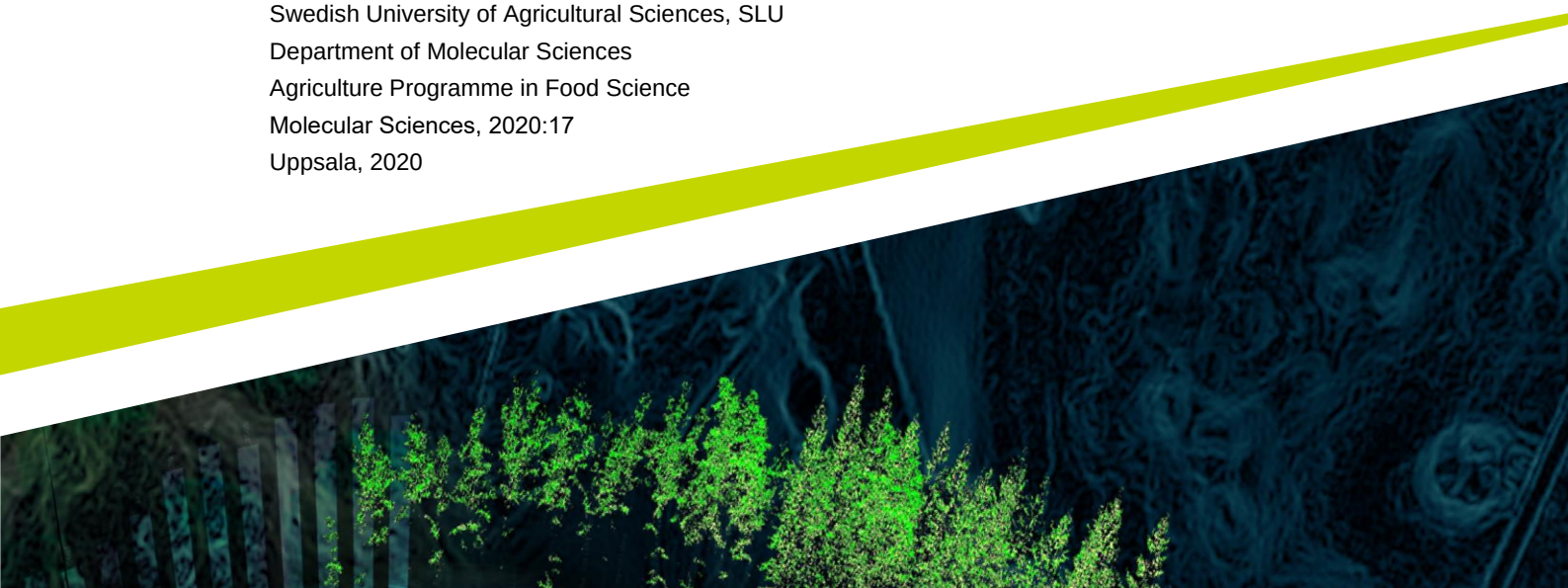
Composition and processability of milk from older cows

– A pilot study on milk quality differences between young and older cows

Sammansättning och processegenskaper hos mjölk från äldre kor – en pilotstudie om skillnader i mjölkkvalitet mellan unga och äldre kor

Gabriella Apelthun

Master Thesis Food Science • 30 hp
Swedish University of Agricultural Sciences, SLU
Department of Molecular Sciences
Agriculture Programme in Food Science
Molecular Sciences, 2020:17
Uppsala, 2020



Composition and processability of milk from older cows – a pilot study on milk quality differences between young and old cows.

Sammansättning och processegenskaper hos mjölk från äldre kor - en pilotstudie om skillnader i mjölkkvalitet mellan unga och äldre kor

Gabriella Apelthun

Supervisor: Monika Johansson, SLU, Department of Molecular Science
Examiner: Åse Lundh, SLU, Department of Molecular Science

Credits: 30 hp
Level: A2E
Course title: Master Thesis in Food Science
Course code: EX0877
Programme/education: Agriculture Programme – Food Science
Course coordinating dept: Department of Molecular Sciences

Place of publication: Uppsala
Year of publication: 2020
Title of series: Molecular Sciences
Part number: 2020:17

Keywords: Raw milk quality, lactation number, gel firmness, rennet coagulation time, Swedish Holstein, Swedish Red

Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences
Department of Molecular Science

Archiving and publishing

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. When you have approved, metadata and full text of your thesis will be visible and searchable online. When the document is uploaded it is archived as a digital file.

☒ YES, I hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work. https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/

☐ ~~NO, I do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.~~

Abstract

Most Swedish dairy cow's life expectancy is five years, which is 2.5 lactations. The most common reason for dairy cows being culled are e.g. impaired fertility, mastitis, or low milk yield. Increased cow longevity is associated with animal welfare and would reduce the emission intensity per unit of milk solids per cow. The objective of this study was to investigate the differences in raw milk quality (e.g. composition, SCC, pH, and plasmin- and plasminogen derived activity) and processability of milk (e.g. curd yield, ethanol stability, gel firmness, rennet coagulation time) from cows with different number of lactations, i.e. old cows compared to young cows, and breeds. Milks samples from SRB and SLB cows were collected for this study, eight from each breed. Milk from young SLB cows, had a significant harder gel firmness and shorter rennet coagulation time compared to milk from older cows, however, it was not observed within milk from SRB cows. Within SRB, the difference in SCC was significant between milk from older and younger cows. There was no significant difference in ethanol stability between lactation numbers, but SLB had significantly higher ethanol stability compared to SRB. In milk from SRB cows, the content of β_{A1}-caseins was significantly higher than in milk from SLB cows, whereas SLB milk had significantly higher content of β_{A2}-caseins. Further research with repeated analysis and more individuals is required, to be able to make any conclusions on how the number of lactations of cows affect the processability and quality of milk.

Keywords: raw milk quality, lactation number, gel firmness, rennet coagulation time, Swedish Holstein, Swedish Red

Sammanfattning

De flesta mjölkkor i Sverige har en förväntad livslängd på 5 år, vilket motsvarar 2,5 laktationer. Den vanligaste orsaken till att mjölkkor slaktas är bl. a. försämrad fertilitet, mastit eller låg mjölkproduktion. En ökad livslängd hos kor förknippas med ökad djurvälfärd och skulle också bidra till en minskning av växthusgasutsläppen per enhet mjölk torrsubstans per ko. Syftet med denna studie var att undersöka skillnader i mjölkråvarans kvalitet (dvs. sammansättning, SCC, pH, plasmin– och plasminogen erhållen aktivitet) och mjölkens processegenskaper (dvs. etanol stabilitet, gelstyrka, koaguleringstid, ostutbyte) hos kor med olika antal laktationer (unga och gamla) och ras. SRB och SLB kor valdes ut för att delta i studien, åtta från varje ras. I mjölk från SLB kor var skillnaden i gelstyrka och koaguleringstid signifikant mellan mjölk från äldre och yngre kor, dock var skillnaden inte signifikant för mjölk från SRB kor. Skillnaden i SCC mellan mjölk från äldre och yngre kor var signifikant hos mjölk från SRB. Det fanns ingen signifikant skillnad mellan antal laktationer och etanol stabilitet, däremot hade SLB signifikant högre etanol stabilitet jämfört med SRB. Ett signifikant högre innehåll av β_{A1}-kasein noterades i SRB, medan SLB hade signifikant högre innehåll av β_{A2}-kasein. Ytterligare studier med fler individer och upprepade analyser krävs för att kunna göra någon signifikant slutsats av hur antalet laktationer påverkar mjölkens sammansättning och processegenskaper.

Nyckelords: mjölkråvarans kvalitet, laktations nummer, gelens fasthet, koaguleringstid, Svensk Röd och vit boskap, Svensk Holstein

Table of contents

List of tables

List of figures

Abbreviations

G_{20}	Gel firmness after 20 minutes
Cn No	Casein number
PG	Plasminogen
PL	Plasmin
PM	Pooled milk samples
RCT	Rennet coagulation time
SCC	Somatic cell count
SLB	Swedish Holstein
SRB	Swedish Red
TS	Total solids
WP	Whey protein

1. Introduction

An increasing number of consumers and companies find sustainability important. There is a growing will and an attempt to act and take responsibility for the planet's resources and climate. The global dairy production plays one role in climate emission, where cows produce greenhouse gases, e.g. methane, generated from feed intake (Röös, 2019). Methane is the main greenhouse gas from cows and is of great concern because of its ability to increase the temperature of the atmosphere. Sustainable dairy production needs to be economically defensible while focusing on how to reduce climate emission and to keep good animal welfare.

Swedish dairy cows on average have 2.5 lactation cycles which means that the average life expectancy is 5 years (Cattle statistics, 2020). Dairy cattle have naturally a much longer life expectancy, around 20 years (De Vries and Marcondes, 2020). The most common reasons for dairy cows being culled in Sweden are e.g. impaired fertility, mastitis, or low milk yield (Cattle statistics, 2020). Increased cow longevity is associated with increased animal welfare (Langford and Stott, 2012) and animal welfare is becoming increasingly important for consumers when buying milk (de Graaf *et al*., 2016). In a UK survey, the researchers found that consumers have an interest in animal welfare and are willing to pay more for food products associated with good cow dairy welfare (Ellis *et al*., 2009).

Culled dairy cows are replaced by heifers but many farmers are unaware of the true cost of rearing dairy heifers from birth to calving (Boulton *et al*., 2017). According to Boulton *et al*. (2017), it takes approximately 1.5 lactations until the rearing costs of the cow is repaid. During the period of rearing the heifer is non-productive and the main emissions comes from the maintenance which is a larger proportion of the total emissions. The total emission quota of the dairy cow decreases when she begins to produce milk. Improved health and fertility of cows lead to an increase in overall survival and a positive impact on the profitability (Bell and Wilson, 2018). Increased longevity of dairy cows would thus reduce the emission intensity per unit milk solids and per cow. In addition, dairy cows increase their milk yield until the fifth lactation (Langford and Stott, 2012).

The average somatic cell count (SCC) increases with parity (Salsberg *et al*., 1984). SCC is also the most important and common quality incentives in today's payment system (Barbano *et al*., 1991; Murphy *et al*., 2016). The payment is often

based on specific levels of SCC where lower levels are connected to higher payment. SCC is related to the health of the cow and elevated levels thereby indicate that there might be negative changes in the quality of their raw milk. High SCC can be a sign of mastitis, a disease that affects the udder health of the cow (Nilsson, 2017). White blood cells from the blood increase in the udder to fight the infection and leave the udder via milk and can be detected and quantified by analyses. Mastitis causes changes in milk composition because blood components are leaking from the blood into the milk (Walstra, 2006). The milk yield may also decrease due to mastitis. The milk in Sweden is currently classified and paid based on the concentration of total milk fat and protein per kg where protein is valued higher (Wedholm *et al*., 2006b; Nilsson 2017). Other quality parameters that are rewarded by the payment system are e.g. low total bacteria counts, bacterial spores, and taste –and odour defects (Nilsson 2017). Yet, it is not known how or if the processability and other qualities of raw milk are affected by the number of lactations.

1.1 Aim and objective

The objective of this study was to investigate the differences in raw milk quality (e.g. gross composition, SCC, pH, and plasmin- and plasminogen derived proteolytic activity) and processability of milk (e.g. curd yield, ethanol stability, gel firmness, rennet coagulation time) in cows with different number of lactations, i.e. old cows compared to young cows and breeds. The definition of an old cow was in this study a cow with three or more lactations during her lifetime, whereas a young cow was a cow with two or fewer lactations. This study aimed to examine whether older cows can be retained in dairy production of benefits other than animal welfare, reduced climate emission, and economy.

2. Background

2.1.1. Major dairy cow breeds in Sweden

The most common dairy cow breed in Sweden today is the Swedish Holstein (SLB) (Nilsson, 2017; Cattle Statistic, 2020). The Swedish Red (SRB) was more common until a few years ago. SRB cows are red or red-brown with white elements and the SLB is black (Widebeck, 2000). SLB cows produce on average 10 551 kg milk per year compared to the SRB which produces 9 245 kg milk per year (Cattle statistic 2020). However, the average fat and protein contents are higher in milk from SRB cows than in SLB milk. Milk from SRB has on average 4.40 % fat and 3.70 % protein while milk from SLB cow on average contains 4.11 % fat and 3.52 % protein (Cattle Statistic 2020). SRB cows also have better general health than SLB cows (Cattle Statistic 2020; Nilsson 2017). In 2019, the average yield for SLB cows was 10 790 ECM kg milk per year whereas SRB cows on average produced 9 910 ECM kg milk per year (Cattle statistics 2020). ECM stands for Energy Corrected Milk which means that the milk is standardized (i.e. fat, protein, and/or lactose) to the same energy value. This equation is used to make it easier to compare milk from different cows and herds (Nilsson 2017).

2.1.2. Milk composition and milk components

Milk is a complex liquid and its main components are water, protein, fat, lactose, and minerals (Walstra, 2006). Fresh cow milk contains on average, 4 % fat, 3.3 % protein (of which 2.6 % is casein), and 4.6 % lactose (ibid.). The milk composition and protein profile vary due to genetic factors, such as breed and individuals, stage of lactation, cow's health, and feed (Walstra, 2006; Wedholm *et al*., 2006b; Nilsson, 2017). Other factors that may have an impact on the milk composition include the age of the cow, season (Nilsson 2017), milking frequency (Løvendahl and Changunda, 2011), and milking systems (Johansson *et al*., 2017). These factors affect the milk quality which directly affects the coagulation properties of the milk, cheese yield, and the final quality of the product.

The fat content in the milk is easiest to control compared to the mentioned components above (Nilsson, 2017). It is influenced mainly through the feed and in

the long run by breeding selection. The protein content in milk is harder to control than the fat content because of genetics, but there is a connection between the components. A higher fat content causes to some extent, increased protein content (Nilsson 2017). The availability of amino acids in the feed and the activity in the rumen stomach of the cow needs to be adequate to produce proteins. The lactose concentration in milk is harder to control due to its important role in milk secretion, based on its osmotic activity, it controls the water flow (Nilsson, 2017). Lactose is synthesized by the mammary epithelial cells and regulates the fluid transport from the blood into the alveoli by the osmotic pressure (McManaman *et al*., 2006). The Golgi vesicles in the epithelial cells secrete the lactose and water is drawn from the blood into the mammary gland and alveoli, forming the milk serum phase. Good udder health is therefore important since the lactose concentration in milk is decreased by the damage of the epithelial cells and the milk yield may be affected (Nilsson, 2017; Walstra 2006). Inflammation in the udder (e.g. mastitis) will cause low molar-mass components from the blood to leak into the milk (Walstra, 2006). This will change the milk composition and lower the lactose content, because of the higher contents of dissolved salts in blood serum.

Protein profile in milk

Of the proteins in the milk, approximately 80% is casein, and 20% serum (whey) proteins (Walstra, 2006). Caseins are present in micelles and are divided into four subgroups, α_1- casein, α_2-casein, β-casein, and κ-casein (Walstra 2006). The subgroups exist in many variants (ibid.). According to Farell *et al.* (2004), β-casein is most common followed by α-casein and κ-casein. The whey proteins are explained as proteins left in the milk serum after precipitation of casein (Farell *et al*., 2004). Most whey proteins are heat sensitive to temperatures above 60°C, due to their globular structure (Walstra, 2006). The major whey proteins are- β-lactoglobulin (β-LG), α-lactalbumin (α-LA), blood serum albumin (BSA), lactoferrin and remaining proteins are immunoglobulins (Ig) and proteas peptones (Walstra 2006).

2.1.3. Importance of milk quality

The raw milk quality is of great importance for the dairy industry. The composition is central for the value of milk and it directly affects its processability in dairy products (Lindmark-Mansson *et al*., 2003). Sweden is together with Denmark, the dominant milk-producing countries in Scandinavia. In 2019, about 27% of the total milk volume produced in Sweden was produced as consumption milk (Jordbruksverket, 2020). Remaining milk was used in the production of e.g. sour milk, cheese, powdered milk, cream, and butter. Calcium is an important component for the cheese-making i.e. the milk clotting process (Walstra, 2006).

When rennet is added to the milk it cleaves off a part of the κ-casein from the micelle surface and in the presence of calcium ions causes the casein micelles to aggregate and form a gel. One common method for quality measurements of the milk is the ethanol stability test (Horne and Muir, 1990). It is an ethanol-induced coagulation test, that indicates what ethanol concentration needed to cause the milk proteins to precipitate. The purpose of the ethanol test is to predict milk heat stability (Chavez *et al*., 2004) and if the milk is suitable for thermal processing e.g. ultra-high temperature and milk powder.

Somatic cells and plasmin activity

Somatic cells found in milk are macrophages, lymphocytes, leukocytes, and polymorphonuclear cells (Murphy *et al*., 2016). SCC is often used as an indicator of whether the cow has mastitis or not since the disease causes somatic cells to migrate from the blood into the milk. Nilsson (2017) defined a healthy cow with a cell count, below 100 000 cells ml/milk. Murphy *et al*. (2016) defined low SCC to be <250 000 cells/mL and high SCC >500 000cells/ml. However, even healthy cows may have a high number of SCC because it increases by age and with the later stage of lactation (Walstra, 2006). Since milk quality is negatively affected by mastitis where cheese yield is often reduced when using raw milk with increased SCC (Murphy *et al*., 2016). It has also been shown that milk with increased SCC generally has elevated the activity of plasmin (Walstra, 2006; Murphy *et al*., 2016).

