A comparative study of innovation rennets from Swedish ruminants: calves, kids, and lambs

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Degree project/Independent project • 30 hp

Swedish University of Agricultural Sciences, SLU

Department of Molecular Sciences Agriculture Programme - Food Science

Molecular Sciences, 2023:32

Uppsala, 2023

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Credits:	30 credits
Level:	A2E
Course title:	Master thesis in Food Science
Course code:	EX0877
Program/Education:	Agricultural program – Food Science
Course coordinating dept:	Department of Molecular Sciences
Place of publication:	Uppsala
Year of publication:	2023
Copyright:	All images used are with permission from the copyright owner.
Part number:	2023:32

Keywords: *Ruminant milk, Swedish bovine rennet, Swedish kid rennet, Swedish lamb rennet, Artisanal cheese production, Rheological properties, Curd yield, Gel firmness, Innovation, Milk coagulation time*

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Abstract

The study aimed to examine Swedish innovation rennets (lamb and kid) and bovine rennets (pepsin and chymosin) in combination with species-specific milk from goat, sheep, and cow. This was accomplished by evaluating the gross composition, casein and whey protein content, casein number of the milk types, as well as rheological properties, such as curd yield, coagulation time, and gel firmness.

The milk composition showed that sheep milk had the highest levels for all investigated milk components but the lowest pH, which was 3% lower for cow and goat milk.

Comparing the effect of milk type on curd yield using the different rennets, the sheep milk in combination with chymosin resulted in 9% and 35% higher curd yield compared to cow and goat milk, respectively (p=0.001). Pepsin in combination with goat milk resulted in 28% and 30% lower curd yield than for cow and sheep milk, respectively (p=0.001). The lamb rennet with sheep milk showed 12% and 24% higher curd yield than with cow and goat milk, respectively (p=0.001), and the kid rennet with goat milk had a 37% lower curd yield than with cow and sheep milk, respectively (p=0.001). Comparing the effects of the rennets on the curd yield for each milk type, cow milk with chymosin and lamb rennet resulted in a 5% higher curd yield than with kid rennet (p=0.021). Kid rennet with goat milk showed 15% and 29% lower curd yield compared to chymosin and lamb rennet, respectively (p=0.001). The curd yield from sheep milk with lamb rennet was 10% and 17% higher compared to pepsin and kid rennet (p=0.001).

The effect of milk type on coagulation time showed that cow milk with chymosin was 50% and 60% longer than in goat and sheep milk (p=0.001). Pepsin with cow milk displayed 44% and 54% higher coagulation time than with goat and sheep milk, respectively (p=0.001). Sheep milk with lamb rennet had 46% and 53% shorter coagulation times than with goat and cow milk, respectively (p=0.001). Within the milk types, the effects of rennets showed that lamb rennet, compared to chymosin, pepsin, and kid rennet, resulted in the longest coagulation time, 9%, 26%, and 54% longer, respectively, in combination with cow milk (p=0.001). Goat milk with lamb rennet showed a 48%, 52%, and 72% longer coagulation time compared to chymosin, pepsin, and kid rennet, respectively (p=0.001). Kid rennet with sheep milk resulted in 47%, 51%, and 61% shorter coagulation time than with pepsin, chymosin, and lamb rennet, respectively (p=0.001).

Comparing the effect of milk type on gel firmness showed that chymosin in sheep milk resulted in 84% and 80% higher gel firmness than in cow and goat milk, respectively (p=0.001). Pepsin with sheep milk displayed the highest gel firmness, 83% and 81% higher than for cow and goat milk, respectively (p=0.001). Lamb rennet with sheep milk resulted in 82% higher gel firmness compared to cow and goat milk, respectively (p=0.001). Kid rennet with goat milk showed 49% and 78% lower gel firmness than cow and sheep milk (p=0.001). Within the milk types, the effects of rennets on gel firmness showed that when kid rennet was used in cow milk, gel firmness was 31%, 39%, and 41% higher compared to when pepsin, lamb rennet, and chymosin, respectively, were used (p=0.001). Kid rennet with goat milk showed the lowest gel firmness, 32% lower than for chymosin and pepsin (p=0.002). Sheep milk with kid rennet resulted in 37% and 41% lower gel firmness compared to chymosin and pepsin rennet (p=0.010).

This research indicates that the species-specific innovative rennets from Swedish ruminants can potentially contribute to the optimization of the production of Swedish artisanal cheeses, thereby increasing the added value of the final product.

Keywords: Ruminant milk, Swedish bovine rennet, Swedish kid rennet, Swedish lamb rennet, Artisanal cheese production, Rheological properties, Curd yield, Gel firmness, Innovation, Milk coagulation time

Sammanfattning

Studiens syfte var att undersöka svenskt innovationslöpe från lamm och killing i jämförelse med bovint löpe (pepsin och chymosin) i kombination med mjölk från get, får och ko. I studien utvärderades mjölkens generella sammansättning, innehåll av kasein och vassleproteiner, kaseintal, reologiska egenskaper (koaguleringstid och gelstyrka), samt utbyte av ostmassa.

