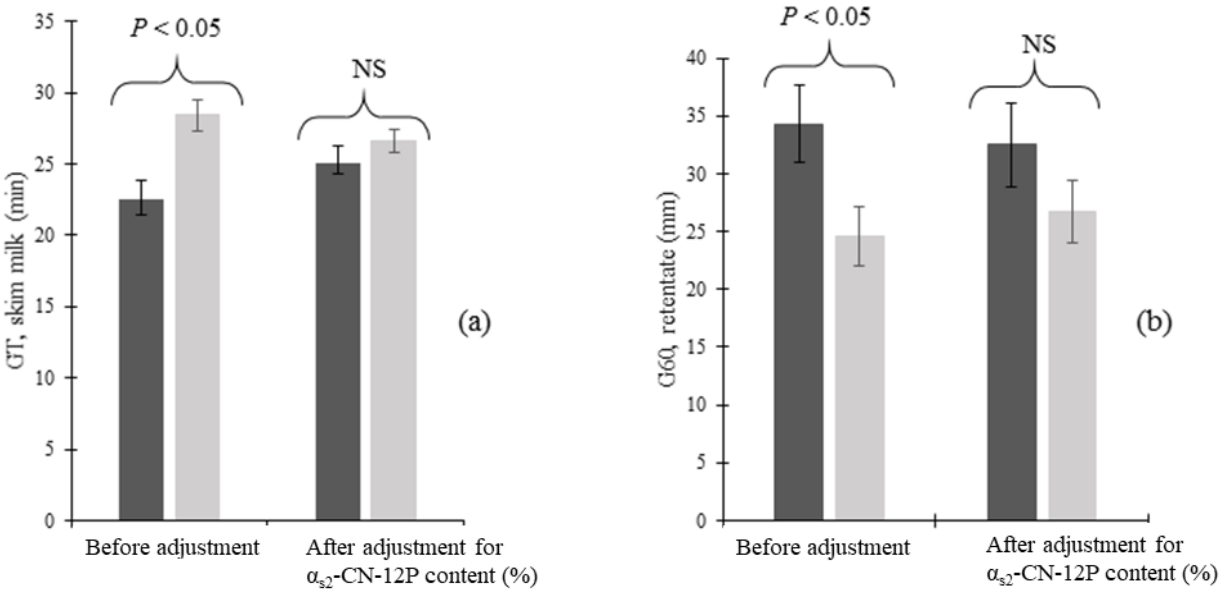
P < 0.05
NS
GT, skim milk (min)
35
30
25
20
15
10
5
0
(a)
Before adjustment
After adjustment for
αs2-CN-12P content (%)
P < 0.05
NS
G60, retentate (mm)
40
35
30
25
20
15
10
5
0
(b)
Before adjustment
After adjustment for
αs2-CN-12P content (%)

Fig. 5

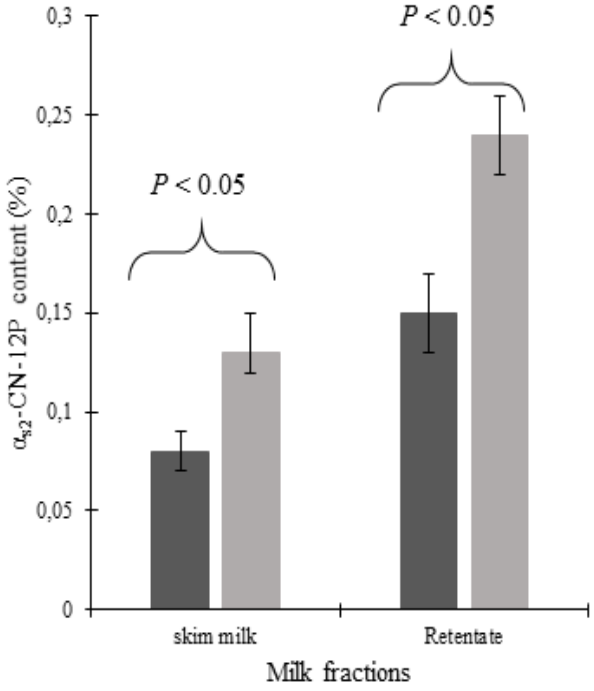
0,3
0,25
0,2
0,15
0,1
0,05
0
α_{s2}-CN-12P content (%)
P < 0.05
P < 0.05
skim milk
Retentate
Milk fractions