Plasmin (PL) is the major endogenous protease in milk (Ismail and Nielsen, 2010). Plasminogen (PG), the inactive precursor of plasmin, is also present in milk (Walstra, 2006). Urokinase is a serine protease, also known as urokinase plasminogen activator (uPA) converting plasminogen to the active form plasmin (Walstra, 2006). Proteolysis, induced by plasmin can have both damaging and beneficial effects on flavour and texture of dairy products (Ismail and Nielsen, 2010). However, it depends on what kind of dairy product and the extent of hydrolysis.

Compared to plasmin, the inactive plasminogen concentrations are higher in milk. Plasminogen migrates from the blood into the udder, where it is transformed into plasmin if plasminogen activators are present (Murphy *et al*., 2016). The udder has a favourable temperature (37℃) and the damage done to the milk proteins by plasmin will most likely occur in the udder before milking (ibid.). Plasmin and plasminogen are heat resistant and can to some extent withstand UHT treatment (ibid.). Plasmin hydrolyses proteins, especially β-caseins and α-caseins, while whey proteins seem not to be affected as much (Walstra, 2006; Murphy *et al*., 2016). When β-casein is hydrolysed by plasmin it results in y-caseins and protease-peptones, and this reaction proceeds even at very low temperatures (Walstra, 2006). The degradation of products from β-caseins is lost in the whey serum during the cheese-making process (Auldist & Hubble, 1998, see Murphy *et al*., 2016). The

amount and activity of plasmin and plasminogen are therefore important in the perspective of milk quality.

Casein content and cheese yield

In cheesemaking, caseins are the main proteins and the crucial components for the coagulation process (Lindmark-Mansson *et al*., 2003; Murphy *et al*., 2016). Elevated SCC has been associated with a decrease of casein in milk. Other factors in the cheese process associated with increased SCC are e.g. increased rennet coagulation time and increased cheese moisture (Barbano *et al*., 1991; Murphy *et al*., 2016). In a study of Swedish dairy milk, between 1970 and 1996 (Lindmark-Mansson *et al*., 2003) showed a decrease in casein content in the milk while the total protein content stayed the same, due to the increase of whey proteins. The decrease of casein content in Swedish dairy milk can be associated with the increased milk yield (Lindmark-Mansson *et al*., 2003). Other explanations could be factors such as breeding, feeding, plasmin activity, and the payment system where the content of fat and protein being overseen by the valuing of increased milk yield (ibid.). Casein number (Cn No %) is defined as total casein out of the total protein, times hundred. A large increase in total protein and a smaller increase in casein results in a lower Cn No. The casein is an important variable since it determines the cheese yield per kilogram of milk protein (Walstra, 2006). The most common definition of cheese yield is the resulting cheese (kg) per 100 kg of milk, with a defined protein and fat content (ibid.). Two of the most important components in cheese yield are fat and protein, however, the casein is often considered as the most central component in cheese manufacturing, because most cheese cannot contain less than 20% casein (Walstra, 2006). Low content of casein affects the cheese production because of its negative effect on the cheese yield (Lindmark-Mansson *et al*., 2003), where more milk per kg cheese is needed (Nilsson 2017). The fat content may vary in the cheese (Walstra, 2006). The cheese yield is of importance for the manufacture since it may determine the profit in the end (Walstra, 2006; Wedholm *et al*., 2006b).

Sustainability and cows

Cows and other ruminants are an important part of the climate debate because of their greenhouse emissions at different stages of their life. Methane is generated when feed is digested by ruminants, and both carbon dioxide and nitrous oxide is produced during feed production (Röös, 2019). Compared to carbon dioxide, methane emission has up to 34 times more effect on the climate. However, methane does not stay in the atmosphere like most of the carbon dioxide, because it is removed after about one decade. If the methane emission is kept on a constant level, i.e. an equilibrium between what is generated and decomposed, an increase in

temperature may be under control. However, an increase in methane emissions will increase temperature (Röös, 2019).

2.1.4. Aim of study

The aim of this study was to investigate the differences in raw milk quality (e.g. gross composition, SCC, pH, and plasmin- and plasminogen derived proteolytic activity) and processability of milk (e.g. curd yield, ethanol stability, gel firmness, rennet coagulation time) in cows with a different number of lactations and breed. Cows with ≥3 or more lactations were defined as old and cows with ≤2 or fewer lactations were defined as young cows.

3. Materials and methods

3.1. Animals and milk sampling

Milk samples from individual cows were collected from the experimental dairy heard at Swedish Livestock Research Centre, Lövsta, Uppsala, Sweden. The selection of cows was based on the number of lactations. Suitable individuals were selected in consultation with the Research and Education Coordinator at Lövsta.

Milk samples were collected from 16 dairy cows. The cows were of two different breeds, Swedish Red (SRB) and The Swedish Holstein (SLB). The cows were divided into two groups based on breed. Each group (n=8) consisted of four younger i.e., ≤ 2 lactations and four older i.e., ≥ 3 lactations (Table 1). The cows participating in this study were in the lactation interval of at least 8 weeks after calving and have 12 weeks until the next one. Milk sampling took place in the morning (between 7-9 AM) for four weeks (Feb-Mar, week 8-11 in 2020). The milk from separate breeds was collected one week and replicated the following week, i.e. biological replicates. The cows were normally machine milked by a milking robot, but to facilitate the collection of milk in this study, the cows were bucket milked by staff at Lövsta Research Center. Around 300-500 ml of milk from each cow was directly poured into a glass bottle and stored in a cooling box until arrival to the laboratory. The study was conducted in the research facilities at Swedish university of agriculture (SLU) in Uppsala, Sweden.

Table 1. Lactation number of young and old cows in SRB and SLB

	SRB (n=8) Lactation number	SLB (n=8) Lactation number
Young	1, 2, 2, 2	2, 2, 2, 2
Old	3, 4, 4, 5	3, 3, 3, 4

3.2. Pooling of milk samples from individual cows

In order to mimic tank milk, the collected fresh milk samples from the eight animals were pooled separately for young respective old cows. Pooled milk aliquots of 200 ml were created, based on five different concentrations of young and old cow's milk (see Table 2). The various concentrations aimed to investigate whether pooling milk with different proportions of milk from younger and older cows affects the result. The five concentrations of pooled milk samples (PM) were used in all analyses. Technical replicates were performed to receive a reliable result.

Table 2. Proportions of pooled milk samples (PM) from young and old cows[1,2]

PM	Milk from young cows (%)	Milk from old cows (%)
1a/1b	0	100
2a/2b	30	70
3a/3b	50	50
4a/4b	70	30
5a/5b	100	0

[1]The definition of an old resp. young cow in this study: old ≥ 3 lactations; young ≤ 2 lactations.
[2]The letters a and b stand for sampling occasion one resp. two.

3.2.1. Milk sample preparation

Milk samples of 50 ml, from each pooled milk, were placed in a centrifuge (Sorvall, Super T21, Sorvall Products L.P., Newton, Connecticut, USA); rotator (ST-H750) and defatted at 3000 RPM at 4˚C for 10 min. After the centrifugation step, the fat layer on the surface of the milk was removed by a cotton stick. The defatted PM samples were used for rheology measurements, ethanol stability test, plasmin and plasminogen derived activity, and protein profile. The whole milk samples were used for gross composition analyses and micro cheeses. Fresh milk was used for rheological analyses, ethanol stability, and micro cheese manufacture. Milk for the remaining analyses was stored at –20 and 4˚C until use.

3.2.2. Analysis of milk gross composition

Individual milk samples were analysed for gross composition and whey protein content at the Department of Animal Nutrition and Management, SLU. Total protein, total casein, total fat, and lactose concentrations were analysed by mid-infrared spectroscopy method (Fourier Transform Infrared Spectroscopy; FTIR); (FOSS Electric A/S (Hilleröd, Denmark). Somatic cell count (SCC) was analysed by electronic fluorescence-based cell counting (Fossomatic Foss FT 120, Hilleröd,

Denmark). The milk gross composition in each pooled milk sample was analysed once, that is one biological replicate.

3.2.3. pH measurement

The fresh pooled milk samples were tempered in a water bath for 15 minutes (min) at 30˚C before measuring the pH of the milk (one biological replicate). The pH was determined using Mettler Toledo, SevenCompact pH meter S210. The pH was measured before the analysis of the coagulation properties.

3.2.4. Detailed analysis of the milk protein profile

Frozen aliquots of defatted milk were thawed and prepared for analysis of the milk protein profile by capillary electrophoresis (CE) (Agilent Technologies 7100, Capillary electrophoresis). This method was performed according to Johansson *et al*. (2013). Individual proteins: α-LA, β-LG, α_{s1}-CN, α_{s2}-CN, β_B-CN, β_{A1}-CN, β_{A2}-CN, and κ-CN, were calculated in percent, based on the peak area expressed in % in the electropherogram. The total casein concentration was defined as the sum of α_{s1}-CN, α_{s2}-CN, β_B-CN, β_{A1}-CN, β_{A2}-CN, and κ-CN. The total whey protein concentration was defined as the sum of α-LA and β-LG. Each pooled milk sample was analysed once by CE, that is one biological replicate.

Preparation of buffers

Sample buffer and Run buffer were prepared according to a standard operating procedure, for the CE analysis.

The Sample buffer containing 0.167M Tris[hydroxymethyl]aminomethane (Triss; Mw 121.14) 0.067M Ethylenediaminetetraacetic acid (EDTA; Mw 372.2), 0.042M 4-Morpholinopropanesulfonic acid (MOPS; Mw 209.26), and w/w 0.05% Hydroxypropyl methylcellulose (HPMC) and 6.3g ion exchange resin (AG 501-X8 Resin, Bio-Rad Laboratories, Inc, CA) was dissolved in 350 ml urea solution of 6 M (Mw 60.06) over the night. After dissolution, the Sample buffer was filtered through a 0.45 µm membrane into 15 ml falcon tubes, aliquots of 13 ml. The Sample buffer aliquots were stored at – 20˚C. On the day of sample preparations and the analysis, 0.017M D, L-dithiothreitol (DTT; Mw 154.25) was added to the Sample buffer to disrupt the disulphide bridges of the milk proteins.

The Run buffer consisted of 0.02M sodium citrate tribasic dihydrate (Mw 294.10), 0.19M citric acid monohydrate (Mw 210.14), and w/w 0.05%M HPMC and was dissolved in 0.35 ml of 6M urea solution. The Run buffer was filtered through 0.45 µm membrane and aliquots of 2 ml were stored at –20˚C together with the Sample buffer aliquots until used for analysis. Chemicals were obtained from Sigma-Aldrich (Sigma-Aldrich, USA) unless otherwise stated.

Milk sample preparation

From each pooled milk sample, 2 ml of defatted milk was thawed in a water bath at 45˚C for 15 minutes. The samples were vortexed and placed in the water bath for another 15 min. From each sample, 150 µl of milk were pipetted into an Eppendorf tube and mixed with 350 µl of the Sample buffer. The sample solution was vortexed and incubated for one hour at room temperature. Samples were then defatted a second time in 10 minutes at 10 000 RPM and 4˚C (Hitachi T15A61-0606). The surface lipid layer on the samples was removed, and the sample was filtered through a 0.45 µm econofilter nylon membrane (Agilent Technologies, Agilent Captiva Econofilter) into a new Eppendorf tube. 30 µl of the filtrate was transferred to the conic vials for the analyses by CE.

3.2.5. Plasmin and plasminogen activity

Preparation of reagents and buffer

The plasmin buffer (pH 7.4) consisted of 20 mM 6-aminocaproic acid (EACE) (Mw 131.17), 53 mM Trizma hydrochloride (Mw 157.6), and 117 mM NaCl (Mw 58.44; VWR Chemicals, Belgium). The buffer was prepared in 600 ml distilled water and pH was adjusted to 7.4 using 1mM and 1M hydrochloride (HCl) and measured by Mettler Toledo, SevenCompact pH meter S210. The plasmin buffer was stored at room temperature.

Plasmin activity was measured using 25 mg chromogenic substrate (Biophen CS-41(03); Hyphen BioMed, Neuville Sur Oise, France) for plasmin and plasminogen. The Chromogenic substrate in the substrate bottle was diluted with 10 ml of distilled water, mixed carefully, and divided into 1 ml aliquots. The substrate solution was stored at 8˚C.

Plasminogen derived activity was measured by plasmin activation with urokinase (U4010-10KU; Sigma-Aldrich, Co., St Louise, MO, USA) from human kidney cells. The 13.1 mg urokinase powder was diluted with 600 µl dH_2O and mixed carefully. The enzyme solution was aliquoted to 100 µl and stored at –20˚C.

Milk sample preparation

Frozen aliquots of defatted milk were thawed and prepared for analysis of plasmin (PL) and plasminogen (PG) derived proteolytic activities in the milk. The method was performed according to Korycka-Dahl *et al.* (1983) and modified by de Vries *et al*. (2015).

For each sample of pooled milk, 320 µl defatted milk sample was transferred into a new 15 ml falcon tube and mixed with 4 680 µl plasmin buffer. To dissociate plasmin and plasminogen from the casein micelles incubation with EACE at room temperature for 2 hours was performed followed by ultracentrifugation. After the

incubation period, 3 900 µl from the sample solution was pipetted into Beckman tubes. The tubes were closed and ultra-centrifuged (Op-tima MAX-XP, Beckman Coulter, Inc., Bromma, Sweden) using RP55T angle rotor, 12 mL×12 at 4˚C for 1h at 100 000 RPM. After the ultracentrifugation step, the serum was divided into 2 ml aliquots and frozen at –20˚C until analysis.

For PL and PG derived activity analysis, a 96-wells microplate was used. In the two first rows, three wells were left blank, i.e. it only contained plasmin buffer. For the proteolytic activity of PL, 650 µl of milk serum and 173 µl of the substrate solution (Biophen) was mixed in an Eppendorf tube and two wells of the plate (two technical replicates) were loaded with 190 µl each. The substrate contains the specific peptide sequence, pyro-Glu-Phe-Lys-pNa-HCl, which is cleaved by PL, leaving the end product p-nitroaniline (pNA), a fluorogenic peptide to be measured (Korycka-Dahl *et al.,* 1983). The formation of pNA is measured as a change in absorbance and reflects the activity of PL. To the remaining volume in the Eppendorf tube, 10.5 µl urokinase was added, to measure PG derived proteolytic activity. It was mixed carefully, and two wells (two technical replicates) were loaded with 190 µl each.

PL and PG derived activity was measured by absorbance at 405 nm, at every third minute in 120 min (41 cycles, 3min/cycle) at 37˚C in a multi-mode microplate reader (FLUOstar Omega, BMG Labtech, Ortenberg, Germany). The activity was expressed as a change in absorbance per time unit ($\Delta A405/\Delta t$).

The PL activity and total activities were calculated from the linear parts of the absorbance curve versus time. The PG derived activity was calculated by the difference between total activity and PL activity. PL and PG derived activity were expressed in the same units (U/mL). One unit equals the amount of urokinase activated PG or PL, that in 1 minute, causes a 0.001 change of absorbance at 405nm, at 37°C (Korycka-Dahl *et al.*, 1983). The PL and PG in each pooled milk sample were analysed by two technical replicates.

3.2.4. Rheological measurements

The rheological properties were measured using a rheometer (Bohlin CVOR 150, Malvern instruments) equipped with a cup (Ø 25 mm) and a concentric cylinder (Ø 28 mm) at a height of 40 mm. Peltier element was used to control the temperature (30°C) during the measurements. The method used was previously described by Johansson *et al*. (2015). In short, bovine rennet 75/25 chymosin/pepsin, 180 IMCU (Scandirenn Kemikalia AB, Skurup, Sweden), was added to the skimmed milk at a concentration of 0.18 IMCU/ml. The rennet coagulation time (RCT) was measured from the addition of the rennet until elastic modulus (G') reached 1 Pa. The gel firmness ($G_{20,}$ Pa) was determined twenty minutes from the rennet addition. The RCT and G_{20} in each pooled milk sample were analysed by two technical replicates.

3.2.5. Ethanol stability test

Ethanol stability is defined as the highest ethanol concentration that can be added to the sample without causing visual coagulation of the milk proteins when equal volumes of milk and aqueous ethanol solution (v/v) are mixed (Davies and White, 1958). Ethanol concentrations ranging between 48-96% in 2% increments were prepared and 99.5% EtOH was used as a stock.

In an Eppendorf tube, 0.5 ml of defatted milk and 0.5 ml of each ethanol dilution were vortexed and incubated for 30 min, before determining the concentration which will give rise to coagulation. During each sampling occasion, one biological replicate of the ethanol stability test was carried out on each pooled milk sample.

3.2.6. Micro-cheese production

The manufacturing of the micro cheeses was executed according to Othmane *et al.* (2002) and Högberg (2016), with some modifications (see Figure 1). In this method, the curd yield and whey concentration were measured after rennet-coagulation and centrifugation steps. Four technical replicates of each pooled milk sample were performed to ensure a whey volume that would suffice for the protein analysis.

