International Dairy Journal 145 (2023) 105736

Contents lists available at ScienceDirect

International Dairy Journal

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

Influence of different genetic polymorphisms of α_{S1}- and κ-casein on Havarti-type cheese: Effects on cheese-making efficiency and cheese quality

Martine Andrea Olsen [a, 1], Isaya Appelesy Ketto [a, b, 1], Jorun Øyaas [c], Ahmed Abdelghani [a], Kristine Svartbekk Myhrer [d], Siv Borghild Skeie [a, *]

[a] Faculty of Chemistry, Biotechnology and Food Science (KBM), Norwegian University of Life Sciences (NMBU), P.O Box 5003, N-1432 Ås, Norway
[b] ASAS Dairies Farm LTD, P.O Box 562, Iringa, Tanzania
[c] TINE SA, Postboks 25, 0051 Oslo, Norway
[d] Norwegian Institute of Food and Aquaculture Research (NOFIMA), P.O Box 210, N-1431 Ås, Norway

ARTICLE INFO

Article history:
Received 27 February 2023
Received in revised form
16 June 2023
Accepted 16 June 2023
Available online 23 June 2023

ABSTRACT

The effect of the composite genotypes of α_{S1}-κ-casein (BBAA, BBBB, BCAA) on renneting properties and yield during cheese-making of a Havarti-type cheese was investigated. Milk with α_{S1}-κ-CN BCAA showed a shorter renneting time, while milk with α_{S1}-κ-CN BBAA obtained the highest cheese yield. 24 h after starter addition, different protein profiles were found between the cheeses with different α_{S1}-κ-CN genotypes. After 5 months of ripening, cheese with α_{S1}-κ-CN BBBB contained more free amino acids than α_{S1}-κ-CN BCAA. Cheese with α_{S1}-κ-CN BCAA expressed a higher intensity of sweetness and lower intensity of hardness than the α_{S1}-κ-CN BBBB and BBAA cheeses. Cheeses with α_{S1}-κ-CN BBAA had a higher intensity of sunlight flavour than cheeses with the two other α_{S1}-κ-CN genotypes and were assessed to be less juicy than α_{S1}-κ-CN BBBB cheeses. The composite genotypes of α_{S1}-κ-casein affect milk coagulation, cheese yield and cheese maturation and are highly relevant for the dairy industry.

1. Introduction

Numerous investigations have been made on the effects of milk protein genetic polymorphism on the rennet coagulation properties (time from rennet addition until start of coagulation, coagulation rate and gel firmness) of individual milk samples at laboratory scale (Gustavsson et al., 2014; Hallén, Allmere, Näslund, Andrén, & Lundén, 2007; Jensen et al., 2012; Jõudu, Henno, & Värv, 2007; Jõudu et al., 2009; Ketto et al., 2017; Poulsen et al., 2013). From these studies, there is a general agreement that the B-variant of κ-casein (κ-CN) and β-lactoglobulin (β-LGB) and the BC genotype of α_{S1}-casein (α_{S1}-CN) are associated with better coagulation properties. The B-variant of β-casein (β-CN) is usually represented at a low frequency in the cow population, but in the studies mentioned above, several report that milk with this variant shows good coagulation properties. The BB genotype of κ-CN and β-LGB is associated with higher cheese yield compared with the AA genotype (Fox, Guinee, Cogan, & McSweeney, 2017a).

Renneting time and cheese yield are important indicators of cheese-making efficiency. Cheese yield calculations are, on principle, based on how much cheese is obtained from a specific amount of milk, and expressed as the amount of cheese (kg) produced from 100 kg of milk. It is also possible to predict cheese yield based on milk composition and the desired composition of cheese. These kinds of calculations are useful both for the industry to keep track of the efficiency and profitability of their production, and also for researchers to study the efficiency when making changes in the raw material or in the cheese-making process (Lucey & Kelly, 1994).

A review by Skeie (2007) stated that identification of the casein genotypes that are correlated with both improved cheese-making properties, cheese yield and cheese quality would provide possibilities for selective breeding of cows producing milk for cheese manufacture. Some studies on the effect of single-protein loci on the cheese-making properties of various cheeses have been established, i.e., Cheddar, Mozzarella, Parmigiano-Reggiano, Sevicia, Asiago, Caciotta and Montansio cheeses (Bonfatti et al., 2011;

* Corresponding author.
E-mail address: siv.skeie@nmbu.no (S.B. Skeie).
[1] Shared first authorship, both authors contributed equally.

https://doi.org/10.1016/j.idairyj.2023.105736

Walsh et al., 1998a; Zambrano, Eraso, Solarte, & Rosero, 2010). However, there is limited published information on the effects of casein composite genotypes (α_{S1}-κ-CN) on the cheese-making properties, cheese yield, and especially cheese quality. Mayer, Ortner, Tschager, and Ginzinger (1997) made Edam-type cheese from pooled milk of Brown cattle with genotype combinations of β-CN, κ-CN and β-LGB. Depending on the composite genetic variants, they observed large differences in the fat content of whey, curd fines and cheese yield. Milk with the composite genotype (β-κ-CN, β-LGB) A^2B-AA-AA showed the highest cheese yield, whereas milk with the composite genotype (β-κ-CN, β-LGB) A^2A^2-AA-AA gave the lowest cheese yield. In milk from Norwegian Red (NR), the A^2-variant is dominating with a frequency of 79.7%, while the B-variant has a frequency of 1.2% (Ketto et al., 2017). Moreover, the B-variant is most frequent for α_{S1}-CN while the C-variant represents 8.9% of the frequency in the NR population. For κ-CN, the A and B variants are equally distributed. In the above-mentioned studies, the focus has been on β-CN, κ-CN and β-LGB, and very few studies have focused on the influence of α_{S1}-CN on cheese yield and cheese quality.