Figure legends

Fig. 1. Effect of κ-CN genotypes (■ κ-CN AA and ■ κ-CN BB) on the RCT of the retentate before and after adjustment for α_{s2}-CN-10P, α_{s2}-CN-11P, α_{s2}-CN-12P, β-LG and α-LA contents (a) and a_{30} before and after being adjusted for α_{s2}-CN-12P content in the statistical model (b); RCT= rennet-clotting time in minutes, a_{30} = Curd firmness at 30 minutes and NS = Non-significant.

Fig. 2. Effect of β-LG genotypes (■ β-LG AB and ■ β-LG BB) on the 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 and ■ BB) and similar genotypes for α_{s1}-CN, β-CN and β-LG (i.e., BC, A^2A^2 and BB, respectively).

Fig. 4. Effect of κ-CN genotypes (■κ-CN AA and ■ κ-CN BB) on the GT of the skim milk (a) and G60 of the retentate (b) before and after adjustment for the α_{s2}-CN-12P content. GT = gelation time and G60 = acid gel firmness at 60 min and NS= Non-significant.

Fig. 5. Variation in α_{s2}-CN 12P content between the κ-CN genotypes (■ AA and ■ BB) in skim milk and retentate.

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

Table 2:

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

	Fractions of milk		
Milk composition (%)	Skim milk (Before UF)	Retentate (After UF)	Permeate (After UF)
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)			
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 presents are the means ± standard deviation. ND = Not detected, NA= Not analyzed

Table 3

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

		Content of milk proteins, %										
Genotypes		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 presents 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]

		Skim milk (Before UF)			Retentate (After UF)		
Protein		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 presents 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), a_{30} = curd firmness at 30 min (mm).

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])

		Skim milk (Before UF)			Retentate (After UF)		
Protein		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 presents 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) and G60 = acid gel firmness at 60 minutes (mm))

Supplementary Table S1

Effect of milk protein genotypes on the content of milk proteins of the skim milk (before UF-concentration) [a]

		Content of milk proteins, %										
Genotypes		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	1.21 ± 0.05	0.83 ± 0.04	0.25 ± 0.01	0.31 ± 0.03	0.03 ± 0.01	0.16 ± 0.01	0.11 ± 0.01	1.17 ±0.05	0.28 ± 0.02	0.12 ± 0.01	0.29 ± 0.01
	BC	1.24 ± 0.10	0.84 ± 0.07	0.26 ± 0.01	0.27 ± 0.05	0.03 ± 0.01	0.14 ± 0.02	0.10 ± 0.02	1.06 ± 0.09	0.31 ± 0.03	0.10 ± 0.01	0.29 ± 0.01
p-value		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
κ-CN	AA	1.18 ± 0.08	0.80 ± 0.05	0.25 ± 0.02	0.25 ± 0.04	0.03 ± 0.01	0.14 ± 0.02	0.08 ± 0.01	1.01 ± 0.08	0.29 ± 0.03	0.11 ± 0.01	0.27 ± 0.02
	BB	1.28 ± 0.07	0.86 ± 0.04	0.27 ± 0.01	0.33 ± 0.03	0.03 ± 0.01	0.16 ± 0.02	0.13 ± 0.01	1.14 ± 0.06	0.30 ± 0.02	0.11 ± 0.01	0.30 ± 0.02
p-value		NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
β-LG	AB	1.26 ± 0.08	0.86 ± 0.05	0.27 ± 0.02	0.29 ± 0.04	0.03 ± 0.01	0.15 ± 0.02	0.11 ± 0.02	1.13 ± 0.07	0.27 ± 0.03	0.11 ± 0.01	0.33 ± 0.02
	BB	1.26 ± 0.07	0.81 ± 0.04	0.25 ± 0.01	0.28 ± 0.03	0.03 ± 0.01	0.15 ± 0.02	0.10 ± 0.01	1.14 ± 0.06	0.31 ± 0.02	0.11 ± 0.01	0.25 ± 0.02
p-value		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**

[a] Values presents are the Least square means ± standard error, NS=Non-significant, *$P < 0.05$ and **$P < 0.01$