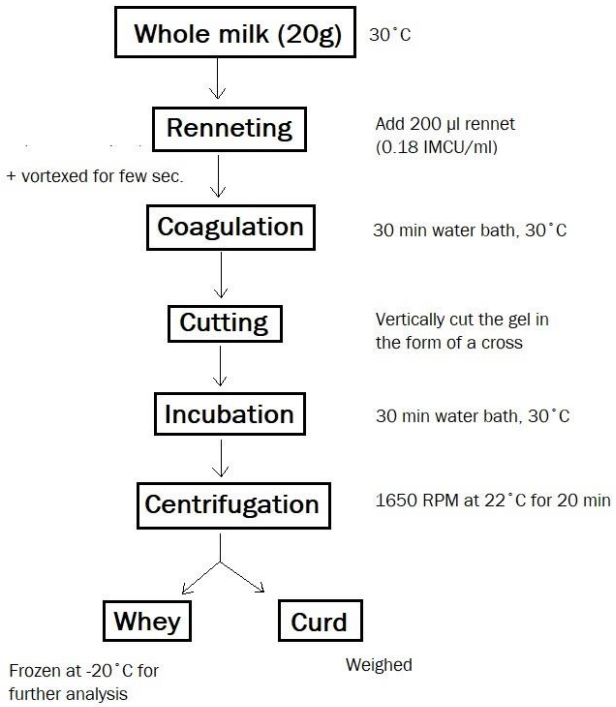


Figure 1. Flow chart for the manufacturing of micro cheese, with inspiration from Othmane *et al.* (2002) and Högberg (2016).

Material preparation

Rennet solution with a strength of 18 IMCU was prepared from an original rennet with a strength of 180 IMCU. The composition of rennet was 75% chymosin and 25% bovine pepsin (Scandirenn Kemikalia AB, Skurup Sweden).

Milk sample preparations

The fresh whole pooled milk (PM) was stored at 4˚C prior analysis. A pre-weighed 50 ml colonial falcon tube was used to weigh 20 grams of whole milk and warmed in a water bath at 30˚C for 30 min. 200 µl rennet (strength 18 IMCU) was added, giving 0.18 IMCU/ml milk. To ensure even distribution of rennet in the milk, the sample was vortexed for a few seconds before the coagulation was carried out in a water bath at 30˚C for 30 min. After 30 min, the obtained gel was vertically cut, in the form of a cross. To allow syneresis, the gel was incubated for another 30 min in a 30˚C water bath.

The colonial falcon tube containing the gel (curd + whey) was centrifuged at 1650 RPM (Sorvall, Super T21, Sorvall Products L.P., Newton, Connecticut, USA) at 22˚C for 20 min. The expelled whey was transferred to a new, pre-weighed 50 ml colonial falcon tube and the curd and the whey were weighed separately. The whey was frozen at –20˚C for further analyses of the protein content. The curd was weighed and the weight of the colonial falcon tube was subtracted.

To determine the casein content in the milk, the whey was analysed by mid-infra-red technique. The total protein content in the milk minus the protein in whey is roughly equal to the casein content of the milk. The casein number (Cn No%) was calculated as (casein/total protein) *100. The total individual curd yield was determined by expelling the whey from the curd and weigh the curd in the already weighed falcon tube. The curd was calculated in percent (per 100 grams).

3.3. Statistical analysis

Pearson’s correlations were performed for milk samples from both SRB and SLB cows. Two-sided regression analyses were performed on quality variable and milk proteins in the pooled milk samples to investigate whether it was a difference between them. Significant levels in this current study were the following values: 0.05, 0.01, and 0.001. Statistical analysis was performed using software Minitab® Version 18.1 (Minitab Inc., in the United States). Graphical illustrations were made in Microsoft® Excel® version 1908.

4. Result

4.1. Milk gross composition

The milk gross composition for the two sampling occasions is shown in Tables 3 and 4. Most of the components in the milk from SRB and SLB cows was similar between the two sampling occasions. However, milk from SLB had some samples of milk that stood out because of their high content of totals solids and fat in both of the sampling occasions. The SCC in milk from both SRB and SLB cows was relatively lower in the second sampling occasion.

Table 3. Milk composition, SCC and pH in both sampling occasions in PM from SRB cows[1, 2]

PM-SRB (% milk from young cows)	TS%	Fat%	Lactose%	Protein%	Casein%	WP%	Cn No.%	SCC*10^3/ mL	pH
1a (0%)	12.90	4.18	4.57	3.52	2.58	0.94	73.30	535.00	6.64
1b	13.46	4.70	4.56	3.52	2.60	0.92	73.86	257.00	6.59
2a (30%)	12.74	4.06	4.57	3.44	2.53	0.91	73.55	392.00	6.63
2b	12.84	4.20	4.53	3.42	2.54	0.88	74.27	177.00	6.60
3a (50%)	12.52	3.93	4.56	3.35	2.47	0.88	73.73	287.00	6.64
3b	13.18	4.50	4.56	3.41	2.55	0.86	74.78	147.00	6.62
4a (70%)	12.36	3.80	4.56	3.28	2.43	0.85	74.09	177.00	6.69
4b	12.64	4.21	4.45	3.26	2.42	0.84	74.23	90.00	6.62
5a (100%)	12.20	3.68	4.58	3.19	2.38	0.81	74.61	18.00	6.68
5b	12.31	3.87	4.49	3.21	2.39	0.82	74.45	10.00	6.60

[1]The contents are in percent per 100 grams. [2]Abbreviations: PM=pooled milk sample; TS=total solids; WP=whey protein; Cn No=casein number; SCC=somatic cell count. The letters a=sampling occasion one; b= sampling occasion two; 1–5=pooled milk samples with different proportions (%) of milk from young cows.

Table 4. Milk composition, SCC and pH in both sampling occasions in PM from SLB cows[1, 2]

PM-SLB (% milk from young cows)	TS%	Fat%	Lactose%	Protein%	Casein %	WP%	Cn No.%	SCC*10^3 / mL	pH
1a (0%)	12.80	4.53	4.54	2.94	2.20	0.74	74.83	634.00	6.59
1b	12.89	4.13	4.7	3.22	2.44	0.78	75.78	196.00	6.64
2a (30%)	13.11	4.73	4.57	3.02	2.25	0.77	74.50	469.00	6.57
2b	13.27	4.57	4.71	3.23	2.42	0.81	74.92	173.00	6.65
3a (50%)	14.66	6.20	4.60	3.09	2.30	0.79	74.43	487.00	6.58
3b	13.55	4.87	4.73	3.25	2.42	0.83	74.46	176.00	6.65
4a (70%)	13.72	5.17	4.62	3.15	2.35	0.80	74.60	229.00	6.58
4b	13.81	5.13	4.74	3.26	2.41	0.85	73.93	144.00	6.64
5a (100%)	16.43	7.93	4.59	3.13	2.30	0.83	73.48	53.00	6.57
5b	14.17	5.51	4.76	3.27	2.38	0.89	72.78	68.00	6.64

[1]The contents are in percent per 100 grams. [2]Abbreviations: PM=pooled milk samples; TS=total solids; WP=whey protein; Cn No=casein number; SCC=somatic cell count. The letters a=sampling occasion one; b= sampling occasion two; 1–5=pooled milk samples with different proportions (%) of milk from young cows.

The mean values of gross composition, SCC, and pH, from the two milk sampling occasions, in both breeds, are shown in Tables 5–6. In SRB milk, total solids (TS) was numerically lower in PM with a higher proportion of milk from younger cows, but it was only significant for PM5 ($p<0.05$). TS in SLB milk was, however, significantly higher in milk from younger cows (PM5; $p<0.05$) compared to milk from older cows (PM1), see Table 6.

Table 5. Mean values of milk composition, SCC, and pH in PM from SRB cows (n=2). The significant differences between milk from old (PM1) and young cows (PM5) are shown with an asterisk in the column of PM[1,2,3]

Mean ±SD	PM1 (0%)	PM2 (30%)	PM3 (50%)	PM4 (70%)	PM5 (100%)
Object					
TS %	13.18±0.28	12.79±0.05	12.85±0.33	12.50±0.14	12.26±0.06*
Fat%	4.44±0.26	4.13±0.07	4.22±0.29	4.01±0.21	3.78±0.10
Lactose%	4.57±0.01	4.55±0.02	4.56±0.00	4.51±0.06	4.54±0.05
Protein%	3.52±0.00	3.43±0.01**	3.38±0.03***	3.27±0.01***	3.20±0.01***
Casein%	2.59±0.01	2.54±0.01	2.51±0.04*	2.43±0.01**	2.39±0.01***
WP%	0.93±0.01	0.90±0.02	0.87±0.01**	0.85±0.01**	0.82±0.01***
Cn No%	73.58±0.28	73.91±0.36	74.26±0.52	74.16±0.07	74.53±0.08
SCC*10^3/mL	396.00±139	284.50±108	217.00±70	133.50±44.0	14.00±4.00*
pH	6.62±0.03	6.62±0.02	6.63±0.01	6.66±0.04	6.64±0.04

*[1]The percentage in parentheses in PM1–5, indicates the proportion of milk from young cows in the PM. The values are presented in percent per 100 grams milk. [2]Abbreviations: SD=standard deviation; PM=pooled milk samples; TS=total solids; WP=whey protein; Cn No=casein number; SCC=somatic cell count. [3]Level of significance: *= p≤0.05; **= p≤0.01; ***= p≤0.001.*

Table 6. Mean values of milk composition, SCC, and pH in PM from SLB cows (n=2). The significant differences between milk from old (PM1) and young cows (PM5) are shown with an asterisk in the column of PM[1,2,3]

Mean ±SD	PM1 (0%)	PM2 (30%)	PM3 (50%)	PM4 (70%)	PM5 (100%)
Object					
TS %	12.85±0.05	13.19±0.08	14.11±0.56	13.77±0.05	15.30±1.13*
Fat%	4.33±0.20	4.65±0.08	5.54±0.67	5.15±0.02	6.72±1.21*
Lactose%	4.62±0.08	4.64±0.07	4.67±0.07	4.68±0.06	4.68±0.09
Protein%	3.08±0.14	3.13±0.11	3.17±0.08	3.21±0.06	3.20±0.07
Casein%	2.32±0.12	2.34±0.09	2.36±0.06	2.38±0.03	2.34±0.04
WP%	0.76±0.02	0.79±0,02	0.81±0.02	0.83±0.03	0.86±0.03*
Cn No%	75.30±0.47	74.71±0.21	74.45±0.01	74.26±0.34	73.13±0.35**
SCC*10^3/mL	415±219	321±148	332±156	187±43	61±8
pH	6.59±0.03	6.59±0.04	6.60±0.04	6.60±0.03	6.59±0.04

*[1]The percentage in parentheses in PM1–5, indicates the proportion of milk from young cows in the PM. The values are presented in percent per 100 grams milk. [2]Abbreviations: SD=standard deviation; PM=pooled milk combinations; TS=total solids; WP=whey protein; Cn No=casein number; SCC=somatic cell count. [3]Level of significance: *= p≤0.05; **= p≤0.01; ***= p≤0.001.*

There was no significant difference in lactose content between the PM 1–5 for SRB nor SLB. Milk from young SRB cows (PM5) had a numerical lower content of TS, fat, total protein, whey protein, casein, and SCC, compared to milk from old cows (PM1), however, it was only significantly ($p<0.05$) lower content of total protein, casein, whey protein, compared to milk from old cows (see Appendix, Table 1a). Milk from young SRB cows had significantly ($p<0.05$) lower SCC compared to milk from older cows, however, no significant difference in SCC was observed for SLB (see Tables 1a and 1b in Appendix).

In SLB, whey protein and fat were numerically higher in PM with a higher proportion of milk from younger cows, the difference was only significant ($p<0.05$) in PM with milk from 100% young cows (see Appendix, Table 1b). With an increased proportion of young milk, the Cn No was numerically higher in SRB and numerical lower in SLB. However, the difference in Cn No was not significant in SRB milk, and in milk from SLB, the decrease was only significant ($p<0.05$) for milk from young cows (see Appendix, Tables 1a, b). The pH varied between 6.6 and 6.7 with no significant difference independent on the breed or PM.

Comparison of SRB and SLB

Milk from old SLB cows (PM1) had a significantly lower content of whey protein ($p<0.05$) compared to milk from old SRB cows. PM with 30% milk from young SLB cows (PM2) had significantly lower total protein, casein ($p<0.01$), and whey protein ($p<0.001$) content compared to the corresponding sample from SRB, but

SLB had significantly ($p<0.01$) higher casein number. Milk from old SLB cows had significantly higher ($p<0.05$) SCC compared to milk from old SRB cows (see Appendix, Table 3c).

4.2. Analysis of protein profile

The protein profile was estimated and expressed in percent, by calculating the proportion the detected peak area of a milk protein make up out of the total area of all detected peaks (See Figure 2).

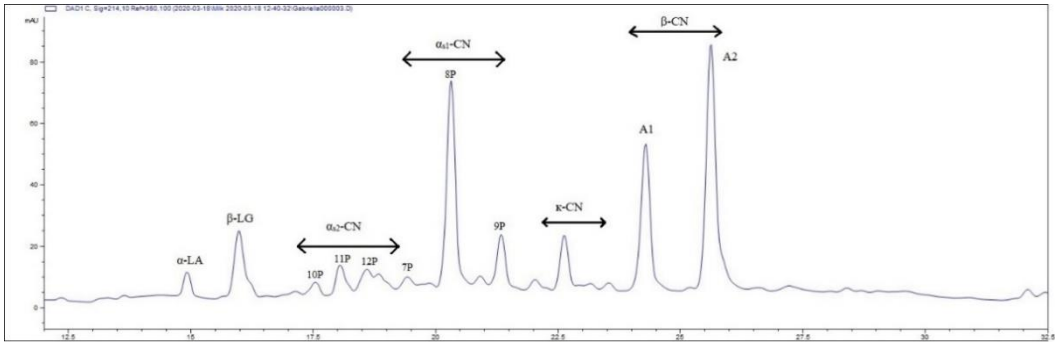


Figure 2. Protein peak distribution; a representative capillary electrophoresis electropherogram.

The comparison of the individual milk proteins between the two sampling occasions is shown in Tables 7–8. The relative contents of the milk proteins, α-LA, and α_{s2}-CN were numerical lower whereas the relative content of β_{A2}-CN was numerically higher in PM from SRB during the second sampling occasion (Table 7).

Table 7. The relative content (%) of individual proteins of each PM from SRB cows[1]

PM-SRB (% milk from young cows)	α-LA	β-LG	α_{s2}-CN	α_{s1}-CN	κ-CN	β_{B}-CN	β_{A1}-CN	β_{A2}-CN	Total WP	Total casein
1a (0%)	1.97	7.54	8.16	24.21	7.08	4.34	15.05	29.59	9.51	88.43
1b	1.82	7.19	7.05	23.35	6.91	4.35	15.94	30.42	9.01	88.01
2a (30%)	2.04	8.48	8.55	22.60	6.52	4.73	15.84	28.58	10.52	86.81
2b	2.00	6.89	7.10	23.34	7.81	4.25	15.75	28.81	8.89	87.05
3a (50%)	2.07	6.94	7.51	24.38	6.97	4.71	16.62	28.50	9.01	88.68
3b	2.03	6.87	6.49	23.51	8.02	4.35	16.57	28.65	8.91	87.59
4a (70%)	2.07	7.12	6.97	23.88	6.53	4.77	17.32	27.57	9.19	87.04
4b	1.96	7.01	5.97	24.24	7.97	4.46	16.80	28.54	8.97	87.96
5a (100%)	2.24	6.60	6.71	23.42	8.33	4.64	17.78	26.83	8.84	87.71
5b	2.02	7.69	6.37	23.11	6.20	4.75	17.41	29.96	9.71	87.80

[1]Abbreviations: PM=pooled milk combinations; WP=whey protein. The letters a=sampling occasion one; b=sampling occasion two; 1–5=pooled milk samples with different proportions (%) of milk from young cows.

Table 8. The relative content (%) of individual proteins of each PM from SLB cows[1]

PM-SLB (% milk from young cows)	α-LA	β-LG	α_{s2}-CN	α_{s1}-CN	κ-CN	β_B-CN	β_{A1}-CN	β_{A2}-CN	Total WP	Total casein
1a (0%)	1.97	7.24	7.10	21.73	6.02	4.15	5.90	42.22	9.20	87.10
1b	2.18	7.35	5.71	22.83	8.01	4.00	7.50	39.11	9.54	87.17
2a (30%)	2.35	8.29	6.68	21.38	5.36	4.51	4.71	45.18	10.64	87.82
2b	2.04	7.55	5.39	22.70	8.09	4.26	6.02	40.46	9.58	86.92
3a (50%)	2.09	7.55	6.07	22.19	6.40	4.60	3.52	44.83	9.64	87.61
3b	1.95	7.23	3.72	22.61	7.94	3.98	5.13	43.28	9.18	86.67
4a (70%)	2.31	7.43	5.57	22.17	7.64	4.89	3.59	42.43	9.74	86.29
4b	2.05	7.83	4.03	22.10	8.59	4.41	4.05	42.92	9.88	86.11
5a (100%)	2.40	7.84	5.59	22.92	8.21	4.93	1.88	42.74	10.24	86.27
5b	1.86	7.76	3.77	21.13	9.05	4.95	3.00	41.99	9.62	83.89

[1]Abbreviations: PM=pooled milk samples; WP=whey protein. The letters a=sampling occasion one; b=sampling occasion two; 1–5=pooled milk samples with different proportions (%) of milk from young cows.

In milk from SLB cows (see Table 8), the relative contents of κ-CN and β_{A1}-CN were higher, and the relative content of α_{s2}-CN was lower during the second sampling occasion. For the other proteins, no clear pattern was observed.