The objective of the current study was, therefore, to make a Havarti-type cheese from pooled milk with the A^2A^2 genotype of β-CN in combination with the two most frequent genotypes of α_{S1}-CN (BB and BC) and κ-CN (AA and BB) in milk from NR. This gave three different α_{S1}-κ-CN composite genotypes (BBAA, BCAA and BBBB) and the effects on cheese-making properties, yield, and sensory quality were investigated.

2. Materials and methods

The cows were genotyped according to the procedure described by Ketto et al. (2017) using paired-end sequencing of blood samples.

2.1. Experimental design

Evening and morning milk from fifteen individual NR cows were collected from the Animal Production and Experimental Unit at the Norwegian University of Life Sciences (Ås, Norway).

The cows had the same genetic polymorphism for β-CN (A^2A^2) but differed in α_{S1}-CN (BB and BC) and κ-CN (AA and BB), resulting in three groups of α_{S1}-κ-CN composite genotypes (BBAA, BBBB, and BCAA). A similar genetic polymorphism for β-LGB within the three groups was aimed for. However, this was not possible within the university herd and therefore the number of cows within the three α_{S1}-κ-CN composite genotype groups had the following β-LGB genotype: Group BBAA (1 AA, 2 AB, 2 BB), group BBBB (0 AA, 4 AB, 1 BB) and group BCAA (1 AA, 1 AB, 3 BB). All cows included in the experiment were fed the same silage and concentrate. However, their lactation stage was random due to the limited number of cows with the combinations aimed for. The number of cows in each lactation stage was: BBAA; mid: 3, late: 2, BBBB; mid: 4, late: 1 and BCAA; early: 4, mid: 1. The average milk yield per day at the start of the study was: BBAA (29.33 ± 4.85 kg), BBBB (30.46 ± 3.07 kg) and BCAA (31.29 ± 10.02 kg).

The milk from the individual cows was transported from the Animal Production and Experimental Unit to the dairy pilot plant at the Norwegian University of Life Sciences and pooled according to their α_{S1}- and κ-CN composite genotypes (BBAA, BBBB, and BCAA) before treatment and cheese-making. Milk and cheese with the different α_{S1}- and κ-CN composite genotypes are further denoted as BBAA, BBBB, and BCAA milk or cheese.

2.2. Cheese production

A Havarti-type cheese was manufactured over a period of 15 days, with, in total, 5 production days with one or two vats produced on each production day; in total 9 vats of cheese. This resulted in 3 cheese vats (defined as replicate blocks 1–3) produced from each of three types of milk (BBAA, BBBB and BCAA). For each replicate block, the milk with the specified composite genotypes was randomised between the two 200 L cheese vats used. In each vat, 100 L of cheese-milk was standardised to 4.18 ± 0.04% (w/w) fat and pasteurised at 62 °C for 30 min. The cheese-milk was cooled to 32 °C, before addition of the starter culture (CHN-19, Chr. Hansen, Copenhagen, Denmark) at 2% (v/v) concentration. Rennet (Chy-Max Plus, Chr. Hansen) was added 30 min after starter addition, at 25 mL 100 L^{-1}. The coagulum was cut when optimum firmness was obtained, this was determined by an experienced cheesemaker, and the time from the rennet addition to the time when the coagulum was cut was recorded as renneting time (min). After cutting, the grains were stirred for 50 min at 32 °C, and then 45% (v/v) whey was drained off and replaced with 35% (v/v) water. The temperature of the curd was increased to 38 °C and the curd was scalded for 20 min, with a double stirring intensity. After scalding, the cheese curd was drained and transferred to the moulds (1 L) and turned every 20 min, in total 4 times. The moulds were removed, and the cheeses were salted in brine (25 °Be) for 1 h. The cheese was stored overnight at room temperature and then vacuum sealed in plastic bags. The cheese was ripened at 16 °C for 4 weeks and then at 5 °C for 4 months. The pH profile during cheese-making was monitored using a pH meter (PHM61; Radiometer, Copenhagen, Denmark).

2.3. Milk and cheese analyses

The chemical composition (fat, lactose, total protein, casein and total solids (TS)) of the cheese-milk was analysed using a MilkoScan FT1 (Foss Electric A/S, Hillerød, Denmark). Cheese TS, protein and fat were measured 24 h after the start of cheese-making. Total solids of cheese was measured according to IDF standard 4A (IDF, 1982) with a slight modification as the samples were pre-dried at room temperature (not using sand) for 20 h prior to drying in the oven. Fat was analysed by the Gravimetric method according to IDF Standard 250 (ISO 23319) (IDF, 2022). Protein was determined by measuring total nitrogen by the Kjeldahl method according to IDF standard 20-1 (ISO 8968-1) (IDF, 2014) using factor 6.38 to calculate the protein from the total nitrogen.