Resultatet av mjölkens sammansättning visar att fårmjölk innehöll högsta mängden för alla undersökta mjölkkomponenter, men dess pH var 3% lägre än för ko- och getmjölk.

När effekten av mjölktyp undersöktes avseende utbyte av ostmassa, gav fårmjölk i kombination med chymosin 9% och 35% högre mängd jämfört med ko- och getmjölk (p=0,001). Pepsin med getmjölk resulterade i 28% och 30% lägre utbyte än med ko- och fårmjölk (p=0,001). Lammlöpe med fårmjölk gav 12% och 24% högre utbyte än med ko- och getmjölk (p=0,001), och killinglöpe med getmjölk 37% lägre utbyte än med ko- och fårmjölk (p=0001). Effekterna av löpe inom mjölktyp visade att komjölk med chymosin och lammlöpe resulterade i 5% högre utbyte av ostmassa än med killinglöpe (p=0,021). Killinglöpe med getmjölk gav 15% och 29% lägre utbyte jämfört med chymosin och lammlöpe (p=0,0001). Utbytet av ostmassa från fårmjölk med lammlöpe var 10% och 17% högre jämfört med pepsin och killinglöpe (p=0,001).

Effekten av mjölktyp på löpenas koaguleringstiden visade att chymosin med komjölk resulterade i 50% och 60% längre koaguleringstid än med get- och fårmjölk (p=0,001). Pepsin med komjölk uppvisade 44% och 54% längre koaguleringstid än med get- och fårmjölk (p=0,001). Lammlöpe med fårmjölk hade 46% och 53% kortare koaguleringstid än med get- och komjölk (p=0,001). Inom mjölktyp hade lammlöpe, jämfört med chymosin, pepsin och killinglöpe, den längsta koaguleringstiden, 9%, 26% respektive 54%, i komjölk (p=0,001). I getmjölk hade lammlöpe 48%, 52% och 72% längre koaguleringstid jämfört med chymosin, pepsin och killinglöpe (p=0,001). Fårmjölk med killinglöpe resulterade i 47%, 51% och 61% kortare koaguleringstid än med pepsin, chymosin och lammlöpe (p=0,001).

I studier av effekten av mjölktyp på gelstyrkan för de olika löpena resulterade chymosin i fårmjölk i 84% respektive 80% högre gelstyrka än i ko- och getmjölk (p=0,001). Pepsin med fårmjölk uppvisade 83% och 81% högre gelstyrka än med ko- och getmjölk (p=0,001). Lammlöpe med fårmjölk resulterade i 82% högre gelstyrka jämfört med ko- och getmjölk (p=0,001). Killinglöpe med getmjölk visade 49% och 78% lägre gelstyrka än med ko- och fårmjölk (p=0,001). Studier av effekterna av löpe inom mjölktyp visade att när killinglöpe användes i komjölk var gelstyrkan 31%, 39% och 41% högre än med pepsin, lammlöpe och chymosin (p=0,001). Killinglöpe uppvisade den lägsta gelstyrkan med getmjölk, 32% lägre än med chymosin och pepsin (p=0,002). Fårmjölk med killinglöpe resulterade i 37% och 41% lägre gelstyrka jämfört med chymosin- och pepsin (p=0,010).

Denna studie indikerar att artspecifikt innovationslöpe från svenska idisslare skulle kunna bidra till ett ökat mervärde i produktionen av svenska hantverksostar.

Nyckelord: Idisslare mjölk, svensk bovint löpe, svenskt killinglöpe, svenskt lammlöpe hantverksmässig osttillverkning, reologiska egenskaper, ostmassa, gelstyrka, innovation, mjölkkoaguleringstid

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Abbreviations

ANOVA	Analysis of Variance
CF	Curd Firming
CMP	Caseinomacropeptide
CT	Coagulation Time
CY	Curd Yield
GF	Gel Firmness
IDF	International Dairy Federation
IMCU	International Milk Clotting Units
Leu	Leucine
MCA	Milk Coagulating Activity
MCP	Milk Coagulating Properties
MCU	Milk Clotting Unit
MUFA	Monounsaturated Fatty Acids
Phe	Phenylalanine
PUFA	Polyunsaturated Fatty Acids
RCT	Rennet Coagulation Time
REMCAT	Relative Milk Clotting Activity Test
SD	Standard Deviation
SFA	Saturated Fatty Acids
SLU	Swedish University of Agricultural Sciences
SNF	Solid Not Fat
Tyr	Tyrosine
UFA	Unsaturated Fatty Acids
Val	Valine

Introduction

Cheesemaking dates to around 6000 B.C. Its sources of origin differ. One focuses on the discovery of naturally fermented milk in warmer temperatures due to the actions of lactic acid bacteria and ideal pH levels (McSweeney 2007). The other is the discovery of curds and whey rather than the expected milk stored in a young ruminant's stomach used as a carrying pouch (IDFA 2022). Whichever coincidental event led directly to its origin, the general milk clotting activity has since developed into a series of processing steps with many biochemical alterations and contributing factors (Walstra et al. 2006). As cheese manufacturing expanded in response to its popularity, so did the demand for milk-clotting enzymes. Animal rennet was an early popular choice of enzyme because it coagulates milk rapidly at its natural pH without further degrading its proteins. The action of rennet during cheese making is to induce the hydrolysis of protein in milk to initiate other processes that lead to curd formation. Commercial production of rennet began in 1847 (Garg & Johri 1994). Different dairy milk and animal rennet species have distinctive characteristics, such as composition and coagulation, which affect rheological properties and thus impact cheese production and the final product. Therefore, evaluating their effects is essential.