Mean values of milk proteins in PM

The mean relative values of individual milk proteins from both sampling occasions in SRB and SLB are shown in Tables 9–10. The relative content of β_{A1}-CN in milk from SRB cows was significantly higher in PM3 ($p<0.05$), PM4 and PM5 ($p<0.01$) compared to milk from older cows (PM1).

Table 9. Mean values of the relative content (%) for individual proteins in PM from SRB cows (n=2). The significant differences between milk from old (PM1) and young cows (PM5) are shown with an asterisk in the column of PM[1,2,3]

SRB Means±SD	PM1 (0%)[2]	PM2 (30%)	PM3 (50%)	PM4 (70%)	PM5 (100%)
Object %					
α-LA	1.90±0.08	2.02±0.02	2.05±0.02	2.01±0.05	2.13±0.11
β-LG	7.36±0.17	7.68±0.79	6.91±0.03	7.07±0.05	7.14±0.55
α_{s2}-CN	7.61±0.56	7.82±0.73	7.00±0.51	6.47±0.50	6.54±0.17
α_{s1}-CN	23.78±0.43	22.97±0.37	23.94±0.43	24.06±0.18	23.27±0.15
κ-CN	7.00±0.08	7.16±0.64	7.50±0.53	7.25±0.72	7.27±1.06
β_B-CN	4.34±0.00	4.49±0.24	4.56±0.18	4.61±0.16	4.69±0.05
β_{A1}-CN	15.49±0.44	15.80±0.05	16.60±0.02*	17.06±0.26**	17.60±0.19**
β_{A2}-CN	30.00±0.42	28.69±0.12	28.57±0.08	28.05±0.48	28.40±1.57
Total whey protein	9.26±0.25	9.70±0.81	8.96±0.05	9.08±0.11	9.27±0.43
Total casein	88.22±0.21	86.93±0.12*	88.17±0.55	87.50±0.46	87.76±0.04

[1]The percentage in parentheses in PM1–5, indicates the proportion of milk from young cows in the PM.
*[2]Abbreviations: SD=standard deviation; PM=pooled milk sample. [3] Level of significance: *= p≤0.05; **= p≤0.01; ***= p≤0.001.*

Table 10. Mean values of the relative content (%) for individual proteins in PM from SLB cows (n=2). The significant differences between milk from old (PM1) and young cows (PM5) are shown with an asterisk in the column of PM[1,2,3]

SLB Means±SD	PM1 (0%)	PM2 (30%)	PM3 (50%)	PM4 (70%)	PM5 (100%)
Object %					
α-LA	2.07±0.11	2.19±0.16	2.02±0.07	2.18±0.13	2.13±0.27
β-LG	7.29±0.06	7.92±0.37	7.39±0.16	7.63±0.20	7.80±0.04
α_{s2}-CN	6.40±0.69	6.04±0.64	4.90±1.17	4.80±0.77	4.68±0.91
α_{s1}-CN	22.28±0.55	22.04±0.66	22.40±0.21	22.14±0.03	22.02±0.89
κ-CN	7.01±1.00	6.73±1.36	7.17±0.77	8.12±0.48	8.63±0.42
β_B-CN	4.07±0.07	4.38±0.12	4.29±0.31	4.65±0.24	4.94±0.01*
β_{A1}-CN	6.70±0.80	5.37±0.66	4.33±0.81*	3.82±0.23	2.44±0.56**
β_{A2}-CN	40.67±1.55	42.82±2.36	44.05±0.77	42.67±0.25	42.36±0.38
Total whey protein	9.37±0.17	10.11±0.53	9.41±0.23	9.81±0.07	9.93±0.31
Total casein	87.14±1.55	87.37±0.45	87.14±0.47	86.20±0.09	85.08±1.19

[1]The percentage in parentheses in PM1–5, indicates the proportion of milk from young cows in the PM
*[2]Abbreviations: SD=standard deviation; PM=pooled milk sample. [3]Level of significance: *= p≤0.05; **=p≤0.01; ***= p≤0.001.*

In SLB (see Table 10), the opposite was observed, a higher proportion of milk from young cows had a lower relative content of β_{A1}-CN, and it was significant for PM3, PM4 ($p<0.05$) and PM5 ($p<0.01$). In SLB, the relative content of β_B-CN was significantly higher ($p<0.05$) in milk from young cows (PM5) compared to milk from old cows (see Table 10). No significant differences between PM for total whey proteins in Tables 9 and 10, were observed (see Appendix, Tables 2a, b). The total casein was not significantly changed either in SRB or in SLB. The total whey protein concentration varied between 9–10% and total casein concentration varied between 86–88% in both breeds. More statistics for PM and milk proteins in SRB and SLB are shown in Appendix, Table 2a–b.

Comparison of SRB and SLB

Milk from SLB cows contained more of β_{A2}-caseins, while milk from SRB cows had a higher concentration of β_{A1}-caseins. Milk from SLB cows had a significantly lower content of β_{A1}-CN and significantly higher content of β_{A2}-CN than milk from SRB cows compared (see Appendix, Tables 3a–c). Milk from young SLB cows had significantly higher ($p<0.05$) concentration of β_B-CN compared to milk from young SRB cows (see Appendix Table 3c).

4.3. Plasmin and plasminogen-derived activity

Plasmin and plasminogen-derived activity in SRB milk

The comparison of plasmin and plasminogen derived activities between the two sampling occasions is shown in Figure 3a–b and Figure 4a–b. There was no obvious pattern observed between the two sampling occasions in the plasmin (PL) and plasminogen (PG) derived activities either for SRB nor SLB. However, there was a numerical difference in PL activity between milk from old and young cows.

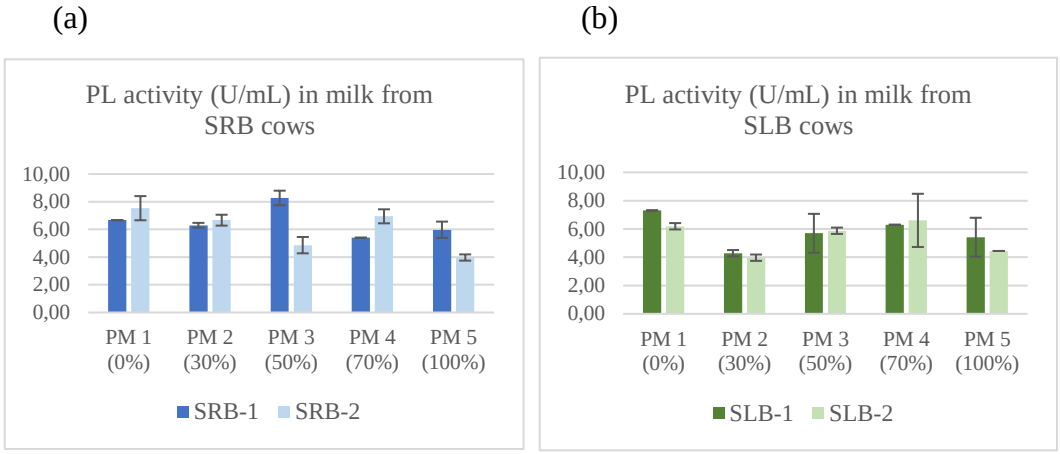


Figure 3. Plasmin activity (U/mL) in milk from SRB (a) and SLB (b) cows (n=2). PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM. Standard deviation is indicated.

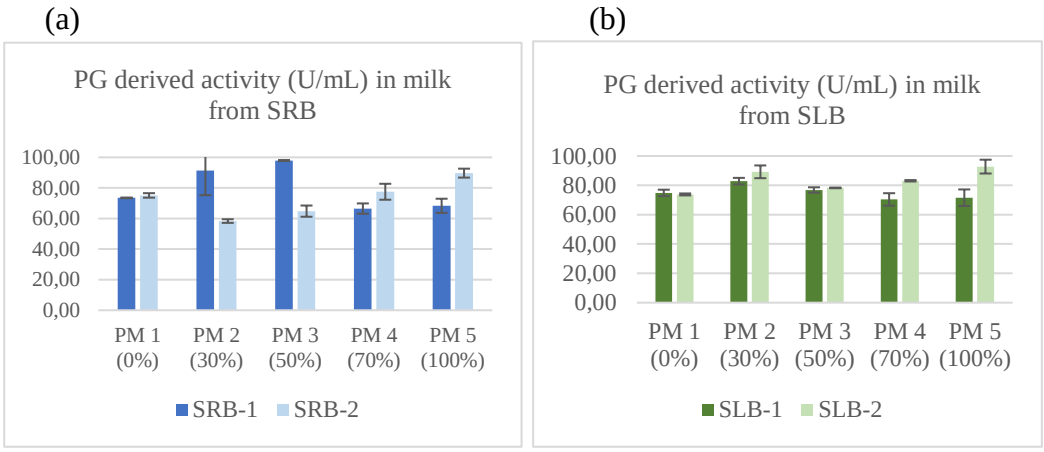


Figure 4. Plasminogen activity (U/mL) in milk from SRB (a) and SLB (b) cows (n=2). PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM. Standard deviation is indicated.

Plasmin and plasminogen derived activity in both breeds

The mean values of PL and PG derived activity, from the two sampling occasions in both breeds are shown in Figures 5a–b. In both breeds, there was a numerical difference in PL activity between milk from old and young cows. The PL activity in milk from young cows (PM5) was 30% and 27% lower for SRB and SLB respectively, compared to milk from old cows (PM1). However, the difference in PL activity was only significant ($p<0.05$) for SLB (See Table 11).

(a) (b)

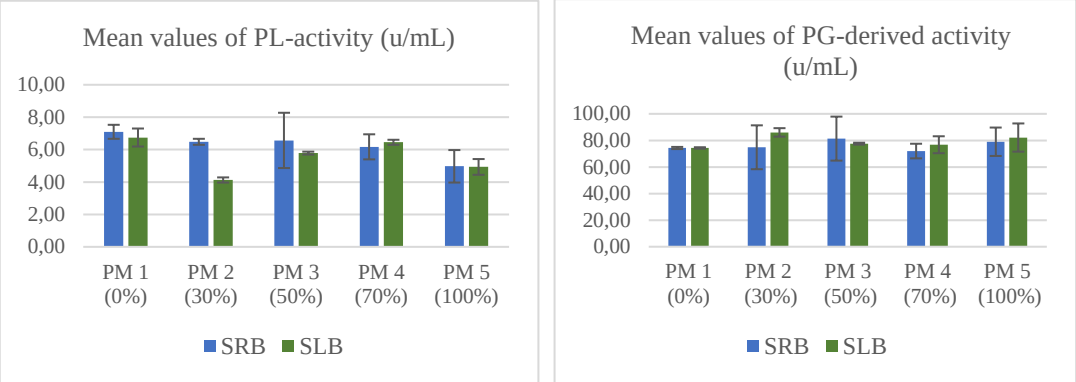


Figure 5. Mean values of plasmin (PL) and plasminogen (PG) activity (U/mL) in milk from SRB and SLB cows (n=4). PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM. Standard deviation is indicated.

The PG derived activity in milk from the two breeds was similar within the PM. No significant difference in PG between milk from old and young cows was observed, neither in SRB nor in SLB milk (see Table 11). Other statistics of PM in SRB and SLB are shown in Appendix, Tables 1a–b. There was no significant difference between PM from the two breeds (see Appendix, Tables 5a–c).

Table 11. Coefficients and p-values for PL, PG and total PL/PG (U/mL) in milk from SRB and SLB cows (n=4)[1,2,3]

	Coefficient (p-value)			
	SRB		SLB	
Dependent variable	PM1 (Intercept)	PM5	PM1 (Intercept)	PM5
PL	7.10	-2.13 (0.182) ns.	6.75	-1.82 (0.014)*
PG	74.30	4.70 (0.789) ns.	74.34	7.83 (0.378) ns.
Total PL/PG	81.40	2.60 (0.887) ns.	81.08	6.02 (0.480) ns.

*[1]PM1 is compared to PM5 and the significance is shown in the column of PM. [2]Abbreviations: PM=pooled milk samples; PL=Plasmin; PG=Plasminogen. [3]Level of significance, ns= not significant; *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $P \leq 0.001$.*

4.4. Rheological measurements

Gel firmness

The comparison of gel firmness between the two sampling occasions is shown in Figure 6 a–b. The gel firmness (G_{20}), in milk from SRB and SLB, was determined twenty minutes from the addition of rennet and expressed in pascal, Pa. The G_{20} in the different PM of milk from old and young cows of the SRB breed did not vary as much compared to milk from SLB (see Figure 6 a–b).

(a) (b)

Figure 6. The gel firmness (Pa) after 20 minutes in milk from SRB (a) and SLB (b) cows (n=2). PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM. Standard deviation is indicated.

The mean values of gel firmness from the two sampling occasions in both breeds are shown in Figure 7. A numerical stronger gel was observed in PM 1–3 in SRB compared to SLB, in contrast to a softer gel in PM 4–5. It was a greater variation in G_{20} within the pooled SLB milk samples compared to SRB (see Figure 7). G_{20} in the PM from older– (PM1) compared to younger cows (PM5) differed with 31% and 226% for SRB and SLB, respectively (see Figure 7). In milk from SLB cows, the average G_{20} from the two sampling occasions was significantly higher ($p<0.001$) in PM from young cows (PM5) compared to old cows (PM1) (see Table 12). No significant difference in G_{20} was observed between PM in SRB. Other statistical results showing PM and G_{20} in SRB and SLB can be found in Appendix Table 1a–b.

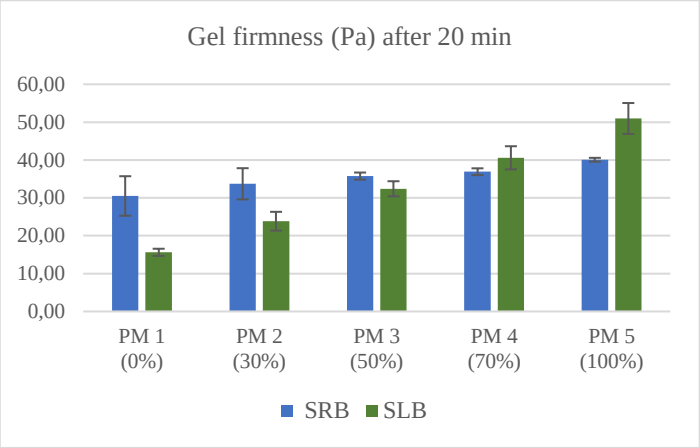


Figure 7. The mean values (n=4) of gel firmness (Pa) in milk from SRB and SLB cows. PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM. Standard deviation is indicated.

Rennet coagulation time for milk from SRB and SLB

The comparison of rennet coagulation time between the two sampling occasions is shown in Figure 8 a–b. The rennet coagulation time (RCT) was expressed in seconds from the addition of rennet until G' (elastic modulus) reached 1 Pa. The longest RCT, for both SRB and SLB, was observed in samples with milk from older cows. Milk samples containing higher proportions of milk from young cows had a numerical lower RCT.

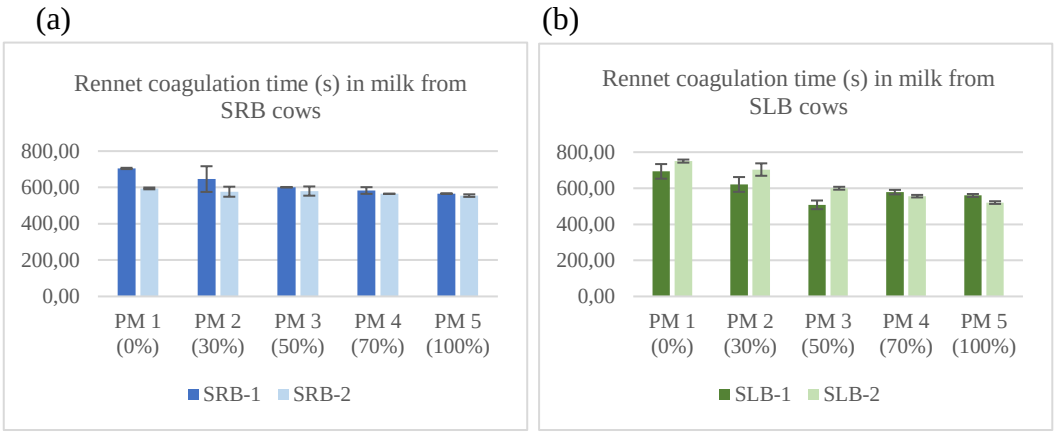


Figure 8. The rennet coagulation time (s) in milk from SRB (a) and SLB (b) cows (n=2). PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM. Standard deviation is indicated.

The mean values of RCT for milk from the two sampling occasions for both breeds are shown in Figure 9. SRB had a smaller difference within the PM compared to SRB, where the milk from older– (PM1) and younger cows (PM5) differed with

14% and 25% within SRB and SLB respectively (see Figure 9). For SLB, there was a significant difference in RCT between the PM from older and younger cows ($p<0.01$) but not in SRB (See Table 12). As the proportion of milk from young cows increased, the RCT decreased in the milk of SLB cows and the decrease was significant also between pooled milk from only old cows and milk consisting of 50% and 70% ($p<0.05$), and 100% ($p<0.01$) milk from younger cows (Appendix, Table 1b).