Predicted cheese yield (PY) was calculated using the Van Slyke formula (eq. (1)) (Fox, Guinee, Cogan, & McSweeney, 2017a); actual cheese yield (Ya) was calculated according to Banks (2007) where Ya was determined as the ratio of the cheese weight (in kg) before brining to the sum of the weight of cheese milk and starter culture (eq. (2)); yield efficiency (YE) (eq. (3)) using Ya and PY was calculated according to Fox et al. (2017a):

$$\text{PY (Van Slyke)} = \frac{(0.93\ \text{F} + \text{C} - 0.1) \times 1.09}{100 - \text{W}} \times 100 \quad (1)$$

where: F, fat in milk (%, w/w); C, casein in milk (%, w/w); W, desired water content in cheese (46%, w/w); 0.1 is the constant for loss of cheese fines in whey; 1.09 is a constant representing other solids included in the cheese.

$$\text{Ya} = \frac{\text{Weight of cheese}}{(\text{Weight of milk} + \text{weight of starter})} \times 100 \quad (2)$$

$$\text{YE} = \frac{\text{Ya}}{\text{PY (Van Slyke)}} \times 100 \quad (3)$$

Samples for analysis of free amino acids (FAAs) were frozen and stored at −20 °C until analysis by HPLC as described by Martinovic et al. (2013). Derivatisation with o-phthalaldehyde (OPA) was done according to Bütikofer and Ardö (1999). Analysis of organic acids was done according to the method described by Skeie et al. (2008) using HPLC. The protein fractions and their relative concentrations in 24 h cheese and ripened cheese were determined by capillary electrophoresis (CE) (Ketto et al., 2017), where peak identification was achieved by comparing with previously published electropherograms (Ardö, McSweeney, Magboul, Upadhyay, & Fox, 2017; Otte, Zakora, Kristiansen, & Qvist, 1997).

2.4. Descriptive sensory analysis

Cheeses were evaluated by a trained sensory panel of 10 trained assessors at the Norwegian Institute of Food, Fisheries, and Aquaculture Research (NOFIMA, Aas, Norway) after 5 months of ripening. Descriptive sensory profiling was made according to the generic descriptive analysis as described by Lawless and Heymann (2010). The analysis was done according to ISO standards 8589 and 8586 (ISO, 2007, 2012) for the design of the test room and the selection and training of assessors, respectively. The sensory panel is trained, checked, and tested before each project as described by Larssen, Monteleone, and Hersleth (2018). The assessors agreed upon a list of 8 aromas, 11 taste/flavour attributes and 7 texture attributes during the term generation phase as described in Supplementary material Table S1. In the pre-test session, the judges were trained in the definition of the attributes by testing samples that were considered extreme with respect to selected attributes typical for the cheese.

The samples were presented in cubes 2 × 2 × 1 cm, and each assessor was served two cheese cubes holding the temperature of 18 °C ± 1 °C. The samples were evaluated by the assessors at an individual speed, and the sensory attributes were evaluated using a 15 cm non-structured continuous line scale with the left side of the scale corresponding to the lowest intensity and the right side corresponding to the highest intensity. During analysis, 9 samples were evaluated in two replicates, in 5 serving sessions. All samples and replicates were served in a randomised order. EyeQuestion (Logic8, Elst, The Netherlands) was used for direct recording of data and the software transformed the responses into numbers between 1 = low intensity and 9 = high intensity. The panel was asked to rinse their palates between the samples using hot and cold water in addition to unsalted crackers.

2.5. Statistical analysis

Significant effects (P <0.05) on the milk composition, renneting time, cheese yield, cheese composition, proteolytic (caseins and amino acids) and sensory data due to the α_{S1}-κ-CN composite genotype (BBAA, BCAA, and BBBB) were found using the mixed procedure of SAS (SAS Enterprise Guide 8.3, SAS, Cary, NC, USA). The composite genotype (n = 3) was used as a fixed factor, while replicate block (n = 3) and vat number (n = 2) were used as random factors.

The covariance structure used was variance components. Least Square Post Hoc (Tukey) was used to test differences between means (all pairwise differences). For the sensory data, the mean of 10 assessors was used in the statistical calculations.

Principal component analysis (PCA) was used to analyse the relationship between the α_{S1}-κ-CN composite genotypes and the protein profile in 24 h cheese and FAA composition in ripened cheese using the factoextra package (Kassambara & Mundt, 2020) in RStudio (R Core Team, 2022) (RStudio 2022.02.2, Boston, MA, USA).

3. Results

The α_{S1}-κ-CN composite genotype significantly affected the composition of cheese milk (Table 1). BCAA milk had a higher content of lactose and lower content of protein compared with both BBAA and BBBB milk. Moreover, BCAA milk had a lower content of casein compared with BBAA milk. The TS of the cheese milk differed between the different α_{S1}-κ-CN composite genotypes where BBAA milk had the highest and BBBB milk the lowest.

The pH development during cheese-making was similar in all vats, the average pH in milk at the start of cheese-making was 6.59 (±0.02) and the pH in cheese 24 h after starter addition was 5.06 (±0.11). The mean TS of the cheeses 24 h after starter addition was 55.96% (w/w) (±0.98). No significant difference was found in pH, protein, fat and total TS content (Table 1), which indicates that the cheese-making procedure was standardised between the treatments and replicates.