1.1 Aim and objective

This thesis aimed to generate data regarding the applicability of innovative and conventional rennets in combination with milk from different species. Swedish innovation rennets (lamb and kid) along with bovine rennet (chymosin and pepsin) were evaluated in combination with goat, sheep, and cow milk from Swedish dairy farms to examine curd yields and rheological properties. The objectives included:

- Examine the coagulation, rennet by rennet, using milk from the different species
- Examine the coagulation, milk type by milk type, using the different rennets
- Evaluate the results from rheological measurements and mini cheese production using statistical analysis.

2. Background

2.1 Rennet

One of the fundamental elements of making cheese is converting milk into curd. An essential step in this process involves coagulating milk by clotting enzymes. Renneting is the traditional method used to make this process possible, and it involves adding an extract, i.e. rennet, with the enzyme chymosin, to milk to promote coagulation (Moschopoulou 2011). Most proteolytic enzymes can coagulate milk. Some examples, in addition to chymosin and pepsin extracted from animals, include cardosins extracted from plants and proteolytic enzymes isolated from bacteria. Plant proteases as extracts have been involved in cheesemaking for centuries (Shah et al. 2014). Proteases such as cardosins from *Cynara sp.* from thistle-like plants can have the same milk clotting activity as calf rennet. One notable drawback of plant coagulants in cheese production is lower cheese yields (Liburdi et al. 2019). For microbial rennet, *Bacillus amyloliquefaciens is* one microorganism that can be used as a coagulant for producing certain cheese types. Its characteristics are low thermostability, high milk clotting activity, and easy denaturation. One disadvantage of this microbial rennet for cheese production is its higher proteolytic activities (An et al. 2014). Higher proteolytic activity can cause the cheese to become too soft (Crabbe 2004). Animal rennet is produced in different forms, such as liquid, powder, or paste. The liquid and powder forms are frequently used in cheese production. Bovine rennet is the most commonly used in commercial production, while lamb and kid (young goat) rennet are used more by cheese artisans (Moschopoulou 2011). Animal rennet can be made by taking the fourth stomach, the abomasum, of unweaned ruminants, most of which are calves, and extracting its enzymes (Jacob et al. 2011).

2.1.1 Chymosin and pepsin

Rennet consists of the proteolytic enzymes chymosin (EC 3.4.23.4) and pepsin (EC 3.4.23.1), which are both classified as aspartic proteinases (Jacob et al. 2011), a class of proteases with optimal activity in acidic environments. Chymosin is present in the true stomach of newborn ruminants and it is already produced during gestation. Younger ruminants contain low amounts of the pepsin enzyme, but it becomes the main proteinase in adult ruminants (Fox et al. 2004). Chymosin can cleave between the amino acids phenylalanine and methionine in κ-casein's 105 and 106 (Phe_{105}-Met_{106}) bonds. Although these enzymes are similar, pepsin can break down caseins and hydrolyze colostrum immunoglobulins, thus explaining why it is not abundant in newborn ruminants (Walstra et al. 2006). Due to chymosin and pepsin's role in milk clotting and the differences in their proteolytic activity, their ratios in rennets are important (Moschopoulou 2011). The age and diet of ruminants significantly influence their enzyme production, thus changing the composition of the rennet (Lundh 2022). Rennet from a one-month-old milk-fed calf could have a relative concentration of 92% chymosin and 8% pepsin in the stomach, whereas a cereal-fed calf between the ages of one and two years old could have a 6% chymosin and 94% pepsin concentration (Lundh 2022). The activity of a rennet which consists of both chymosin and pepsin, corresponds to that of 100% chymosin, provided the content of pepsin does not exceed 25% (Fox et al. 2004). There are, however, factors that affect the activity of all enzymes, such as temperature, pH, and ionic strength (Walstra et al. 2006).