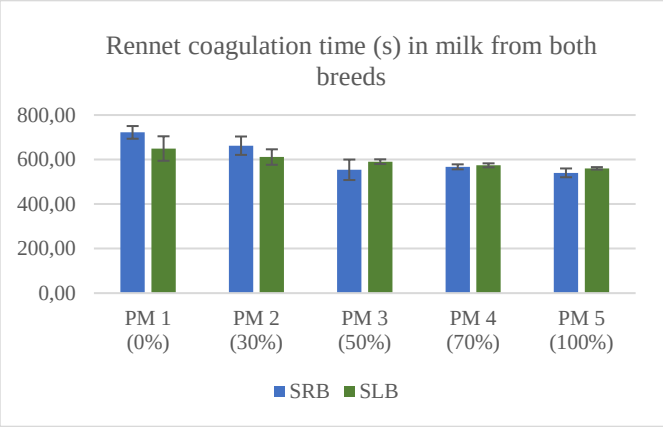


Figure 9. The mean values (n=4) of rennet coagulation time (s) in milk samples from SRB and SLB. PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM. Standard deviation is indicated.

Table 12. Coefficient and p-values for RCT (s) and G_{20} (Pa) in milk from SRB and SLB cows (n=4)[1,2,3]

	Coefficient (p-value)			
	SRB		SLB	
Dependent variable	PM1 (Intercept)	PM5	PM1 (Intercept)	PM5
RCT	649.50	-103.70 (0.058) ns.	722.00	-182.00 (0.010)**
G_{20}	30.50	9.41 (0.08) ns.	15.62	35.35 (0.000)***

*[1]PM1 is compared to PM5 and the significance is shown in the column of PM. [2]Abbreviations: PM=pooled milk samples; RCT=rennet coagulation time; G_{20}= gel firmness. [3]Level of significanc: ns= not significant; *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $P \leq 0.001$.*

The correlation between RCT and gel firmness was strongly negative and significant within SRB (-0.99; $p<0.001$) and SLB (-0.84; $p<0.01$) see Appendix, Tables 7a, and 8a. Other statistical results about PM and RCT in SRB and SLB can be found in Appendix Table 1a–b.

4.5. Ethanol stability

The comparison of the ethanol (EtOH) stability between the two sampling occasions is shown in Figure 10 a–b. In milk from both SRB and SLB cows, the EtOH induced coagulation occurred earlier in the milk collected the second time. The highest values for EtOH stability, above 80%, were observed in milk from the SLB breed (Figure 10b). In milk from SRB, the highest value observed was 71% (PM5; See Figure 10a).

(a) (b)

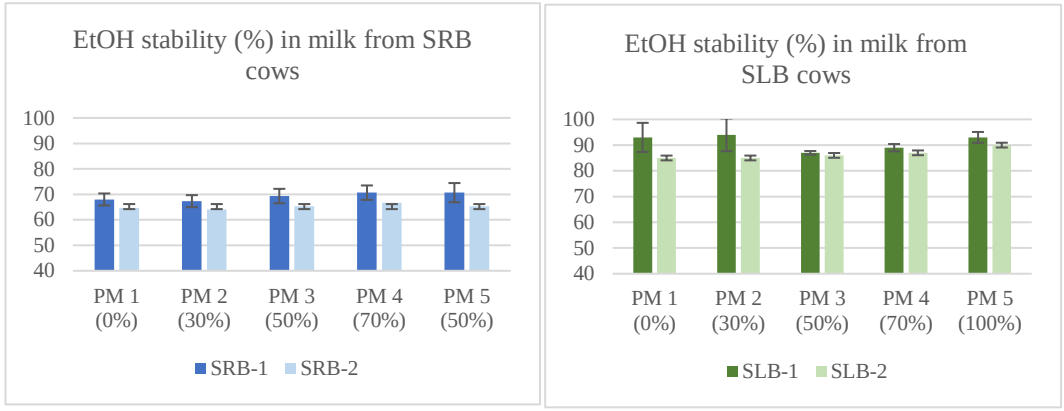


Figure 10. The Ethanol stability (%) in pooled milk samples from SRB (a) and SLB (b) during sampling occasion 1 and 2. PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM. Standard deviation is indicated.

The mean values of EtOH stability from two sampling occasions in both breeds are shown in Figure 11.

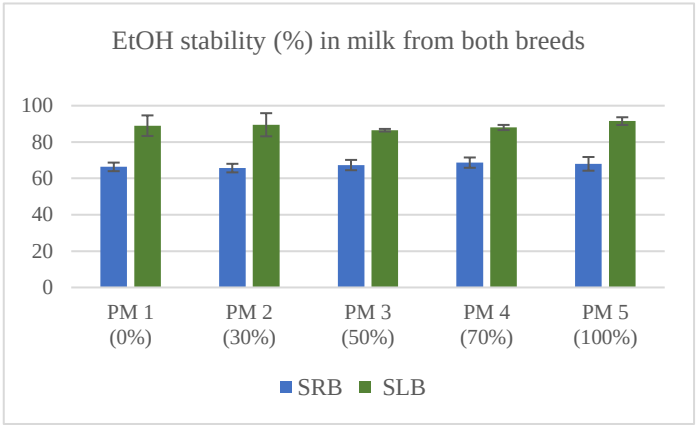


Figure 11. The mean values of ethanol stability (%) in PM from SRB and SLB cows. PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM (n=2). Standard deviation is indicated.

For both SRB and SLB, the PM from older cows had lower average EtOH stability values compared to milk from younger cows. PM from older SLB cows had an EtOH stability of 89% compared to 92% in pooled milk from younger cows. For SRB, the corresponding values were 66% and 68% for old and young cows respectively.

The difference in EtOH stability between milk from young and old cows in each breed was not significant (see Table 13). However, the EtOH stability differed between the breeds. The PM within SLB had on average 22% higher EtOH stability than SRB (see Figure 11) and values were significantly higher ($p<0.05$) than milk from SLB (see Table 14). More statistics between PM in both SRB and SLB are presented in Appendix, Table 5a–b.

Table 13. Coefficient and p-values for the ethanol stability (%) in milk from SRB and SLB (n=2)[1,2,3]

Coefficient (p-value)			
Breed	Dependent variable	PM1 (Intercept)	PM5
SRB	EtOH	66.50	1.50 (0.631) ns.
SLB	EtOH	89.00	2.50 (0.0558) ns.

[1]PM1 is compared to PM5 and the significance is shown in the column of PM5. [2]Abbreviations: PM=pooled milk samples; EtOH= ethanol stability. [3]Level of significance: ns= not significant; *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $P \leq 0.001$.

Table 14. Coefficient and p-values for the ethanol stability (%) between milk from SRB and SLB (n=2)[1,2,3]

Coefficients (p-value)				
	SRB		SLB	
Dependent variable	PM1 (Intercept)	SLB- PM1	PM1 (Intercept)	SLB-PM5
EtOH	66.50	22.50 (0.034) *	68.00	23.50 (0.020)*

[1]PM1 is compared to PM5 and the significance is shown in the column of PM5. [2]Abbreviations: PM=pooled milk samples; EtOH= ethanol stability. [3] Level of significance: ns= not significant; *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $P \leq 0.001$

4.6. Micro cheese production and curd yield

The comparison of the curd yields between the two sampling occasions is shown in Table 15. The curd yield was determined after separating the whey from the curd and expressed in percent per 100g milk. The curd yield decreased in the second sampling occasion in both SRB and SLB milk.

Table 15.The means values of curd yield (%) in PM from SRB and SLB cows, from each sampling occasions (n=4)[1,2]

Means±SD	Curd yield (%)	
PM (% milk from young cows)	SRB	SLB
1a (0%)	70.24±0.64	62.90±0.52
1b	64.84±2.29	57.13±3.48
2a (30%)	69.48±0.90	65.35±0.98
2b	59.26±0.79	57.81±0.54
3a (50%)	67.99±2.09	62.08±1.03
3b	55.06±2.31	60.05±0.05
4a (70%)	62.85±2.98	65.61±0.51
4b	55.75±2.80	57.08±1.85
5a (100%)	55.75±3.55	60.58±1.53
5b	51.80±1.30	61.58±0.55

[1]Abbrevations: PM=pooled milk sample; SD=standard deviation; The letters a=sampling occasion one; b= sampling occasion two. [2]The mean values of each PM (n=4) are presented in percent per 100 grams of milk. The percentage in the parentheses in PM1–5, indicates the proportion of milk from young cows in the PM.

The mean values of the curd yield for PM from both breeds are shown in Table 16. In milk from SRB, the values for curd yield decreased numerically with an increasing proportion from milk from young cows whereas, in milk from SLB the curd yield was more constant independent on type of PM. However, there was no significant difference in the curd yield between the PM, neither for SRB nor for SLB. More statistics is shown in Appendix, Tables 1a–b.

Table 16. Mean values of curd yield (%) in PM from SRB and SLB cows (n=8)[1, 2,3]

Means ±SD	Curd yield (%)	
PM (% milk from young cows)	SRB	SLB
1 (0%)	67.54±2.70 ns.	60.01±2.89 ns.
2 (30%)	64.37±5.11 ns.	61.58±3.77 ns.
3 (50%)	61.53±6.46 ns.	61.06±1.01 ns.
4 (70%)	59.30±3.55 ns.	61.34±4.27 ns.
5 (100%)	53.78±1.98 ns.	61.08±0.50 ns.

*[1]Abbrevations: PM=pooled milk sample; SD=standard deviation. [2] The mean values of each PM (n=8) are presented in percent per 100 grams of milk. The percentage in the parentheses in PM1–5, indicates the proportion of milk from young cows in the PM. [3] Level of significance: ns= not significant; *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $P \leq 0.001$*

5. Discussion

5.1. Milk gross composition and protein profile

Milk gross composition

Most studies investigating the milk composition of different breeds have shown that SRB tends to have higher fat concentration than SLB milk (Wedholm *et al*. 2006a; Cattle Statistic 2020). In the current study, the opposite result was observed, with SLB milk having a higher fat concentration in almost all pooled milk samples (PM) compared to SRB milk. However, one sample, SLB-5a had 7.93% fat which is odd. One explanation could be the human factor, i.e. the milk was not mixed properly before analysis and the bigger cream fraction was captured. The higher content of TS in milk from SLB cows was probably due to the deviating values of fat content in the PM from SLB.

Comparing the average values of the protein content in milk from SRB and SLB cows in the current study, the pattern of total protein and casein contents differed between the two breeds. Milk from SRB had a higher numerical content of total proteins compared to milk from SLB, which is in agreement with the Cattle Statistic (2020). Milk from older SLB cows (SLB-PM1) had significantly lower ($p<0.05$) content of WP compared to milk from older SRB cows (SRB-PM1).

Within SRB, PM with higher proportions of milk from younger cows (PM3–5) had significantly lower contents of total protein, WP, and casein ($p<0.001$) compared to milk from older cows (PM1). The opposite was observed in SLB, where total protein, WP, and casein contents increased numerically with a higher proportion of milk from younger cows. However, the higher content was only significant for WP ($p<0.05$) in PM5. The difference in protein contents is likely due to the proportion of milk from young versus old cows, in the different concentrations of PM.

Besides the genetic factor, the feed can be one explanation for why there was a difference in the total protein content between SRB and SLB. The protein content in the milk is mainly controlled through the availability of amino acids in the feed and a proper activity in the rumor stomach (Nilsson, 2017). The casein to the WP

ratio in our study (about 90:10) did not correspond to the ratio 80:20, which is presented in the literature (Walstra, 2006). One explanation could be that in our analyses, not all of the WP was detected. This fact will shift the ratio of both caseins and WP.

In agreement with Wedholm *et al*. (2006a) the average lactose concentration in our study was higher in SLB compared to milk from SRB. However, the difference in lactose content between the breeds was not significant in our study (see Tables 3a–c in Appendix). The synthesis of lactose requires a coenzyme, which is the whey protein alpha-lactalbumin (α-LA) (Walstra, 2006). However, in our study, the levels of α-LA did not correlate positively with the levels of lactose.

Protein profile in PM

In our study, SLB had a higher concentration of total β-casein compared to SRB. Wedholm *et al.* (2006a) also observed a higher concentration of β-CN in SRB compared to SLB milk. Wedholm *et al.* (2006a) further explained that one reason for the lower concentration of β-casein in SLB milk could be due to protein degradation since they detected a higher level of amino terminals in SLB compared with SRB milk in their study. One explanation to a lower β-CN in SLB in this study could be protein degradation due to high SCC. In our study, many milk proteins increased with higher proportions of milk from younger cows, which also had lower SCC. In SRB, α-LA, κ-CN, β-CN B, and β_{A1}-CN increased in milk with a higher proportion of milk from young cows (PM3–5), but it was significant for only β_{A1}-CN ($p<0.01$). In SLB, a higher relative content of α-LA, β-LG, κ-CN, β-CN, and β_{A2}-CN was observed in milk with a higher proportion of milk from young cows (PM3–5), however, it was significant for only β-CN ($p<0.05$). This may indicate that milk from younger cows may have had a lower proteolytic activity, which may have been favorable to many of the milk proteins. Furthermore, in our study, SLB and SRB milk differed in concentrations of β_{A1} and β_{A2}- caseins. SLB milk had a significantly higher relative content of β_{A2}-caseins whereas SRB milk had a significantly higher relative content of β_{A1}-caseins. This is in agreement with Wedholm *et al*., (2006a) who showed that in Swedish herds, the β-CN genotype A1 was more common in SRB than in SLB cows.

Somatic cell count

In agreement with the literature (Salsberg *et al*., 1984; Walstra, 2006), the milk from older cows (PM1) contained higher SCC/ml compared to the milk from young cows (PM5). This was true for both breeds, but the difference in SCC was only significant in milk from SRB cows ($p<0.05$). Milk from SLB cows had higher numerical values of SCC than milk from SRB cows, but it was only significantly higher in milk from younger cows ($p<0.05$; see Appendix, Table 3a, b, c). Two

samples of pooled milk (SRB-1a and SLB-1a) had SCC above $500*10^3$/mL (see section 4.1.3, Tables 5 and 6). This partly explains the high SCC numbers in PM1 (see section 4.1.3. Tables 7 and 8) of the average value within the breeds. In SRB, one cow within the group of old cows was diagnosed with mastitis after the collection of milk during the first sampling occasion. This most likely explains the high SCC in the pooled milk sample SRB-1a. The reason for high SCC in SLB-1a is not known. The cows were considered healthy at the time of sampling. Previous literature (Nilsson, 2017) has reported that SLB herds generally have more health problems compared to SRB. According to Cattle Statistics (2020), more SLB cows were culled compared to SRB cows, and the most common culling reason within SLB was udder disease. The average age of SLB cows at culling was 62 months, i.e. about 5 years.

High SCC is known to have enhanced activity of plasmin (Walstra, 2006) but no significant correlation between SCC and PL or PG was observed in this study.

5.2. Plasmin and plasminogen derived activity

Along with previous results, the milk samples in our study contained more PG than PL derived activity (Korycka-Dahl *et al*., 1983). The PL activity in SRB milk was lower in milk from young cows (PM5), 4.97 compared to 7.10 U/mL in milk from older cows (PM1). The same was observed in SLB, 4.93, and 6.75 U/mL in milk from young (PM5) old cows (PM1), respectively. The lower PL activity in milk from younger cows was only significant for SLB ($p<0.05$; see Appendix, Table 1a, and 1b). However, the values for both PM1 and PM5 in both breeds were higher than the values presented in Karlsson *et al.* (2017), who observed average PL activity of 3.09 (outdoor period) respectively 3.35 U/mL (indoor period). The milk in the study by Karlsson *et al.* (2017), was silo milk from many individuals which may explain the lower PL activity in their study compared to ours. However, the lactation stage of the cows may affect the PL levels in milk (Korycka-Dahl *et al*., 1985). In this study, milk from young and old cows was pooled into PM and it is therefore not possible to compare individuals and lactation stage. However, it is possible to compare PL activity between the breeds with the average lactation stage of old (n=4) respectively young (n=4) cows. SRB cows were on average in later stages of lactation compared to SLB cows. The older SRB cows were on average 5.5 months in lactation compared to 4.5 months in young SRB cows. Older SLB cows were on average 2.2 months in the lactation stage compared to 2.5 months in young SLB cows. This difference in lactation stages of milk from young and old cows between the breeds may explain the lower PL activity in SLB tank milk (see section 4.1.5, Figure 11a).

SCC increases with lactation number and is often associated with enhanced activity of plasmin (Salsberg *et al*., 1984; Walstra 2006). This is in agreement with our study, where higher PL activity was observed in PM with higher proportions of milk from older cows, which also had higher SCC. Despite that, there was no significant correlation between SCC and PL in SRB nor SLB. PL plays a significant role in the breakdown of caseins, which are important proteins in the coagulation process (Ismail and Nielsen, 2010). Especially β-caseins are more accessible to PL hydrolyses (Walstra 2006). This is in agreement with our observations, where the milk from the old cows in both breeds showed lower relative content of β-CNs. In SRB milk, the relative content of β_{A1}-CN was significantly higher ($p<0.01$) in PM5 compared to PM1. In SLB, the relative content of β_B-CN was significantly higher ($p<0.05$) in PM5, while the relative content of β_{A1}-CN was significantly lower ($p<0.01$) in PM5 compared to PM1. There was no significant difference in the PG derived activity between PM in milk from SRB and SLB cows. However, the PG values in milk from old cows (PM1) in our study were 74.30 and 74.34 U/mL in SRB and SLB respectively and in milk from young cows (PM5), 79.00, and 82.17 U/ml in SRB respectively SLB. The values in our study were below Karlsson *et al*. (2017) values 86.69 (outdoor) and 93.08 U/mL (indoor). The higher amount of PG than PL confirms previous studies (Korycka-Dahl *et al*., 1983) that the proteolytic activity in milk dominates of the inactive PG. The lower content of PG gives a lower amount of PL (Korycka-Dahl *et al.*, 1983) however, this was not observed in our study.