The renneting time was significantly (P <0.02) longer (~9 min) for BBAA milk compared with BCAA milk (Table 2). The calculated PY gave an estimate that milk with BBAA would attain a higher cheese yield compared with milk with BCAA and BBBB and this was confirmed by Ya. BBAA milk gave 0.6 kg more cheese per 100 L milk than BCAA milk (P <0.02) and 0.5 kg more cheese per 100 L milk than BBBB milk (P = 0.05). The protein content of the whey only reflected the protein content of the milk and did not seem to be influenced by differences in cheese yield.

Differences were found in the protein profile of the 24 h cheeses, and the PCA plot (Fig. 1) shows a clear grouping between the cheeses. BCAA cheeses are located on the left side and were associated with higher levels of α_{S1}-CN 9P and 8P (P <0.05) and β-CN A2 (P <0.01) while BBBB and BBAA cheeses are located on the right side and were associated with higher levels of α_{S2}-CN (P <0.01), para-κ-CN (BBAA > BBBB/BCAA) and γ-caseins.

Further, in ripened cheese, the same composite genotype groups still had a remarkably similar protein profile (results not shown), while there were clear differences between cheeses with different composite genotypes (Fig. 2). BCAA cheeses had a higher area of intact β-CN A2 and a lower peak area for two proteins/peptides which were most probably α_{S1}-CN 9P and γ-CN (marked with "x" and "z" respectively). Moreover, BCAA cheese had a different profile of the protein/peptides marked with "o", this is possibly γ-CN and α_{S1}-CN 8P. Differences in the content of FAAs in ripened cheese were found between the cheeses as illustrated in the PCA shown in Fig. 3. BCAA cheese had a significantly lower content of total FAA (40.27 ± 5.25 μmol g^{-1}) compared with BBAA

Table 1
Effects, mean and standard deviation (SD), of the αS1-κ-CN composite genotypes (BBAA, BBBB and BCAA) on milk and 24 h cheese composition (%, w/w).[a]

Component	α_{S1}-κ-CN genotypes						P-value
	BBAA		BBBB		BCAA		
	Mean	SD	Mean	SD	Mean	SD	
Cheese milk							
Fat	4.19	0.01	4.14	0.04	4.17	0.06	NS
Lactose	4.68^{b}	0.02	4.59^{b}	0.07	4.85^{a}	0.04	0.003
Protein	3.78^{a}	0.03	3.66^{a}	0.06	3.43^{b}	0.05	0.006
Casein	2.82^{a}	0.02	2.74^{ab}	0.07	2.60^{b}	0.04	0.02
Total solids	13.63^{c}	0.05	13.19^{a}	0.11	13.32^{b}	0.09	0.002
24 h cheese							
Total solids	56.38	1.63	55.67	0.56	55.84	0.69	NS
Protein	21.82	0.93	21.09	0.63	20.71	0.34	NS
Fat	29.61	0.62	29.79	0.59	30.30	0.81	NS

[a] Fat, lactose, protein, casein and total solids in milk measured by MilkoScan FT1 (Foss Electric A/S, Hillerød, Denmark). In rows, significant differences between means are shown by different superscript letters (P < 0.05).

Table 2
Effects, mean and standard deviation (SD), of the αS1-κ-CN composite genotypes (BBAA, BBBB and BCAA) on experienced renneting time and different yield calculations.[a]

Parameter	α_{S1}-κ-CN genotypes						*P*-value
	BBAA		BBBB		BCAA		
	Mean	SD	Mean	SD	Mean	SD	
Renneting time (min)	30.00[a]	0.00	26.67[ab]	2.88	21.00[b]	1.73	0.02
PY (kg cheese 100 L^{-1} milk)	12.88[a]	0.02	12.63[b]	0.08	12.41[b]	0.14	0.007
Ya (kg cheese 100 L^{-1} milk)	13.81[a]	0.11	13.35[ab]	0.23	13.18[b]	0.07	0.016
YE (%)	103.41	0.85	101.97	2.41	102.50	1.70	NS

[a] Renneting time is the time from rennet addition to cutting of the gel as observed by an experienced cheese-maker; PY is the predicted cheese yield (eq. (1)); Ya is the actual cheese yield (eq. (2)); YE is the yield efficiency (eq. (3)). In rows, significant differences between means are shown by different superscript letters ($P < 0.05$).

cheese (48.06 ± 8.83 μmol g^{-1}) and BBBB cheese (55.91 ± 8.40 μmol g^{-1}) ($P = 0.03$). BCAA is located on the right side and BBBB cheese is located on the left side in. All differences in the content of FAA between the cheeses are shown in Supplementary material Table S2, but briefly, cheese with κ-CN BB (BBBB cheese) differed from cheese with κ-CN AA (BBAA and BCAA) due to significantly higher concentration of five FAAs (Ser, Gln, Thr, Ala and Met). Cheese with α_{S1}-CN BC (BCAA) differed from cheese with α_{S1}-CN BB (BBBB and BBAA) by having a significantly lower concentration of six FAAs (Ser His, Gly, Ile, Leu and Lys). In accordance with the FAA results, the content of DL-pyroglutamic acid also differed significantly between the cheeses: with BCAA < BBAA < BBBB (0.28 ± 0.04, 0.36 ± 0.05 and 0.43 ± 0.07 μmol g^{-1} respectively).