2.1.2 Rennet clotting

Cheese producers rely on an enzyme's ability to have a high ratio of milk clotting activity (MCA). Moschopoulou (2011) explains that the International Dairy Federation (IDF) uses the Relative Milk Clotting Activity Test (REMCAT) on standardized milk to measure the MCA of rennet in International Milk Clotting Units (IMCU). This is how much rennet in mL or gram is needed to cause milk clotting based on a time of 40 minutes and milk at a temperature of 35ºC (Moschopoulou et al. 2007). Adding rennet to milk initiates a milk-clotting reaction that produces curds (Damodaran & Parkin 2017). Milk clotting is promoted when chymosin cleaves the caseinomacropeptide (CMP) from the κ-casein, which causes the casein micelles to become unstable and aggregate. Pepsin contributes to hydrolyzing the bonds of phenylalanine (Phe), tyrosine (Tyr), leucine (Leu), or valine (Val) residues. Thus, in combination, chymosin and pepsin are ideal for cheese production efficiency (Fox et al. 2004). Nearly 75% of the global cheese production market uses rennet-coagulated curds. The syneresis properties of curds coagulated by rennet are more substantial than those coagulated by acid, which results in a more stable product. Chymosin has a higher stability when pH values in milk stay between 5.3 and 6.3, and pepsin has the most stability in milk at a pH of 4.5 (Beynon & Bond 2001). If the pH and temperatures of the milk become too high or too low, it can cause inactivation of the enzymes (Fox et al. 2004). Calves excrete inactive chymosin, or pro-chymosin, in the abomasum, which remains inactive until auto-proteolysis. In milk, chymosin and pepsin can be activated by lowering the pH by adding calcium ions to create an acidic environment. Increasing the temperature to > 40ºC will inactivate these enzymes (Fox et al. 2004).

2.1.3 Production of rennet

An early method for extracting rennet was reconstituting salted ruminant stomachs in whey to draw out the enzymes. This method is still used by some artisan cheese makers and in the traditional production of feta cheese, where kid and lamb stomachs are used instead of calf stomachs (Jacob et al. 2011). Today, different rennet producers may have variations in beginning and ending activation steps, but the general steps are comparatively similar. There are some initial factors to consider during commercial rennet manufacturing; for example, concerning commercial extraction, the type of machinery and filters play an essential role in rennet production (Olsson 2022, personal communication). The extraction process begins with frozen abomasum stomachs, where unweaned calves, lambs, or kid's stomachs can be used. The stomachs are thawed, followed by the breakdown of the stomachs to ensure that all the desired enzymes can be extracted. Placing the stomachs in salt water is the most efficient way to extract the enzymes. The rennet production process continues with a pressing out of all the liquid, which can then be decanted through a centrifuge to guarantee a pure extract. After the extraction, the rennet needs to be activated, which is done by lowering the pH to 1.6-1.8 for about 15 minutes and then returning to its original pH of 5.8. Once the activation is complete, it goes through filtrations. Some producers use vacuum filters or pressure filters based on filtering through perlite or diatomite filter aids. The final filtration is usually at the sterile filter level. Rennet commonly contains 18% salinity to create an ideal enzyme environment and prevent bacteria growth. The rennet is then stored at a refrigerated temperature between 2ºC and 8ºC until it is ready for use (Olsson 2022, personal communication).

2.2 Milk attributes from different dairy species

Factors such as year-round breeding will result in marginal changes in the dairy silo milk composition due to slight variations, whereas seasonal breeding contributes to experiencing more compositional changes (Park et al. 2017). At the end of lactation, there will be a decrease in lactose and an increase in fat, protein, and total solids (Brozos et al. 1998). Other factors that influence the composition of cow, goat, and sheep milk include their environment, diet, lactation number, breed, the condition of their udders, and management (Park et al. 2007). When using milk samples drawn on consecutive days, it is essential to note that there can be variations. Property and composition variations can exist even within a single milking (Walstra et al. 2006). There are characteristic differences between the milk from different dairy species and distinctions in composition and physical properties in milk also affect their rennetability, thus creating variation during cheese making (Ramos & Juarez 2011).

2.2.1 Sheep milk

Sheep milk is mainly used to produce cheese, and it is uncommon for it to be used for direct consumption (Pazzola 2019). It has an average fat percentage of 7.9%, shown in Table 1, which is twice as high as that of cow and goat milk. Sheep milk's average content of lactose and protein is 4.9% and 6.2%, respectively, which is higher than the contents of cow and goat milk. Sheep milk's average density is slightly higher, 1.037g/ml, compared to that of goat milk, 1.034 g/ml, and cow milk. 1.031 g/ml. Sheep milk is also shown to have a higher viscosity than that of goat and cow milk (Park et al. 2007). Pazzola's study of milk coagulation properties (MCP) showed that sheep's rennet coagulation time (RCT) is estimated to be around 8.6 minutes. A shorter RCT is typically associated with a firmer curd, which can contribute to an increased curd yield (CY) (Pazzola 2019). The rennetability of sheep milk, as with all milk types, is affected by its physio-chemical properties, such as its pH and the size of its casein micelles (Ramos & Juarez 2011).

2.2.2 Goat milk

Goat milk is used for both direct consumption and cheese production (Pazzola 2019). Compared to cow and sheep milk, goat milk has the second-highest percentages of fat at 3.8% and protein at 3.4%, but a lower content of casein, on average 2.4%, and lactose at 4.1%. The viscosity and density measurements in Table 1 are proximate to those of cow's milk. Pazzola (2019) showed that the average RCT time for goat milk is about 13 minutes, and the CY for goats can range from 13.6% to 17.8%, depending on the breed. Moreover, the fat and protein content of the milk may likely affect the CY percentage, with higher content resulting in a higher percentage.