5.3. Rheological measurements

Gel firmness and rennet coagulation time

In cheesemaking, milk that can form a firm gel in a short time is desirable (Ikonen *et al*., 2004). In SLB, milk from young cows had a significantly stronger gel compared to milk from old cows. Likewise, in SRB, the gel firmness (G_{20}) increased in PM with a higher proportion of milk from young cows, however, it was not significant. Milk from SLB cows showed greater variability in G_{20} among the PM compared to SRB. Both the strongest and weakest gel was observed in SLB. Non-coagulating milk from SRB has been reported by e.g. Poulsen *et al*. (2017) but this phenomenon was not observed in our study and the lowest gel firmness was observed in SLB. Wedholm *et al.* (2006b) categorized a G' value below 15 Pa as "poorly coagulation milk". In milk examined in this study, only one sample showed a gel strength below this value, indicating that the coagulation properties of the milk in this study were not negatively affected. Another interesting aspect is that low κ-CN content in milk has been associated with poor milk coagulation (Wedholm *et al*., 2006b). In this study, the κ-CN content varied between the PM for both breeds,

and the κ-CN content increased with an increasing proportion of milk from young cows. Despite that the increase was not significant, it might explain why the strongest gel was obtained in milk from young cows. Increased content of total casein may influence the curd firmness according to Joudu *et al.* (2008). However, it was not confirmed in our study.

The RCT for PM from older cows was longer than for younger cows both breeds. The average PM1 in SRB had an RCT of 10.8 min and the average PM1 in SLB had an RCT of 12.0 min. The obtained values agreed with the average RCT, 12.3 min of milk from Holstein cows, in a study by Okibgo *et al*. (1985), where the milk quality was classified by SCC, where abnormal milk had an SCC > 500, 000/ml. Abnormal milk showed a significantly longer RCT and weaker curd than milk with a lower SCC (Okigbo *et al*., 1985). It was observed that G_{20} was negatively correlated to RCT in both SRB (-0.99; $p<0.001$) and SLB (-0.84; $p<0.01$) (see Appendix, Tables 7a, and 8a) and the decrease in RCT generally associated to an increase in G_{20} (Appendix, Table 1a–b). According to Joudu *et al*. (2008), the RCT decreased and the curd firmness increased by a higher concentration of milk proteins, casein, casein fractions, and casein number. In this study, a higher amount of casein in the PM was associated with shorter RCT and a stronger gel with milk from both breeds. In milk from SRB cows, the κ-CN, β_B-CN, β_{A1}-CN was numerically higher in milk from younger cows (PM5), which also had shorter RCT and stronger gel. In SLB, the same was observed, except for an increase in β_{A2}-CN instead of in β_{A1}-CN in PM5. The casein number, however, decreased significantly in SLB-PM ($p<0.01$) and increased (not significant) in SRB-PM with shorter RCT and stronger gel. The casein number was significantly correlated to both G_{20} and RCT within both breeds (see Appendix Table 6a–8a).

Both the protein content and SCC could be factors affecting the coagulation properties. The total casein content was higher in both SRB and SLB during the second sampling, while the SCC was lower (see section 4.1.3, Tables 5–6). In SLB numerical values of total protein content increased, along with the lower SCC. It was a significant positive correlation ($p<0.05$) between SCC and casein in milk from SRB but a negative significant correlation ($p<0.05$) in SLB (see Appendix, Tables 6a, and 7a). SCC and total protein content were positively correlated in milk from SRB ($p<0.01$) and negatively correlated in milk SLB cows ($p<0.001$). Milk with high SCC often has elevated plasmin activity (Walstra, 2006). SCC is also a quality parameter of the raw milk and related to the health of the cow, and can be a sign of mastitis (Nilsson, 2017). Mastitis decreases the milk yield, and contents of casein –and lactose in milk (Walstra, 2006). Barbano *et al.* (1991) and Ikonen *et al.* (2004) suggested that non-coagulating milk could be avoided if low SCC is selected in genetic improvement. However, Wickstrom *et al*. (2009) and Leitner *et al*. (2008) reported that RCT was not correlated to the SCC and likewise no relationship between SCC and gel firmness was observed (Wickstrom *et al*., 2009). It is

therefore likely that the coagulation properties are affected by many other different factors.

In milk from old cows, the gel firmness was lower, and the RCT longer, however, it was only significant for SRB. When comparing the results with previous studies (Okibgo *et al*., 1985; Wedholm *et al*., 2006b), the values obtained of milk from old cows, were not exaggerated in some way. However, ionic calcium content was not measured in the current study, which would have been interesting, since a low content of ionic calcium can affect the coagulation properties negatively (Gustavsson *et al*., 2014).

5.4. Ethanol stability and pH

The ethanol stability test was performed on the PM to indicate the heat stability of the milk proteins. If the milk has a lower pH, the coagulation occurs at lower ethanol concentrations (Walstra 2006). Chavez *et al*. (2004) classified low ethanol stability as 72% (v/v) or less and high ethanol stability as 78% (v/v) or more. In this current study, the ethanol stability of the milk from SRB was always below 72%. In SLB, all pooled milk samples scored above 78% in the ethanol stability test. In the study by Karlsson *et al*. (2017), the average value of ethanol stability was 79 and 81 percent during the indoor and outdoor periods, respectively. Lower pH of the milk and increased content of free calcium ions will decrease the stability of caseins in the milk, and a lower ethanol concentration is required to cause coagulation of the milk (Walstra, 2006). The pH of fresh milk is 6.7 and, in this study, the average pH was 6.6 in both SRB and SLB. There was no significant difference between the breeds or the PM. The pH was also in agreement with Okigbo *et al*. (1989) that classified normal milk with a pH close to 6.6. The difference in ethanol stability can be due to the variation in the concentration of ionized calcium and its interactions with other milk components (Davies and White, 1958). The low ethanol stability in SRB may be due to the content of ionized calcium which unfortunately was not studied. The method to evaluate at which ethanol concentration the milk coagulates is subjective which may affect the result in the end.

5.5. Curd yield

Johnson *et al*., (2001) found that milk with longer RCT had a higher cheese yield. This was most clearly observed for SRB in our study which also had a significant correlation ($p<0.01$) between the RCT and curd yield, however, it was not observed within milk from SLB cows. The highest curd yield was observed in milk from old

SRB cows, where the curd yield decreased with an increased proportion of milk from young cows. However, the difference in curd yield between milk from young and old cows was not significant neither in SRB nor SLB.

Milk from SRB, with a higher proportion of milk from old cows, had a higher content of total protein and casein but also more of whey proteins, causing a lower casein number (Cn No). Low casein content is negative for the cheese yield according to Lindmark-Mansson *et al*. (2013) and a high Cn No is of great importance in cheese making (Walstra, 2006). Low casein content is also associated with high SCC (Wickstrom *et al.*, 2009). In our study, the Cn No in milk from SRB cows had a significant correlation with the curd yield (-0.93; $p<0.001$), however, this was not observed within SLB. The highest Cn No within SRB was observed in milk from young cows (which had lower SCC) but it was not significant. The opposite was observed in SLB, where the highest Cn No was in milk from old cows, which was significant ($p<0.01$). The result from SRB is in agreement with Wickstrom *et al*. (2009) which reported a lower casein number found in milk with higher SCC.

The differences in curd yields between the PM and the breeds depends on many factors. The calcium ion activity, pH, and fat content are some important factors (Walstra, 2006). The mico cheeses in our study were made by defatted milk. However, the curd yield is normally affected by the fat content which determines how much the curd shrinks (Walstra, 2006). How and when the curd is cut can also affect the curd yield because more fat and caseins may be lost to the whey solution (Johnson *et al*., 2001).

5.6. General discussion

The composition and processability of the milk differed between breeds and lactation numbers, however, there was no strong evidence showing differences linked to age. The statistics in this study were limited due to the amount of data collected and that is why we had to use regression analysis instead of e.g. ANOVA. The chosen levels of significance, 5%, 1%, and 0.1%, are the most common. A higher significance level could have been included, i.e. 10% to identify important variables and correlation, which otherwise may be missed due to the small number of individuals in the study.

5.7. Further research

This pilot study can be used as guidance for further research within this subject. Increased testing and replicates, as well as more individuals, are required, to make

a strong conclusion of how the number of lactations of the cow affects the processability and quality of milk. To understand how the milk composition differs between lactation numbers and how the composition affects the processability, more replicates are needed. Further studies may also consider modifying pooled milk combinations and the definition of old and young cows.

6. Conclusion

In this study, the processability, i.e. ethanol stability, rennet coagulation time (RCT), gel firmness, and curd yield, and the quality of raw milk, i.e. pH, gross composition, SCC, and plasmin– and plasminogen derived activity in milk from cows of different breeds and lactation numbers have been studied. This study aimed to get insight into whether the lactation number in cows affected the gross composition and processability of the milk. The results showed that milk from older cows, i.e. cows who had 3≥ lactations, had a numerical longer RCT and softer gel firmness compared to milk from younger cows (2≤ lactations), yet these results were only significant for SLB. However, the milk from older cows had rheological properties classified as normal. No significant difference between the ethanol stability and lactation number was observed, but milk from SLB cows had significantly higher ethanol stability compared to milk from SRB. There was no significant difference in pH or curd yield between milk from young and old cows. However, in SRB, pooled milk with a higher proportion of milk from older cows had a higher numerical curd yield and had a significantly higher content of total protein, casein, and whey protein. Milk from SLB had a significantly higher relative content of β_{A2}-caseins whereas SRB milk had a significantly higher relative content of β_{A1}-caseins. Milk from older cows had higher somatic cell count (SCC) and PL activity compared to milk from young cows. However, the observed difference in SCC in milk was only significant for SRB. Older SLB cows had significantly higher SCC compared to the equivalent in SRB. The higher PL activity in milk from older cows was only significant for SLB. The PG derived activity showed no significant difference between milk from older and younger cows.

In this study, different methods were used to study the differences in milk quality and properties between milk from cows in different lactations. There was a great variety in the results between milk from older and younger cows. The results indicate that there are no major differences between milk from young and old cows. In contrast, the evidence for observed differences is not strong enough to conclude if there is a difference or not, related to lactation number. Further research is therefore needed and should include more individuals, to determine if lactation number matters in any aspect of the raw milk quality.

References

Barbano, D. M., Rasmussen, R. R., & Lynch, J. M. (1991). Influence if milk somatic cell-count and milk age on cheese yield. *Journal of Dairy Science, 74*(2), 369-388. https://doi.org/10.3168/jds.S0022-0302(91)78179-4

Bell, M. J., & Wilson, P. (2018). Estimated differences in economic and environmental performance of forage-based dairy herds across the UK. *Food and Energy Security, 7*(1), 12. https://doi.org/10.1002/fes3.127

Boulton, A. C., Rushton, J., & Wathes, D. C. (2017). An empirical analysis of the cost of rearing dairy heifers from birth to first calving and the time taken to repay these costs. *Animal, 11*(8), 1372-1380. https://doi.org/10.1017/s1751731117000064

Cattle Statistics/Husdjurstatistik. (2020). Växa Sverige, Uppsala. Available: https://www.vxa.se/globalassets/dokument/statistik/husdjursstatistik-2020.pdf

Chavez, M. S., Negri, L. M., Taverna, M. A., & Cuatrín, A. (2004). Bovine milk composition parameters affecting the ethanol stability. *Journal of Dairy Research, 71*(2), 201-206. https://doi.org/10.1017/s0022029904000172

Davies, D. T., & White, J. C. D. (1958). The relation between the chemical composition of millk and the stability of the caseinate complex. 2. Coagulation by ethanol. *Journal of Dairy Research, 25*(2), 256-266. https://doi.org/10.1017/s0022029900009250

de Graaf, S., Van Loo, E. J., Bijttebier, J., Vanhonacker, F., Lauwers, L., Tuyttens, F. A. M., & Verbeke, W. (2016). Determinants of consumer intention to purchase animal-friendly milk. *Journal of Dairy Science, 99*(10), 8304-8313. https://doi.org/10.3168/jds.2016-10886

De Vries R, Van Knegsel A, Johansson M, Lindmark-Månsson H, van Hooijdonk T, Holtenius K, Hettinga K. (2015). Effect of shortening or omitting the dry period of Holstein-Friesian cows on casein composition of milk. Journal Dairy Science 98(12): 8678-8687

De Vries, A., & Marcondes, M. I. (2020). Review: Overview of factors affecting productive lifespan of dairy cows. *Animal, 14*, S155-S164. https://doi.org/10.1017/s1751731119003264

Ellis, K. A., Billington, K., McNeil, B., & McKeegan, D. E. F. (2009). Public opinion on UK milk marketing and dairy cow welfare. *Animal Welfare, 18*(3), 267-282.

Farrell, H. M., Jimenez-Flores, R., Bleck, G. T., Brown, E. M., Butler, J. E., Creamer, L. K., . . . Swaisgood, H. E. (2004). Nomenclature of the proteins of cows' milk - Sixth revision. *Journal of Dairy Science, 87*(6), 1641-1674. https://doi.org/10.3168/jds.S0022-0302(04)73319-6

Gustavsson, F., Glantz, M., Buitenhuis, A. J., Lindmark-Mansson, H., Stalhammar, H., Andren, A., & Paulsson, M. (2014). Factors influencing chymosin-induced gelation of milk from individual dairy cows: Major effects of casein micelle size and calcium. *International Dairy Journal, 39*(1), 201-208. https://doi.org/10.1016/j.idairyj.2014.06.011

Högberg, M. (2016). *Keeping goats and kids together.* Diss. Uppsala: Sveriges lantbruksuniversitet, 1652-6880; 2016:41. ISBN: 978-91-576-8584-1, e ISBN 978-91-576-8585-8

Horne, D.S., Muir, D.D. (1990). Alcohol and heat-stability of milk protein. Journal of Dairy Science, 73(12), 3612-3626. https://doi.org/10.3168/jds.S0022-0302(90)79064-9

Ikonen, T., Morri, S., Tyriseva, A. M., Ruottinen, O., & Ojala, M. (2004). Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. *Journal of Dairy Science, 87*(2), 458-467. https://doi.org/10.3168/jds.S0022-0302(04)73185-9

Ismail, B., & Nielsen, S. S. (2010). Invited review: Plasmin protease in milk: Current knowledge and relevance to dairy industry. *Journal of Dairy Science, 93*(11), 4999-5009. https://doi.org/10.3168/jds.2010-3122

Johansson, M., Akerstedt, M., Li, S. J., Zamaratskaia, G., & Lundh, A. S. (2013). Casein Breakdown in Bovine Milk by a Field Strain of Staphylococcus aureus. *Journal of Food Protection, 76*(9), 1638-1642. https://doi.org/10.4315/0362-028x.Jfp-13-112

Johansson, M., Högberg, M., & Andrén, A. (2015). Relation Between αS1-Casein Content and Coagulation Properties of Milk from Swedish Dairy Goats. *The Open Food Science Journal, 9*(1).

Johansson, M., Lundh, Å., De Vries, R., Sjaunja, K.S., 2017. Composition and enzymatic activity in bulk milk from dairy farms with conventional or robotic milking systems. Journal of Dairy Research. https://doi.org/10.1017/s0022029917000140

Johnson, M. E., Chen, C. M., & Jaeggi, J. J. (2001). Effect of rennet coagulation time on composition, yield, and quality of reduced-fat cheddar cheese. *Journal of Dairy Science, 84*(5), 1027-1033. https://doi.org/10.3168/jds.S0022-0302(01)74562-6

Jordbruksverket. (2020). Marknadsrapport mjölk -och mejeriprodukter, utveckling till och med 2019. *Livsmedelskedjan och exportenheten,* Jönköping.

Joudu, I., Henno, M., Kaart, T., Pussa, T., & Kart, O. (2008). The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. *International Dairy Journal, 18*(9), 964-967. https://doi.org/10.1016/j.idairyj.2008.02.002

Karlsson, M. A., Langton, M., Innings, F., Wikstrom, M., & Lundh, A. S. (2017). Short communication: Variation in the composition and properties of Swedish raw milk for ultra-high-temperature processing. *Journal of Dairy Science, 100*(4), 2582-2590. https://doi.org/10.3168/jds.2016-12185

Korycha-Dahl, M., Ribadeau, B., Chene, N., Marta., J. (1983). Plasmin Activity in Milk. *Journal of Dairy Science*, 66: 704-711.