The BCAA cheese was perceived by the sensory panel to have a higher intensity of sweet taste than BBAA and BBBB cheeses ($P < 0.01$) but had a lower hardness ($P < 0.05$) (Fig. 4). There was more sunlight flavour in BBAA cheese compared with BCAA cheese ($P < 0.05$). Moreover, the BBBB cheese did not seem to differ from the two other cheeses (BBAA and BCAA) in most attributes except that it had a higher degree of juiciness ($P < 0.05$) compared with the BBAA cheese.

4. Discussion

In the present study, a Havarti-type cheese was made from milk with different composite genotypes of α_{S1}-κ-CN (BBAA, BBBB and BCAA). Milk composition, cheese-making properties, yield, ripening and sensory properties of the cheeses were evaluated.

Even though the BCAA milk had a lower casein content than BBAA milk, the BCAA milk obtained a significantly faster renneting time. Usually, a higher milk protein/casein content leads to better coagulation properties (Fox, Guinee, Cogan, & McSweeney, 2017b; Horne & Lucey, 2017; Jõudu, Henno, Kaart, Püssa, & Kärt, 2008). The BC genotype of α_{S1}-CN has earlier been reported to have good coagulation properties (Jensen et al., 2012; Jõudu et al., 2009; Ketto et al., 2017; Poulsen et al., 2013). However, conflicting results of casein content in milk with α_{S1}-CN BC are reported. Devold, Brovold, Langsrud, and Vegarud (2000) found a lower casein content while Jakob (1994) reported a higher content. The result from the current study suggests that other important factors than casein content affect the renneting time of milk. Factors such as protein composition and casein micelle size may have an influence, as explained further. Ketto et al. (2017) found that milk with α_{S1}-CN BC contained smaller micelles compared with α_{S1}-CN BB. Smaller

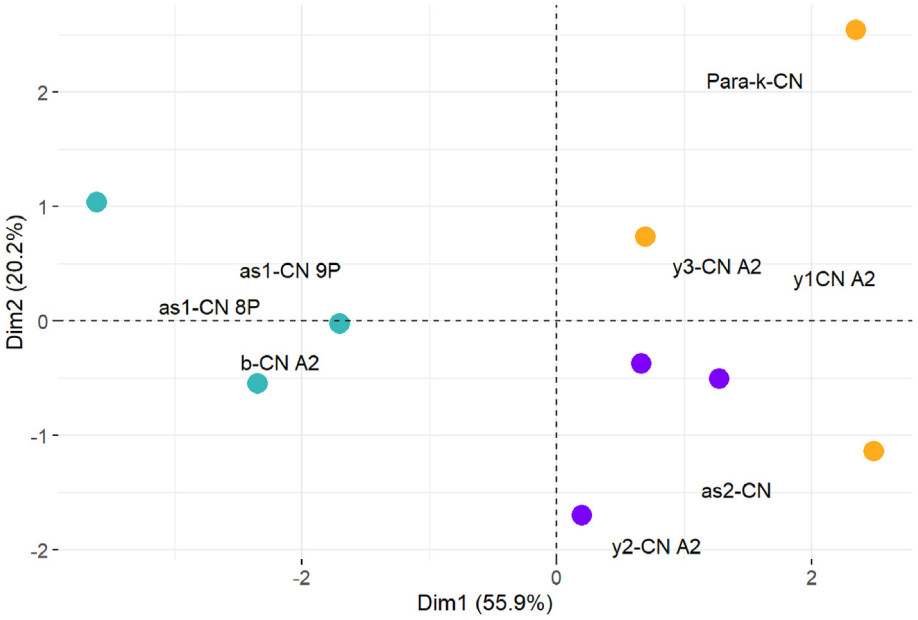

Fig. 1. Principal component analysis of caseins and γ-CN in 24 h cheeses with different composite genotypes of α_{S1}-κ-CN (●, BBAA; ●, BBBB; ●, BCAA). Principal components 1 and 2 explain 55.9 and 20.2% of the variation, respectively (Dim = dimension of the PCA).