2.2.3 Cow milk

Cow milk is versatile and is used for direct consumption, cheese production, butter production, and many other products (Eurostat 2022). Its protein, casein, and fat contents are on average lower compared to sheep and goat milk, whereas the lactose content is higher (Table 1). The density is comparable to sheep and goat milk, but cow milk has the lowest viscosity (Park et al. 2007). CY from cow milk is, on average, 15% (Cipolat-Gotet et al. 2018). According to Pazzola (2019), cow milk's RCT is estimated to be between 10 and 20 minutes. The milk composition affects the coagulation process and the consistency of the final product

Table 1. Composition and properties of cow, sheep, and goat milk

Composition/Properties	Cow	Sheep	Goat
Protein (%)	3.20	6.20	3.40
Casein (%)	2.60	4.20	2.40
Fat (%)	3.60	7.90	3.80
Lactose (%)	4.70	4.90	4.10
pH	6.65 – 6.71	6.51 – 6.85	6.50 – 6.80
Viscosity (C_p)	2.00	2.86 – 3.93	2.12
Density (g/ml)	1.0231 – 1.0398	1.0347 – 1.0384	1.029 – 1.039

Data from (Park et al. 2007). C_p*=centipoise measures viscosity in liquids.*

2.3 Cheesemaking and caseins

Cheesemaking involves different technical steps that impact the milk's structure and define the characteristics of the cheese (Nicosia et al. 2022). Understanding these processing steps is essential, as they affect the cheese yield and quality (Walstra et al. 2006).

When the cheesemakers receive raw milk, there are a few options. Raw milk can be stored at a temperature of 4ºC untreated for about two days (Walstra et al. 2006). If the milk needs to be kept for longer, the cheesemaker may choose to heat treat the milk, which can extend the milk's quality up to 12-48 hours after it has been in storage for 24 hours. Thermization is a process in which milk is heated at 63ºC for 10-15 seconds, and this treatment comes before pasteurization. After thermization, the enzymes in the milk are still active, but the number of psychrotrophic bacteria is reduced (Walstra et al. 2006). Not all cheesemakers pasteurize milk, but when they do, the milk is heated at 72ºC for about 15 to 20 seconds (Rukke et al. 2016). The cheese milk is usually inoculated with a starter culture of lactic acid bacteria which converts lactose into lactic acid through the metabolic process of fermentation. The pre-ripening of the cheese milk aims to initiate starter culture growth, giving the culture some time to start producing lactic acid, which is vital to lowering the pH of the milk. (McSweeney 2007). Lowering the pH will help facilitate the next substantial step in cheesemaking, renneting.

Renneting is the addition of an enzyme to aid in milk coagulation. Proteolytic enzymes, such as pepsin and chymosin, help break down the milk's caseins for cheesemaking (Fox et al. 2004). Most (80%) of the milk protein consist of the four caseins α_{S1}-, α_{S2}-, β-, and κ- casein; proteins which remain stable at 100ºC and withstand a pressure of 100 MPa (Walstra et al. 2006). It is important to note that there are variations in these caseins and their amounts depending on the species of milk. The casein micelles' instability is accelerated with proteolytic enzymes or through acidification. This instability causes the formation of curds (Dalgleish 2011). Since most of the casein stays in the curd, casein content affects the cheese yield more than the fat content (Walstra et al. 2006). The renneting process takes 30 to 45 minutes at a temperature between 30ºC and 32ºC (Hamdy et al. 2022). During this step, the casein micelles aggregate, and a gel starts to form.

The final step includes the expulsion of whey from the gel, also called syneresis. The syneresis rate depends on how the cheesemaker cuts the gel (size of curd grains), stirring, and temperature (Walstra et al. 2006). Finalizing syneresis can take about 30 to 60 minutes, depending on how much moisture and acidity are desired in the cheese. The removal of whey should not be forced, as it will also affect the curd grains. The curd grains are then placed in the molds, pressed, salted, and allowed to ripen. These processing steps must be optimized and adapted for specific types of cheese produced (Janhøj & Qvist 2010).