Langford, F. M., & Stott, A. W. (2012). Culled early or culled late: economic decisions and risks to welfare in dairy cows. *Animal Welfare, 21*, 41-55. https://doi.org/10.7120/096272812x13345905673647

Leitner, G., Silanikove, N., Jacobi, S., Weisblit, L., Bernstein, S., & Merin, U. (2008). The influence of storage on the farm and in dairy silos on milk quality for cheese production. *International Dairy Journal, 18*(2), 109-113. https://doi.org/10.1016/j.idairyj.2007.09.001

Lindmark-Mansson, H., Fonden, R., & Pettersson, H. E. (2003). Composition of Swedish dairy milk. *International Dairy Journal, 13*(6), 409-425. https://doi.org/10.1016/s0958-6946(03)00032-3

Løvendahl, P., Chagunda, M.G.G., 2011. Covariance among milking frequency, milk yield, and milk composition from automatically milked cows. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2010-3589

Mcmanaman, J.L., Reyland, M.E., Thrower, E.C., 2006. Secretion and Fluid Transport Mechanisms in the Mammary Gland: Comparisons with the Exocrine Pancreas and the Salivary Gland. *Journal of Mammary Gland Biology and Neoplasia.* https://doi.org/10.1007/s10911-006-9031-3

Murphy, S. C., Martin, N. H., Barbano, D. M., & Wiedmann, M. (2016). Influence of raw milk quality on processed dairy products: How do raw milk quality test results relate to product quality and yield? *Journal of Dairy Science, 99*(12), 10128-10149. https://doi.org/10.3168/jds.2016-11172

Nilsson, M. (2017). *Mjölkkor*. Andra utgåvans första tryckning. Vinninga: BMM Förlag. ISBN: 978-91-639-2958-8.

Näslund, J., Fikse, W. F., Pielberg, G. R., & Lunden, A. (2008). Frequency and effect of the bovine acyl-CoA : diacylglycerol acyltransferase 1 (DGAT1) K232A polymorphism in Swedish dairy cattle. *Journal of Dairy Science, 91*(5), 2127-2134. https://doi.org/10.3168/jds.2007-0330

Okigbo, L. M., Richardson, G. H., Brown, R. J., & Ernstrom, C. A. (1985). Coagulation properties of abnormal and normal milk from individual cow quarters. *Journal of Dairy Science, 68*(8), 1893-1896. https://doi.org/10.3168/jds.S0022-0302(85)81046-8

Poulsen, N. A., Glantz, M., Rosengaard, A. K., Paulsson, M., & Larsen, L. B. (2017). Comparison of milk protein composition and rennet coagulation properties in native Swedish dairy cow breeds and high-yielding Swedish Red cows. *Journal of Dairy Science, 100*(11), 8722-8734. https://doi.org/10.3168/jds.2017-12920

Röös, E. (2019). *Kor och klimat*. Uppsala. SLU, Epok – Centrum för ekologisk produktion och konsumtion. ISBN: 978-91-576-9673-1.

Salsberg, E., Meek, A. H., & Martin, S. W. (1984). Somatic-cell counts – associated factors and relationship to production. *Canadian Journal of Comparative Medicine-Revue Canadienne De Medecine Comparee, 48*(3), 251-257.

Walstra, P. (2006). *Dairy science and technology* (2nd ed. ed.). Boca Raton: CRC/Taylor & Francis.

Wedholm, A., Hallen, E., Larsen, L. B., Lindmark-Mansson, H., Karlsson, A. H., & Allmere, T. (2006a). Comparison of milk protein composition in a Swedish and a Danish dairy herd using reversed phase HPLC. *Acta Agriculturae Scandinavica Section a-Animal Science, 56*(1), 8-15. https://doi.org/10.1080/09064700600836745

Wedholm, A., Larsen, L. B., Lindmark-Mansson, H., Karlsson, A. H., & Andren, A. (2006b). Effect of protein composition on the cheese-making properties of milk from individual dairy cows. *Journal of Dairy Science, 89*(9), 3296-3305. https://doi.org/10.3168/jds.S0022-0302(06)72366-9Nas

Widebeck, L. (2000). *Mjölkkor*. Natur & Kultur. ISBN: 978-91-273-5300-8.
Wickstrom, E., Persson-Waller, K., Lindmark-Mansson, H., Ostensson, K., & Sternesjo, A. (2009). Relationship between somatic cell count, polymorphonuclear leucocyte count and quality parameters in bovine bulk tank milk. *Journal of Dairy Research, 76*(2), 195-201. https://doi.org/10.1017/s0022029909003926

Acknowledgments

A special thank you to my supervisor, Professor Monika Johansson, for your great support, guidance, advice, and encouragement during my thesis. It has been a privilege to have you as my supervisor. I would also like to thank the employees at Swedish Livestock Research Centre, Lövsta, Uppsala, Sweden, for your friendly service. At last, big thanks to my family for always encouraging me.

Appendix 1- Popular scientific summary

Did you know that the average life length of dairy cows in Sweden today is 5 years? This means that cows give birth to about 2.5 calves and will then be replaced by a heifer (a young female cow that has not delivered a calf yet). Of course, they can live much longer but are often killed because of reasons such as udder disease, low milk yield, or impaired fertility. Well, there are many good reasons for keeping dairy cows longer in life, one is sustainability, another is animal welfare. Dairy cows emit lots of methane through burps and farts, which is generated from their feed intake. Methane is an important greenhouse gas, and the dominating gas related to dairy production. Methane has a large impact on the climate, because an increase in methane emission may lead to a higher global temperature. The global dairy production is often criticized for not doing enough or for being ignorant of the problem.

By increasing the cow's life length, the methane emission per cow, and unit total milk solids (that is, the nutrients in the milk) would decrease. The milk would thereby decrease the climate impact compared to how it is today. Increasing the cow's life length is also associated with good animal welfare, which is an important factor for many consumers when pursuing milk. However, sustainable dairy production needs to be economically defensible at the same time. Research has shown that it may not be very economical to replace a cow with a new heifer to increase the milk yield. The cow starts producing milk after the first calf when it is about 2 years old. So far, the heifer has only been an expense for the farmer, due to the rearing costs, and for the environment, since the cows emit lots of greenhouse gases without producing milk. Not until the cow has given birth to 1.5 calves the farmer can begin to earn money. The growing interest in sustainability in many areas within society shows the possibility to make changes. Climate concerns are addressed not just by consumers, but also by companies and in politics.

If we want the cows to live longer, we need to know if there are any differences in quality between milk from older and younger cows. This is what this thesis aimed to study. The milk must be of the same quality, otherwise, it can be difficult to convince producers of the other benefits. Milk was collected from cows of two

different breeds, SRB and SLB. Eight cows, i.e. four older and four younger, from each breed participated. The composition of the milk was analysed by different methods, to understand the raw milk quality. The processability was studied by measuring the curd yield, ethanol stability, gel firmness, rennet coagulation time of the milk. Excellent milk will form a strong gel in a short time.

The results of this study indicate that there are no major differences between milk from older and younger cows. Milk from older cows in this study had a numerical longer rennet coagulation time and obtained a numerical softer gel than milk from young cows. However, it was only significant for milk from one of the breeds, SLB. There was no significant difference in the stability of milk proteins or pH linked to the age of the cow. Milk from old cows had higher numerical somatic cell count (SCC) compared to milk from young cows, but it was only significant in milk from SRB. It was not very surprising since SCC naturally increases with age. SCC is an important milk quality parameter, because a high SCC may indicate that the cow has mastitis, an udder disease. There was no significant difference in curd yield between milk from young and old cows in SRB nor SLB. Milk from older SRB cows had a significantly higher content of total protein, casein, and whey protein compared to milk from young cows, these differences were, however, not observed in milk from SLB cows.

The observed differences between milk from young and old cows are not strong enough to make significant conclusions. However, this study can be used as guidance for further research within this subject. One recommendation for further studies would be to include more individuals, to be able to draw conclusions of how the age affects the raw milk quality.

Appendix 2- Statistics

Table 1a. Coefficient and p-values in PM1–5 from SRB cows[1,2]

SRB		Coefficients (p-value)			
Dependent variable	Intercept (PM1)	PM2	PM3	PM4	PM5
PL	7.10	-0.62 (0.672)	-0.53 (0.714)	-0.93 (0.529)	-2.13 (0.182)
PG	74.30	0.50 (0.975)	7.10 (0.689)	-2.30 (0.896)	4.70 (0.789)
Tot PL/PG	81.40	-0.10 (0.996)	6.50 (0.719)	-3.20 (0.858)	2.60 (0.887)
SCC	396	-112 (0.405)	-179 (0.205)	-263 (0.086)	**-382 (0.027)***
Total solids	13.18	-0.39 (0.239)	-0.33 (0.309)	-0.68 (0.067)	**-0.93 (0.025)***
Protein	3.52	**-0.09 (0.009)****	**-0.14 (0.001)*****	**-0.25 (0.000)*****	**-0.32 (0.000)*****
Casein	2.59	-0.06 (0.094)	**-0.06 (0.030)***	**-0.17 (0.002)****	**-0.21 (0.001)*****
Whey protein	0.93	-0.04 (0.052)	**-0.06 (0.007)****	**-0.09 (0.002)****	**-0.12 (0.000)*****
Curd yield	67.54	-3.17 (0.649)	-6.01 (0.400)	-8.24 (0.264)	-11.35 (0.143)
Fat	4.44	-0.310 (0.328)	-0.23 (0.467)	-0.44 (0.189)	-0.67 (0.068)
Lactose	4.57	-0.015 (0.762)	-0.01 (0.919)	-0.06 (0.256)	-0.03 (0.640)
RCT	649.50	-38.30 (0.409)	-59.0 (0.223)	-75.50 (0.136)	-103.70 (0.058)
G_{20}	30.50	3.22 (0.487)	5.25 (0.276)	6.41 (0.195)	9.41 (0.08)
Casein number	73.58	0.33 (0.495)	0.68 (0.190)	0.58 (0.250)	0.95 (0.086)
pH	6.62	0.000 (1.00)	0.02 (0.715)	0.04 (0.351)	0.03 (0.548)

[1]Level of significance: *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $P \leq 0.001$. [2]Abbreviations: PM=pooled milk samples; PL=plasmin; PG=plasminogen; SCC=somatic cell count; RCT= rennet coagulation time; G_{20} = gel firmness after 20 min.

Table 1b. Coefficient and p-values in PM1–5 from SLB cows[1]

SLB		Coefficients (p-value)			
Dependent variable	Intercept (PM1)	PM2	PM3	PM4	PM5
PL	6.75	**-2.62 (0.003)****	-0.96 (0.107)	-0.29 (0.575)	**-1.82 (0.014)***
PG	74.34	11.70 (0.208)	3.21 (0.708)	2.40 (0.779)	7.83 (0.378)
Total PL/PG	81.08	9.08 (0.302)	2.25 (0.787)	2.11 (0.800)	6.02 (0.480)
SCC	415	-94 (0.652)	-83 (0.688)	-228 (0.296)	-354 (0.130)
Total solids	12.845	0.35 (0.684)	1.26 (0.176)	0.92 (0.302)	**2.46 (0.028)***
Protein	3.08	0.05 (0.751)	0.09 (0.532)	0.13 (0.394)	0.12 (0.412)
Casein	2.32	0.02 (0.892)	0.04 (0.720)	0.06 (0.593)	0.02 (0.857)
Whey protein	0.76	0.03 (0.405)	0.05 (0.190)	0.07 (0.106)	**0.10 (0.029)***
Curd yield	60.01	1.57 (0.718)	1.05 (0.808)	1.33 (0.759)	1.06 (0.806)
Fat	4.33	0.32 (0.732)	1.21 (0.231)	0.82 (0.396)	**2.39 (0.043)***
Lactose	4.62	0.02 (0.853)	0.05 (0.679)	0.06 (0.584)	0.06 (0.615)
RCT	722.0	-60 (0.245)	**-168 (0.014)***	**-155 (0.019)***	**-182 (0.010)****
G_{20}	15.62	8.22 (0.085)	**16.79 (0.007)****	**24.96 (0.001)*****	**35.35 (0.000)*****
Casein number	75.3	-0.59 (0.246)	-0.856 (0.115)	-1.038 (0.069)	**-2.17 (0.005)****
pH	6.62	-0.005 (0.920)	0.00 (1.00)	-0.005 (0.920)	-0.01 (0.841)

[1]Level of significance: *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $p \leq 0.001$.

Table 2a. Coefficient and p-values of the milk proteins in PM 1–5 from SRB cows[1]

SRB		Coefficients (p-value)			
Dependent variable	Intercept (PM1)	PM2	PM3	PM4	PM5
α-LA	1.90	0.12 (0.255)	0.15 (0.167)	0.12 (0.271)	0.23 (0.056)
β-LG	7.37	0.32 (0.628)	-0.46 (0.628)	-0.30 (0.651)	-0.22 (0.736)
α_{s2}-CN	7.61	0.22 (0.782)	-0.61 (0.450)	-1.14 (0.187)	-1.07 (0.209)
α_{s1}-CN	23.78	-0.81 (0.148)	0.16 (0.750)	0.28 (0.586)	-0.52 (0.328)
κ-CN	7.00	0.16 (0.873)	0.50 (0.628)	0.25 (0.806)	0.27 (0.794)
B_B-CN	4.34	0.15 (0.531)	0.19 (0.431)	0.27 (0.269)	0.35 (0.166)
β_{A1}-CN	15.49	0.31 (0.421)	1.11 (0.025)	1.57 (0.006)	**2.10 (0.002)****
β_{A2}-CN	30.00	-1.31 (0.277)	-1.43 (0.240)	-1.95 (0.129)	-1.60 (0.195)
Total whey protein	9.26	0.44 (0.501)	-0.31 (0.637)	-0.18 (0.777)	0.01 (0.985)
Total casein	88.22	-1.29 **(0.043)***	-0.09 (0.860)	-0.72 (0.192)	-0.47 (0.372)

[1] Level of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $P \leq 0.001$

Table 2b. Coefficient and p-values of the milk proteins in PM 1–5 from SLB cows[1]

		Coefficients (p-value)			
Dependent variable	Intercept (PM1)	PM2	PM3	PM4	PM5
α-LA	2.07	0.12 (0.621)	-0.05 (0.821)	0.11 (0.664)	0.06 (0.817)
β-LG	7.29	0.62 (0.083)	0.10 (0.747)	0.34 (0.298)	0.51 (0.140)
α_{s2}-CN	6.40	-0.37 (0.774)	-1.51 (0.270)	-1.60 (0.244)	-1.72 (0.216)
α_{s1}-CN	22.28	-0.24 (0.771)	0.12 (0.888)	-0.145 (0.863)	-0.258 (0.759)
κ-CN	7.01	-0.29 (0.827)	0.16 (0.904)	1.11 (0.413)	1.62 (0.249)
B_B-CN	4.07	0.31 (0.296)	0.22 (0.452)	0.58 (0.082)	**0.87 (0.02)***
β_{A1}-CN	6.70	-1.33 (0.204)	**-2.37 (0.048)***	**-2.88 (0.025)***	**-4.26 (0.006)****
β_{A2}-CN	40.67	2.16 (0.302)	3.39 (0.130)	2.01 (0.333)	1.70 (0.407)
Total whey protein	9.37	0.74 (0.144)	0.04 (0.923)	0.44 (0.352)	0.56 (0.248)
Total casein	87.14	0.23 (0.797)	-0.00 (1.00)	-0.94 (0.325)	-2.06 (0.062)

[1] Level of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $P \leq 0.001$

Table 3a. Coefficient and p-values of the milk gross composition and milk proteins between PM1–2 from SRB and SLB cows[1, 2, 3]

	Coefficients (p-value)			
Dependent variable	Intercept (SRB-PM1)	SLB-PM1	Intercept (SRB-PM2)	SLB-PM2
PL	7.10	-0.35 (0.666)	6.79	-1.35 (0.156)
PG	74.30	0.04 (0.973)	74.57	5.62 (0.491)
Tot PL/PG	81.40	-0.31 (0.866)	81.36	4.27 (0.580)
SCC	396	19 (0.948)	340	28 (0.845)
Total solids	13.18	-0.34 (0.359)	12.99	0.03 (0.872)
Protein	3.52	-0.44 (0.088)	3.48	**-0.37 (0.003)****
Casein	2.59	-0.27 (0.154)	2.56	**-0.24 (0.009)****
Whey protein	0.93	**-0.17 (0.017)***	0.91	**-0.14 (0.000)*****
Curd yield	67.54	-7.53 (0.197)	65.95	-5.16 (0.160)
Fat	4.44	-0.11 (0.769)	4.29	0.21 (0.324)
Lactose	4.57	0.06 (0.563)	4.56	0.07 (0.157)
RCT	649.50	72.50 (0.362)	630.40	61.8 (0.167)
G_{20}	30.50	-14.88 (0.107)	32.11	**-12.38 (0.019)***
Casein number	73.58	1.72 (0.089)	73.74	**1.26 (0.010)****
pH	6.62	0.00 (1.000)	6.62	-0.003 (0.916)
α-LA	1.90	0.18 (0.312)	1.96	0.18 (0.120)
β-LG	7.37	-0.07 (0.733)	7.52	0.08 (0.852)
α_{s2}-CN	7.61	-1.20 (0.309)	7.71	**-1.50 (0.035)***
α_{s1}-CN	23.78	-1.50 (0.166)	23.38	**-1.22 (0.047)***
κ-CN	7.00	0.01 (0.990)	7.08	-0.21 (0.787)
B_B-CN	4.34	-0.27 (0.063)	4.42	-0.19 (0.259)
β_{A1}-CN	15.49	**-8.79 (0.011)***	15.65	**-9.61 (0.000)*****
β_{A2}-CN	30.00	**10.66 (0.022)***	29.35	**12.40 (0.000)*****

[1]SRB-PM1 is compared to SLB-PM1 and SRB-PM2 is compared to SLB-PM2. [2]Level of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $P \leq 0.001$. [3]Abbreviations, PL=plasmin; PG=plasminogen; SCC=somatic cell count; RCT=rennet coagulation time; G_{20}=gel firmness after 20 min.