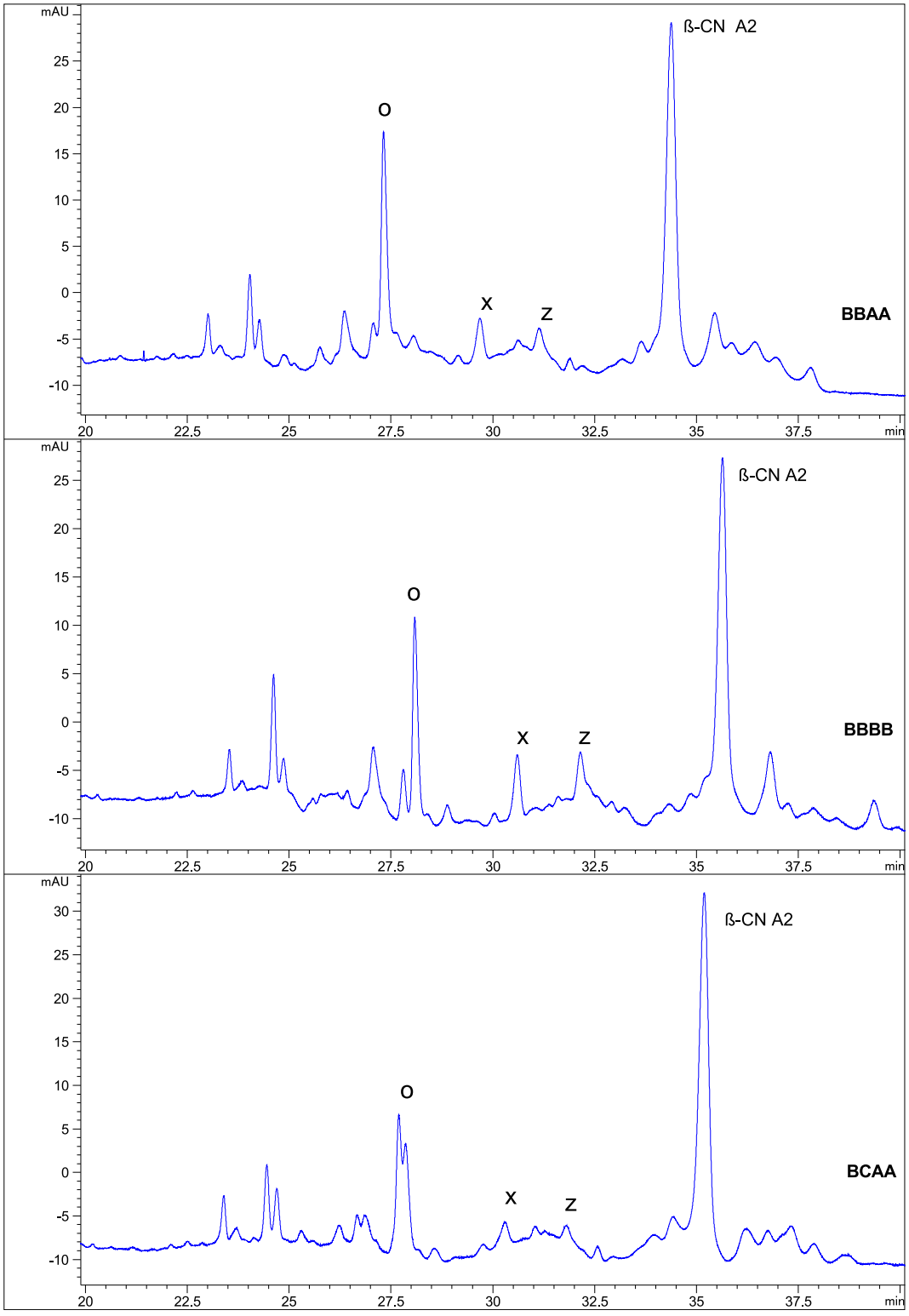

Fig. 2. Protein profiles of cheeses after 5 months of ripening representing the 3 composite genotypes (BBAA, BBBB and BCAA) analysed by capillary electrophoresis (absorbance at 214 nm; mAU).

casein micelles are known to have a positive effect on coagulation properties (Glantz et al., 2010; Logan et al., 2014; Walsh et al., 1998b), probably due to a greater total surface area of the micelles, which then leads to the formation of a stronger network during coagulation. Ketto et al. (2017) also found that milk with α_{S1}-CN BC contained a higher relative concentration of κ-CN, which forms the stabilising layer of the casein micelles, and this might explain why the micelles were smaller in milk with α_{S1}-CN BC.

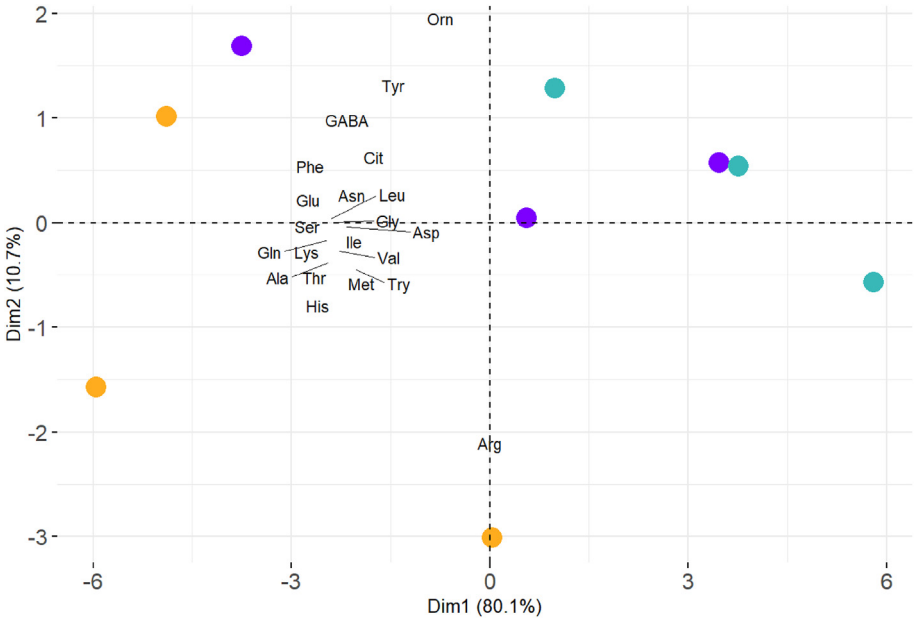

Fig. 3. Principal component analysis of free amino acids in cheese with different composite genotypes of α_{S1}-κ-CN (●, BBAA; ●, BBBB; ●, BCAA) ripened for 5 months. Principal components 1 and 2 explain 80.1 and 10.7% of the variation, respectively (Dim = dimension of the PCA).