2.3.1 Milk coagulation properties

Evaluating the milk coagulation properties (MCP) is fundamental in cheese production (Bittante 2011). The analysis of curd yield, rennet coagulation time, and curd firmness are also the primary interests of this study. RCT is the time it takes once the rennet is added, to the milk to begin gelatinization. Some factors that can affect the RCT in milk are temperature, pH, rennet types, rennet concentrations, and milk composition (such as casein concentrations) (Horne & Banks 2004). If the temperature of the milk is lower than 18ºC, the rate at which the coagulation takes place will be insignificant. Because of the addition of starters in cheese production, the milk temperature is typically maintained at around 30ºC. Similarly, the ideal milk temperature for rennet coagulation is about 30ºC. If the temperatures are too high, around 45ºC or higher, depending on the rennet type and pH, it can cause thermal denaturation of the rennet, preventing coagulation (McSweeney 2007). The rennet activity and protein concentration also influence coagulation. When more than the required amount of rennet is in the milk during cheese making, it can speed up coagulation. Moreover, caseins, together with fat and calcium, make up the milk gel's structure. When higher amounts of caseins are present, the coagulation speeds up, thus making for a faster RCT (McSweeney 2007). The milk's pH also impacts its coagulation times because it affects the enzymes' activity during coagulation. Higher pH levels can slow coagulation, whereas a lower pH helps with the enzyme's activity (McSweeney 2017). Milk contains casein micelles, which are structures made up of negatively charged caseins on the surface. Starter cultures, typically comprised of lactic acid bacteria, will produce and release protons in the milk during the fermentation process, lowering the pH (Farkye 2004). Chymosin also has a negative charge, and lowering the milk pH will somewhat reduce the repulsion between the enzyme and the casein micelle (Lundh 2022).

2.3.2 Factors affecting cheese yield

One factor affecting cheese yield is how milk is handled and stored. Milk stored for too long at cold temperatures before and after transportation to the dairy impairs the coagulation characteristics, resulting in protein and fat loss into the whey and thus affecting the cheese yield negatively. Milk's composition and quality can also influence its cheese yield. In order to determine the udder health of an animal, the somatic cell count (SCC) can be tested in milk to indicate whether there is inflammation in the mammary glands (Hogeveen et al. 2011). High SCC levels could indicate mastitis, an inflammation of the mammary gland and in most cases caused by a bacterial infection (McSweeney 2007). Studies have shown that mastitis is associated with lower levels of casein, lactose, and non-fat solids, while a study with Gyr cows reported that elevated SCC levels at quarter level, was associated with higher levels of whey protein, fat, and total solid content (Malek dos Reis et al. 2013). Zhang et al. (2022) suggested, that from a microbiota and transcriptomic response perspective, for cow's milk to be considered to be from a healthy gland, its SCC should be at or below 100,000 cells/ml, a SCC exceeding 200,000 cells/ml usually indicatin mastitis. Conversely, the Pasteurized Milk Ordinance regulation sets the goat's milk SCC’s limit at about 1,000,000 cells/ml, but SCC's lower than 500,000 cells/ml have less of an effect on the milk's composition (Chen et al. 2010). The proposed threshold for SCC in sheep's milk for healthy mammary glands is 250,000 cells/ml (Albenzio et al. 2019). It is important to note that SCC averages vary depending on the animals' genetics, health and management, age, and lactation stage. The SCC levels in noninfected goat milk are generally higher than in noninfected cow and sheep milk (Albenzio et al. 2019).

Other factors influencing cheese yield can be different rennets, which have different proteolytic activities, affecting milk clotting. Some rennet types can over- or under-hydrolyze casein depending on the curd's contact time with whey and its pH, which is another yield impactor (Mona et al. 2011). The gel firmness and stable protein network are important factors in improving the losses in the whey. During the later steps of cheese production, how the coagulum is cut and stirred can also affect the cheese yield (McSweeney 2007).

2.4 Innovation group

When Sweden stopped producing animal rennet, an innovation group was formed in 2017 based on the uncertainty around rennet production in other countries. The group started work on a project to produce rennet from Swedish ruminants. The rennet would come from Swedish goat kids, lambs, and calves that have only had milk from their species and have been raised and slaughtered in Sweden. The main goal of the innovation group was to “develop, evaluate, and apply a method to produce Swedish rennet for small-scale dairy farms.” The project's financial support comes from the Swedish Agency for Agriculture and the European Agricultural Fund (Journal. Nr. 2019-170-1). The innovation group aims to offer consumers assurance and an ethically manufactured product. The group theorizes that domestic species-specific rennet would create a higher value for the final product and strengthen the competitiveness of Swedish artisan cheese producers. The project would also be able to use the ruminant stomachs that would otherwise be wasted, thus contributing to a more sustainable food chain. The Swedish University of Agricultural Sciences (SLU) is responsible for characterizing the bovine, kid, and lamb rennets by investigating the resulting gel strength, coagulation time, and curd yield when the rennets are used for coagulation of milk.

This master's thesis was conducted within the frames of the project, characterizing rennets (lamb and kid) from the innovation group's project.

3. Materials and Methods

In this laboratory study, goat, sheep, and cow milk from Swedish dairy farms were used in combination with the species-specific rennets. The rennets used in this study were lamb and goat kid rennets from Swedish animals, produced within the innovation project at Sacco System Nordic AB, Skurup, Sweden (Journal. Nr. 2019-170-1), where the bovine chymosin and pepsin were also produced. Chymosin and pepsin were both used as reference rennets. The Swedish dairy farm's raw milk arrived at SLU frozen. After thawing, the milk was aliquoted for mini cheese production, rheology, pH, and gross composition.