Table 3b. Coefficient values and p-values of milk gross composition and milk proteins, between PM3–4 from SRB and SLB cows[1, 2]

	Coefficients (p-value)			
Dependent variable	Intercept (SRB-PM3)	SLB-PM3	Intercept (SRB-PM4)	SLB-PM4
PL	6.57	-0.78 (0.692)	6.17	0.28 (0.753)
PG	81.40	-3.80 (0.839)	72.00	4.74 (0.630)
Tot PL/PG	87.90	-4.60 (0.825)	78.17	5.02 (0.635)
SCC	217	114 (0.571)	133.50	53.0 (0.475)
Total solids	12.85	1.26 (0.191)	12.50	**1.27 (0.013)***
Protein	3.38	-0.21 (0.133)	3.27	-0.07 (0.365)
Casein	2.51	-0.15 (0.173)	2.43	-0.05 (0.277)
Whey protein	0.87	-0.06 (0.115)	0.85	-0.02 (0.515)
Curd yield	61.53	-0.46 (0.950)	59.30	2.04 (0.748)

Fat	4.22	1.32 (0.210)	4.00	**1.15 (0.031)***
Lactose	4.56	0.11 (0.248)	4.51	0.18 (0.165)
RCT	590.50	-36.5 (0.520)	574	-6.7 (0.686)
G_{20}	35.75	-3.34 (0.268)	36.91	3.67 (0.368)
Casein number	74.27	0.19 (0.750)	6.66	0.05 (0.432)
pH	6.63	-0.02 (0.720)	74.16	0.11 (0.789)
α-LA	2.05	-0.03 (0.736)	2.01	0.166 (0.355)
β-LG	6.91	0.49 (0.096)	7.07	0.56 (0.112)
α_{s2}-CN	7.00	-2.10 (0.242)	6.47	-1.67 (0.212)
α_{s1}-CN	23.94	-1.54 (0.085)	24.06	**1.92 (0.009)****
κ -CN	7.50	-0.33 (0.757)	7.25	0.87 (0.421)
B_B-CN	4.53	-0.24 (0.572)	4.61	0.04 (0.910)
β_{A1}-CN	16.60	**-12.27 (0.004)****	17.06	**-13.24 (0.001)*****
β_{A2}-CN	28.57	**15.48 (0.003)****	28.05	**14.62 (0.001)*****

[1]SRB-PM3 is compared to SLB-PM3 and SRB-PM4 is compared to SLB-PM4.

[2]Level of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $P \leq 0.001$.

Table 3c. Coefficient values and p-values of milk gross composition and milk proteins, between PM5 from SRB and SLB cows[1, 2,]

	Coefficients (p-value)	
Dependent variable	Intercept (SRB-PM5)	SLB-PM5
PL	4.97	-0.04 (0.975)
PG	79.00	3.2 (0.853)
Tot PL/PG	83.97	3.1 (0.844)
SCC	14.00	**46.50 (0.032)***
Total solids	12.26	3.04 (0.115)
Protein	3.20	0.000 (1.000)
Casein	2.39	-0.05 (0.380)
Whey protein	0.82	0.05 (0.277)
Curd yield	56.19	4.89 (0.384)
Fat	3.78	2.94 (0.136)
Lactose	4.54	0.14 (0.283)
RCT	545.8	-5.5 (0.824)
G_{20}	39.91	11.06 (0.114)
Casein number	74.53	-1.40 (0.060)
pH	6.64	-0.04 (0.578)
α-LA	2.13	0.00 (0.999)
β-LG	7.14	0.66 (0.354)
α_{s2}-CN	6.54	-1.86 (0.182)
α_{s1}-CN	23.27	-1.24 (0.304)
κ -CN	7.27	1.37 (0.356)
B_B-CN	4.70	**0.25 (0.046)***
β_{A1}-CN	17.60	**-15.16 (0.001)*****
β_{A2}-CN	28.40	**13.97 (0.013)***

[1]SRB-PM5 is compared to SLB-PM5. [2]Level of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $P \leq 0.001$

Table 4. Coefficients and p-values of the ethanol stability in PM 1–5 from SRB and SLB cows[1]

		Coefficients (p-value)				
Breed	Dependent variable	Intercept (PM1)	PM2	PM3	PM4	PM5
SRB	EtOH	66.50	-1.00 (0.747)	0.50 (0.871)	2.50 (0.433)	1.50 (0.631)
SLB	EtOH	89.00	0.50 (0.905)	-2.5 (0.558)	-1.00 (0.812)	2.5 (0.558)

[1] Abbreviations: PM=pooled milk samples

Table 5a. Coefficient and p-values of the ethanol stability between PM1–3 from SRB and SLB cows[1, 2,]

	Coefficients (p-value)					
Dependent variable	Intercept (SRB-PM1)	SLB- PM1	Intercept (SRB-PM2)	SLB-PM2	Intercept (SLB-PM3)	SRB-PM3
EtOH	66.50	**22.50 (0.034) ***	65.50	**24.00 (0.037) ***	67.00	**19.50 (0.011) ***

[1] Abbreviations: PM=pooled milk samples. The intercept is compared to the corresponding pooled milk sample in the other breed. [2]Level of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $P \leq 0.001$.

Table 5b. Coefficient and p-values of the ethanol stability between PM4–5 from SRB and SLB cows[1,2]

	Coefficients (p-value)			
Dependent variable	Intercept (SLB-PM4)	SRB-PM4	Intercept (SRB-PM5)	SLB-PM5
EtOH	69.00	**19.00 (0.014) ***	68.00	**23.50 (0.020) ***

[1]The intercept is compared to the corresponding pooled milk sample in the other breed.

[2]Level of significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $P \leq 0.001$.

Table 6a. Pearson correlations coefficients in SRB pooled milks samples for investigated quality traits[1, 2]

SRB	PL	PG	Total PL/PG	SCC	TS	Protein	Casein	WP	Fat	Lactose	G_{20}	RCT	Curd yield	Cn No.	pH
PL	1.00	0.21	0.30	0.47	0.30	0.43	0.37	0.53	0.25	0.15	-0.30	0.31	0.40	-58	-0.08
PG		1.00	**1.00*** **	0.20	-0.23	0.09	-0.19	0.09	-0.23	-0.12	-0.22	0.20	0.53	-0.43	-0.13
Total PL/PG			1.00	0.24	-0.20	-0.05	0.15	0.14	-0.20	-0.10	-0.24	0.22	0.55	-0.47	-0.14
SCC				1.00	0.43	**0.81****	**0.72***	**0.93*****	0.28	0.47	**-0.97 *****	**0.97*****	**0.86****	**-0.90*****	-0.01
TS					1.00	**0.85****	**0.90*****	**0.69***	**0.98*****	0.17	-0.32	0.37	0.22	-0.20	-0.61
Protein						1.00	**0.99*****	**0.95*****	**0.73***	0.41	**-0.71***	**0.75***	0.62	-0.61	-0.42
Casein							1.00	**0.89*****	**0.80****	0.40	-0.61	**0.65***	0.50	-0.47	-0.46
WP								1.00	0.56	0.41	**-0.84****	**0.87*****	**0.80****	**-0.82****	-0.30
Fat									1.00	-0.04	-0.19	0.23	0.09	-0.08	**-0.69***
Lactose										1.00	-0.40	0.38	0.33	-0.28	0.43
G_{20}											1.00	**-0.99*****	**-0.81****	**0.86*****	-0.05
RCT												1.00	**0.82****	**-0.86*****	-0.02
Curd yield													1.00	**-0.93*****	-0.09
Cn No.														1.00	-0.01
pH															1.00

[1] Coefficient level and level of significance (* $P \leq 0.05$; ** $P \leq 0.01$; ***$P \leq 0.001$)) for interactions between parameters are indicated. [2] Abbreviations, PL=Plasmin; PG=Plasminogen; SCC=Somatic cell count; TS=total solids; WP=whey protein; G_{20}=gel firmness after 20 min; RCT=rennet coagulation time; Cn No=casein number.

Table 6b. Continued

SRB	α-LA	β-LG	α_{s2}-CN	α_{s1}-CN	κ-CN	β_B-CN	β_{A1}-CN	β_{A2}-CN
PL	-0.29	-0.17	0.35	0.33	0.15	-0.26	-0.45	0.06
PG	-0.04	0.54	0.30	-0.02	-0.58	0.61	0.04	0.25
Total PL/PG	-0.06	0.51	0.33	0.03	-0.55	0.57	-0.00	0.25
SCC	-0.34	0.49	**0.88*****	0.15	-0.32	-0.21	**-0.85****	0.31
TS	**-0.76****	0.05	0.16	-0.09	0.10	**-0.74***	**-0.69***	0.61
Protein	**-0.64***	0.30	0.62	-0.05	-0.13	-0.61	**-0.93*****	0.56
Casein	**-0.64***	0.22	0.53	-0.10	-0.04	**-0.68***	**-0.89*****	0.55
WP	-0.60	0.43	**0.76****	0.03	-0.29	-0.48	**-0.93*****	0.53
Fat	**-0.81****	-0.00	-0.03	-0.05	0.15	**-0.76****	-0.60	**0.64***
Lactose	0.30	0.03	**0.64***	-0.17	-0.05	0.05	-0.21	-0.24
G_{20}	0.27	-0.59	**-0.84****	-0.13	0.34	0.13	**0.78****	-0.28
CT	-0.33	0.56	**0.83****	0.14	-0.35	-0.19	**-0.82****	0.36
Curd yield	-40	**0.66***	**0.83****	0.05	**-0.73***	0.15	**-0.64***	0.45
Cn No	0.37	-0.56	**-0.82****	-0.19	0.51	-0.03	**0.69***	-0.33
pH	**0.74***	-0.21	0.12	0.29	0.09	0.52	0.45	**-0.83****
α-LA	1.00	-0.25	-0.03	-0.04	0.30	0.52	0.60	**-0.85****
β-LG		1.00	0.62	-0.52	**-0.71***	0.33	-0.38	0.39
α_{s2}-CN			1.00	-0.18	-0.41	0.09	**-0.66***	0.10
α_{s1}-CN				1.00	0.23	-0.15	-0.01	-0.12
κ-CN					1.00	-0.54	0.10	-0.45
β_B-CN						1.00	0.62	-0.39
β_{A1}-CN							1.00	-0.55
β_{A2}-CN								1.00

Table 7a. Pearson correlations coefficients in SLB pooled milk samples for investigated quality traits[1,2]

SLB	PL	PG	Total. PL/PG	SCC	TS	Protein	Casein	WP	Fat	Lactose	G_{20}	RCT	Curd yield	Cn No.	pH
PL	1.00	**-0.67***	-0.57	0.31	-0.13	-26	-0.17	-0.34	-0.10	-0.23	-0.19	0.10	-0.04	0.28	-0.11
PG		1.00	**0.99*****	-0.23	-0.20	0.41	0.29	0.53	-0.25	0.58	0.26	-0.12	-0.24	-0.40	0.55
Total PL/PG			1.00	-0.21	-0.26	0.41	0.29	0.53	-0.29	0.60	0.25	-0.12	-0.27	-0.40	0.58
SCC				1.00	-0.43	**-0.87*****	**-0.75***	**-0.82****	-0.30	**-0.72***	-0.62	0.20	0.50	0.41	-0.50
TS					1.00	0.10	-0.10	0.45	**0.99*****	-0.12	**0.66***	**-0.65***	0.001	**-0.63***	-0.37
Protein						1.00	**0.95*****	**0.80****	-0.04	**0.95*****	0.44	-0.15	**-64***	-0.23	**0.79****
Casein							1.00	0.57	-0.24	**0.90*****	0.15	0.11	**-0.71***	0.09	**0.83****
WP								1.00	0.35	**0.76***	**0.86*****	-0.59	-0.33	**-0.77****	0.48
Fat									1.00	-0.25	0.62	**-0.65***	0.11	-0.64	-0.49
Lactose										1.00	0.35	-0.07	**-0.66***	-0.21	**0.91*****
G_{20}											1.00	**-0.84****	0.08	**-0.93*****	0.00
RCT												1.00	-0.31	**0.80****	0.27
Curd yield													1.00	-0.15	**-0.76***
Cn No.														1.00	0.07
pH															1.00

[1] Coefficient level and level of significance (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$)) for interactions between parameters are indicated. [2] Abbreviations, PL=Plasmin; PG=Plasminogen; SCC=Somatic cell count; TS=total solids; WP=whey protein; G_{20}=gel firmness after 20 min; CT=coagulation time; Cn No=casein number.

Table 7b. Continued

SLB	α-LA	β-LG	α_{s2}-CN	α_{s1}-CN	κ-CN	β_B-CN	β_{A1} -CN	β_{A2} -CN
PL	-0.08	-0.56	0.14	0.16	-0.09	-0.29	0.16	-0.08
PG	-0.57	0.37	-0.44	-0.48	0.28	0.05	0.01	-0.03
Total PL/PG	**-0.63***	0.31	-0.46	-0.50	0.29	0.00	0.04	-0.05
SCC	-0.01	-0.09	**0.77****	-0.37	**-0.92*****	-0.29	0.30	0.41
TS	-35	0.28	-0.17	0.27	0.31	**0.67***	**-0.84****	0.28
Protein	-0.33	-0.09	**-0.89*****	0.29	**0.89*****	-0.00	-0.03	-0.42
Casein	-0.28	-0.24	**-0.75***	0.44	**0.78****	-0.24	0.25	-0.56
WP	-0.32	0.2	**-0.90*****	-0.08	**0.83****	0.44	-0.54	-0.05
Fat	0.38	0.30	-0.06	0.20	0.18	**0.69***	-0.85**	0.36
Lactose	-0.55	-0.12	**-0.89*****	0.10	**0.82****	-0.16	0.12	-0.43
G_{20}	-0.05	0.37	**-0.66***	-0.25	0.57	**0.74***	**-0.86*****	0.29
RCT	0.02	-0.36	0.41	0.33	-0.17	**-0.71***	**0.89*****	**-0.67***
Curd yield	0.34	0.22	0.46	-0.56	-0.62	0.47	-0.36	0.60
Cn No.	0.16	-0.45	0.50	0.44	-0.40	**-0.72***	**0.85****	-0.38
pH	**-0.69***	0.34	**-0.71***	0.81	**0.66***	0.50	0.46	-0.58
α-LA	1.00	0.46	0.48	0.28	-0.30	0.35	-0.25	0.20
β-LG		1.00	0.06	-0.41	-0.16	0.50	-0.45	0.48
α_{s2}-CN			1.00	-0.06	**-0.82****	-0.07	0.24	0.13
α_{s1}-CN				1.00	0.30	-0.32	0.21	-0.43
κ-CN					1.00	0.19	-0.20	-0.55
β_B-CN						1.00	**-0.88 *****	0.32
β_{A1} -CN							1.00	-0.55
β_{A2} -CN								1.00

Comments to Tables 6–7

Curd yield and SCC

Within SRB (Appendix, Table 6a) and SLB (Appendix, Table 7a) the curds had a strong correlation to the SCC (0.86) with a $p<0.01$. The whey protein and curds within SRB had a strong correlation (0.80) with a $p<0.01$ (Appendix, Table 6a). Within SLB the correlation to the whey protein was lower (-0.72 and $p<0.05$), see Appendix, Table 7a.

Milk gross composition, SCC, and milk proteins

Both within SLB and SRB, the total protein content was significantly strongly correlated to casein– (0.95; $p<0.001$ respectively 0.99; $p<0.001$), and whey protein content (0.80; $p<0.01$ respectively 0.95; $p<0.001$) see Appendix, Table 6–7

Within SLB, a strong negative correlation, -0.86, between SCC and total protein was observed with a significant level at $p<0.001$ (See Appendix, Table 7a). When SCC decreased in the milk from SLB cows, an increase in total protein was observed (see Results 4.1.3, Table 5). For SRB, there was a significant ($p<0.01$) correlation (0.81) between total protein and SCC but no other observation within the TM was seen (see Appendix, Table 6a).

While the SCC decreased, an increase in casein and whey protein content during the second sampling occasion was observed, while the casein number decreased. Within SLB, SCC had a strong correlation which was significant in both whey protein (0.80; $p<0.01$) and casein protein (0.95; $p<0.001$) see Appendix, Table 7a. κ-casein was the protein, within SLB, that had a strong negative correlation (-0.92) and was significant ($p<0.001$) with SCC (see Appendix Table 7b).

PL and PL derived activity

It was observed that plasmin (PL) and plasminogen (PG) had a trend to a negative correlation, (-0.70) within SLB at a significant level $p<0.05$ (see Appendix, Table 7a). There was no significant correlation between PL and PG observed in SRB. However, PG had a significant ($p<0.001$) correlation to the total PL/PG within both breeds (Appendix, Table 6a–7a).

Rheologic properties

Within SLB, gel firmness (G_{20}) significantly correlated to whey protein (0.86; $p<0.001$), see Appendix, Table 7a. G_{20} had no correlation or significance to the casein content. However, in SRB, the total protein, casein and whey protein, all significantly correlated to RCT (Appendix, Table 6a) and no significant correlation

was observed within SLB. Within SLB, both G_{20} and RCT had a significant correlation to β-CN B, β_{A1}-CN, and β_{A2}-CN (see Appendix, Table 7b). Within SRB, it was observed that G_{20} and RCT had a significant correlation to β_{A1}-CN ($p<0.01$; Appendix, Table 6b).