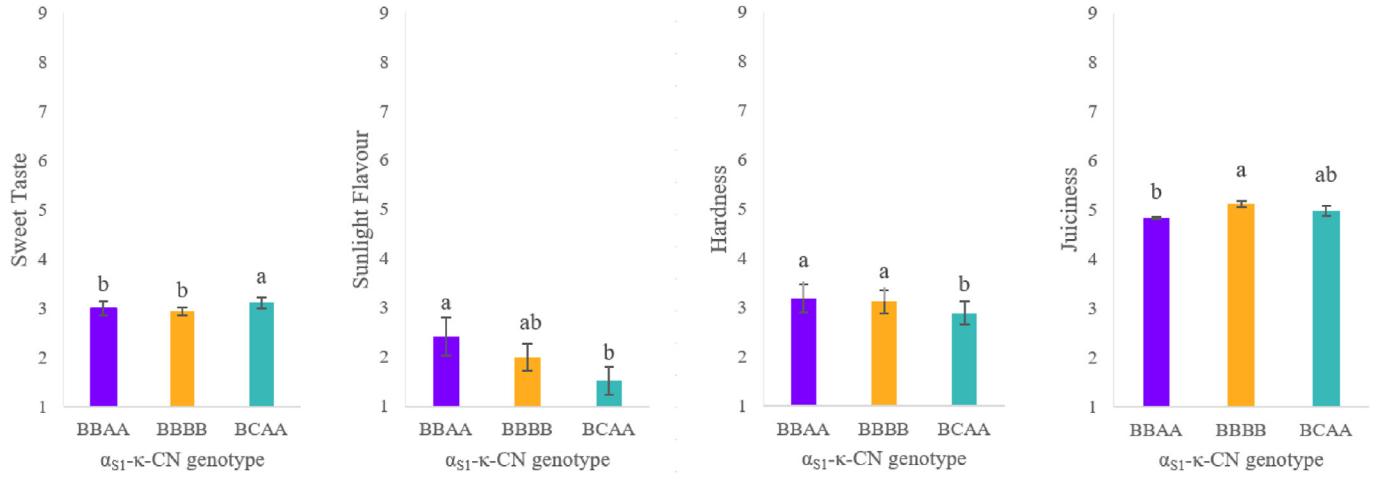

Fig. 4. Effects of the α_{S1}-κ-CN composite genotypes (■, BBAA; ■, BBBB; ■, BCAA) on the sensorial attributes sweet taste, sunlight flavour, hardness and juiciness. Different letters (Tukey groupings) between the α_{S1}-κ-CN composite genotypes indicate significant difference at $P < 0.05$.

In addition to renneting time, cheese yield is another important parameter for cheese-making efficiency. Good coagulation properties are usually connected to a higher cheese yield (Fox et al., 2017a) since the coagulum gets stronger and limits the loss of casein and fat. However, milk with good coagulation properties cannot alone estimate a high cheese yield. This current study confirms the good coagulation properties of α_{S1}-CN BC; however, BCAA milk resulted in a significantly lower cheese yield compared with BBAA. These results underline the importance of analysing more broadly and not just focusing on coagulation properties when selecting genotypes of casein when breeding for an increased efficiency of the cheese-making process. The most important factor affecting cheese yield is the milk composition, more specifically fat and casein (Fox et al., 2017a), therefore the milk casein content directly influences cheese yield. As the milk was fat standardised, the BBAA milk, which had the highest casein content was predicted to, and in fact gave, a significantly higher yield compared with the two other genotypes. In this study the cheese yield was highly correlated with the casein content of the milk.

Aleandri, Buttazzoni, Schneider, Caroli, and Davoli (1990) found that the BB genotype of α_{S1}-CN resulted in higher cheese yield compared with the BC genotype, which confirms the results of the present study, while others have found no differences in cheese yield when comparing the B and C variant (Ng-Kwai-Hang, 2007). This current study did not find any evidence that the κ-CN genotype affected Ya, but other studies (Walsh et al., 1995, 1998a) have reported a higher cheese yield with the κ-CN BB genotype compared with the AA genotype on pilot-scale Cheddar cheese production. In this study, the κ-CN genotype AA in combination with α_{S1}-CN BC resulted in a lower cheese yield, while in combination with α_{S1}-CN BB, the yield increased. This underlines the necessity to evaluate the composite genotypes rather than the individual ones.

The protein composition in 24 h cheese is usually reflected by the protein profile of the milk since the degradation of proteins is limited this early in the ripening process. However, there is already a difference in the degradation of β-CN, since cheese with α_{S1}-CN BB is associated with a higher relative concentration of γ-CN. This indicates that the early proteolysis of β-CN might be affected by differences in α_{S1}-CN genotypes (BC versus BB).