3.1.1 Rennet sample preparation

There were four rennets used for this study. Their concentrations were standardized for mini cheese production and rheological tests. The prepared rennet tubes were refrigerated at a temperature of 4ºC. The bovine chymosin rennet consisted of 75% chymosin and 25% pepsin with an activity of 180 international milk clotting units (IMCU). This rennet was diluted by pipetting 667 μL of the bovine chymosin solution into the Falcon tube along with 9.333 mL of tap water, resulting in 10 mL of bovine chymosin rennet with a concentration of 12 IMCU. Pepsin consisted of 95% pepsin and 5% chymosin and had an activity of 180 IMCU. So, the same dilution process was performed, resulting in the same concentration of 12 IMCU. The remaining Swedish ruminant rennet was the lamb and kid rennet; their activities were 12 IMCU, and no modifications were needed.

3.1.2 Milk sample preparation

The goat, sheep, and cow bulk milk samples came from three Swedish dairy farms and were collected on three different occasions during the same week for each milk type. All the samples were labeled with their milk type and the day they were acquired, which became their batch numbers. As the milk samples were sent frozen to SLU laboratories in one-liter batches, they were kept in the freezer at a temperature of -20ºC until analysis. When the samples were needed, they were placed in the refrigerator to thaw at a temperature of around 4ºC the day before they were used in the laboratory. After the first thawing, the milk was used for measurements of pH, gross composition, and the production of mini cheeses. The rest of the milk samples were aliquoted and re-frozen for future analyses. For the rheology, the milk was defatted before re-freezing. For this, 20 mL of each milk sample was placed in a 50 mL conical Falcon tube. The tubes were centrifuged using the Sorvall Lynx 4000 (ThermoFisher, Langenselbold, Germany). The instrument settings were 4ºC at 3000 RPM for 10 minutes. After the milk was centrifuged, the top layer of fat was removed. From the defatted milk, 1.5 mL was pipetted into 2 mL Eppendorf tubes. The tubes were then frozen at -20ºC until analyzed.

3.2 Milk gross composition

Three batches of whole milk were obtained from each of the dairy species. It is important to note that the gross composition was also analyzed for the whey samples from the mini cheese production. The protein measurements of the whey were used to calculate the casein content and casein numbers used for the evaluations. The gross composition, i.e., the content of fat, protein, lactose, and total solids, was analyzed at the Department of Animal Nutrition and Management, SLU, using Fourier Transform Infrared Spectroscopy (FTIR, Hillerød, Denmark). Other parameters measured with FTIR were saturated fatty acids, unsaturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids along with the myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1C9) and density. The same technique was used to analyze the protein content in the whey. The department also analyzed the somatic cell count with an automated fluorescence-based cell counting device (Fossomatic, Hillerød, Denmark). For each of the three milk types (cow, goat, and sheep), three biological replicates were analyzed for their casein amount, casein number, and protein content in whey. Each biological replicate within the milk types was analyzed three times, resulting in nine replicates, from which the mean values and standard deviations were determined.

3.3 Mini cheesemaking procedure

The process used for making the mini cheeses was modeled after the method from (Othmane et al. 2002) with some adaptations. The flowchart for this procedure is shown in Figure 1. After thawing, the refrigerated whole milk samples were placed in a 30ºC water bath for 30 minutes to begin the initial warming of the milk. Falcon tubes (15 ml) were used to process the mini cheeses. The tubes were weighed, and their weight was recorded in an Excel file. The experimental design is shown in Appendix 2, Figure A1. In total, 216 mini cheese tubes were used in this part of the study. Each milk type was processed in three batches: one for each day the milk was collected (three biological replicates). Each batch was evaluated with four different rennet types, with six technical replicates each; that is, for each milk batch and rennet, the analysis was repeated six times. Each batch of 24 tubes was filled with 10 grams of milk each. The tubes were placed in a 32ºC water bath for 30 minutes for pre-warming. The following steps involved removing the tube rack from the water bath and adding 150 µL of the designated rennet to each tube.

The final rennet concentration in each sample was 0.18 IMCU. The tubes were placed back into the 32ºC water bath for another 30 minutes to allow for coagulation. The curd formation occurred in the tubes, and the curd was cut with a cylindrical, cross-shaped tool that was wiped off after cutting curds formed using each type of rennet. The tubes were then placed back into the 32ºC water bath for another 30 minutes to allow for syneresis to occur. They were then placed in a laboratory centrifuge (Sorvall, Super T21, Sorvall Products L.P., Newton, Connecticut, USA) for 20 minutes at 22ºC at 1650 RPM, which separated the curd from the whey. The whey from the six tubes was poured into a new Falcon tube for each rennet type and weighed. The curd in the tubes was also weighed, and both weights were recorded in Excel. The original empty tube weight was subtracted from the tube weight with the curd. From this, the curd yield percentages could be calculated.