In ripened cheese, differences were observed in protein degradation, especially between cheeses with different genotypes of α_{S1}-CN. However, the electropherogram of ripened cheese is difficult to compare with that of the 24 h cheese as the retention times differ, therefore the peaks are difficult to identify. If the anticipations made on the identification of the peaks are correct, cheese with α_{S1}-CN BB (BBAA and BBBB) has a higher degradation of β-CN, while cheese with α_{S1}-CN BC (BCAA) has a higher degradation of α_{S1}-CN. The left part of the peak marked with "o" in the electropherogram of the BCAA cheese is probably γ-CN that has not been further degraded as seen in BBAA and BBBB cheese, while the right part of the peak most probably is α_{S1}-CN 8P that is more degraded than that of the BBAA and BBBB cheese.

Since ripened BCAA cheese had a higher peak of β-CN and a different protein/peptide profile and a lower concentration of total FAA compared with the ripened BBBB and BBAA cheeses, it is reasonable to assume that the degradation of β-CN is important for the ripening process of this cheese and that the ripening proceeded slower in the BCAA cheeses. The different genetic variants of Norwegian Red differ in milk protein composition (Ketto et al., 2017), which could result in different degradation profiles of the proteins, resulting in differences in FAA content in the cheese if the enzyme activities in the cheese are similar. In this study, the pH in the cheese both at the start of ripening and at the end was similar which would indicate similar enzyme activities.

DL-Pyroglutamic acid is a derivate from glutamine or glutamic acid (Gazme, Boachie, Tsopmo, & Udenigwe, 2019). It is present in many cheese varieties, but especially in long-ripened Italian cheeses such as Grana Padano and Parmigiano Reggiano (Mucchetti et al., 2000). Pyroglutamic acid can be used as an indicator of ripening (Mucchetti et al., 2000), as can the total content of FAAs. The formation of pyroglutamic acid is believed to be more dependent on the starter culture than on the raw milk microflora (Gazme, Boachie, Tsopmo, & Udenigwe, 2019; Mucchetti et al., 2000). However, the same starter culture was used for all cheeses in this current study. There are some indications that there are other factors in addition to the microflora that can affect the formation of pyroglutamic acid in cheese. Olsen, Ferneborg, Vhile, Kidane, and Skeie (2023) found that the protein source in concentrate feed affected the content of pyroglutamic acid in a Gouda-type cheese made using the same starter culture and with similar cheese microbiota in ripened cheese. The result of the current study indicates that also milk protein genetic variants, in addition to the feed for dairy cows and starter culture as found in previous studies, may affect the formation of pyroglutamic acid in cheese and thereby the cheese ripening.

BCAA cheese was experienced by the sensory assessors to be less firm compared with BBAA and BBBB cheeses, even though they did not differ in TS or fat content. This has also previously been reported by Nuyts-Petit, Delacroix-Buchet, and Vassal (1997) who found that Saint-Paulin cheese made with the B variant of α_{S1}-CN was associated with firmer cheese after 45 d of ripening. Moreover, the differences in firmness and juiciness could most probably be related to differences in proteolysis between the cheeses. Although significant differences in some sensory attributes were observed by a trained professional sensory panel, the intensities of these were low. Therefore, consumers can probably not differentiate between the cheeses.

5. Conclusions

Despite the fact that BCAA milk had a lower casein content, making cheeses with this genotype showed a shorter renneting time, but a lower cheese yield compared with BBAA milk. BBAA milk had the highest casein content giving the highest cheese yield. This shows that the superior renneting properties of α_{S1}-CN BC, which have also been reported in previous studies, might not contribute to a higher cheese-making efficiency in total. The results of this experiment add to previous research showing that the genetic polymorphism of casein needs to be taken into consideration regarding cheese-making properties, cheese yield and most probably also cheese quality. Which variant to choose would depend on what properties to emphasise. Using the results obtained in this present study to calculate the influence of the genetic variants on the revenue of a cheese plant producing 30 vats (processing 20,000 kg milk per vat) per day, 3780 kg more cheese could be obtained per day using BBAA milk compared with BCAA milk. However, BCAA milk used 9 min less to coagulate to sufficient firmness than BBAA milk and would result in 4.5 h daily reduced processing time. The cheesemaker needs therefore to decide what to emphasise.

The BCAA cheeses had a lower content of FAA and were less firm than BBAA and BBBB cheeses. Cheeses with α_{S1}-CN BB genotype contained the highest concentration of FAA and a higher content of peptides from proteolysis, indicating a faster ripening. However, it is important to note that the current research was carried out at a pilot scale, using only 100 L of milk, and these results, therefore, need to be confirmed at a larger scale.

Credit author statement

Martine Andrea Olsen: Methodology Validation, Formal analysis, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization. **Isaya Appelesy Ketto**: Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft. **Jorun Øyaas**: Conceptualization, Methodology, Project administration, Funding acquisition. **Ahmed Abdelghani**: Validation, Investigation, Resources. **Kristine Svartbekk Myhrer**: Validation, Investigation, Resources. **Siv Borghild Skeie**: Conceptualization, Methodology, Validation, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Funding

This work was funded by The Research Council of Norway (NFR; Grant number 234114) and TINE SA (Grant number 52114115). The Research Council of Norway infrastructure grants (NFR; Grant numbers: 208674/296083) financed the dairy pilot plant used in this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to thank May Aalberg and Kari Olsen for assisting in milk and cheese analyses. We also acknowledge the technical support from the workers of the dairy pilot plant (Ola Tjåland and Geirfinn Lund). The sensory panel at the Norwegian

Institute for Food and Aquaculture Research (NOFIMA) are acknowledged for conducting the sensory trials.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2023.105736.

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