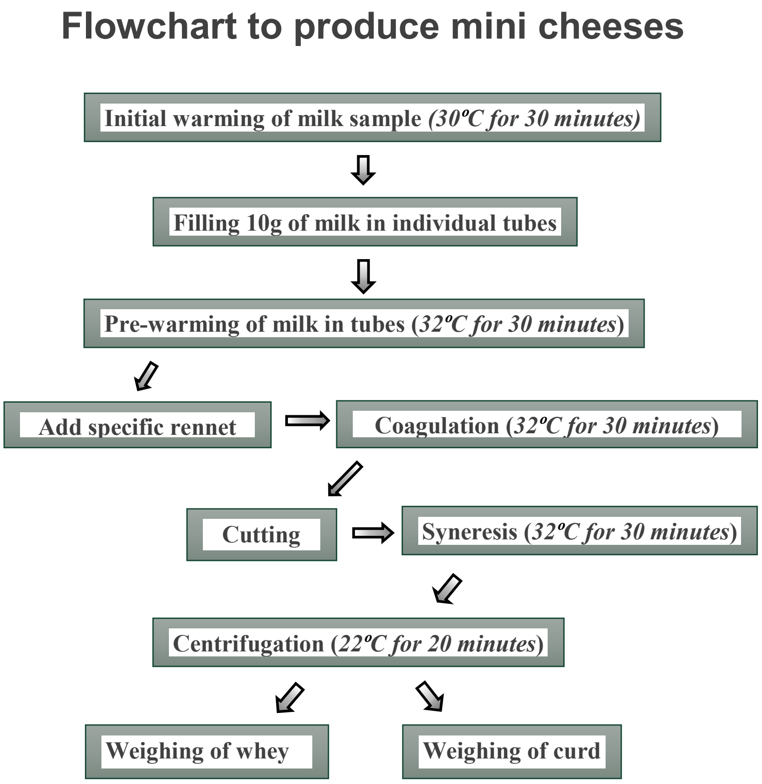

Figure 1. Flow chart to produce the mini cheeses adapted from (Othmane et al. 2002)

3.4 pH measurements

The pH of whole milk was measured at room temperature. Each sample was placed in a 15-mL Falcon tube and measured using a Mettler Toledo^{TM} S210 SevenCompact^{TM} pH meter. The electrode was placed inside the tube with the whole milk and the pH value was recorded.

3.5 Rheological properties

For the rheological properties, frozen defatted milk was used. The milk samples were thawed and placed into a 35ºC water bath. Before measurements, 22.5 µL of rennet was added to the tubes, resulting in a final rennet concentration of 0.18 IMCU in each sample. The samples were processed using a hybrid rheometer (Discovery Hr-3 TA Instruments, New Castle, USA) with a Peltier plate (Hard Anodized Aluminium with Solvent Trap, 40 mm). The instrument was set at a strain of 1%, a frequency of 1 Hz, and a temperature of 35ºC. Then, 1.236 mL of milk was pipetted onto the 2 mm cone and processed for 20 minutes. Three different types of milk were processed in three biological batches each, totaling nine batches. For each batch, four replicates for each of thte four rennets were processed, resulting in 16 technical replicates for each of the nine batches. In total, 144 samples were used for the rheological measurements part of this study.

3.6 Statistical analysis

Statistical analyses were performed using Minitab® Version 19.2021.1.0 (Minitab, LLC., United States). All the obtained values in this study were first entered into Excel and then into the Minitab® program. A one-way analysis of variance (ANOVA) was performed to investigate if there were significant differences in the way the different rennet types affected the coagulation of different milk types, also taking variation between milk batches into account. Tukey's pairwise comparisons examined differences between the rennet types, milk types, and milk batches. The statistical significance was assessed with a 95% confidence interval.

4. Results

4.1 Milk gross composition

The gross composition of cow, goat, and sheep milk is shown in Table 2. The values are averages of the three different milk batches. Sheep milk showed the highest values for all investigated milk attributes except for pH, which was 3% lower than that of cow and goat milk. All milk attributes differed significantly except for the density, SCC, and C16:0.

Table 2. Gross composition of three milk types: cow, goat, and sheep

Milk gross composition				
Parameters	**Cow milk**	**Goat milk**	**Sheep milk**	**P-value**
Density (g/ml)	1.031 ± 0.00	1.028 ± 0.00	1.029 ± 0.00	0.085
Fat (g/100g)	4.15 ± 0.03^{b}	3.77 ± 0.20^{b}	6.66 ± 0.57^{a}	0.001
Lactose (%)	4.76 ± 0.01^{a}	4.18 ± 0.04^{b}	4.30 ± 0.28^{b}	0.010
Protein (g/100g)	3.61 ± 0.03^{b}	3.26 ± 0.11^{b}	5.81 ± 0.82^{a}	0.001
SCC (x10^3cells/ml)	104 ± 2.65	798 ± 365	1035 ± 1402	0.421
Total solids (%)	3.31 ± 0.06^{b}	12.06 ± 0.14^{b}	17.51 ± 1.35^{a}	0.001
SFA (g/100g)	2.75 ± 0.02^{b}	2.69 ± 0.22^{b}	4.32 ± 0.28^{a}	0.001
UFA (g/100g)	1.24 ± 0.01^{b}	0.98 ± 0.11^{b}	2.17 ± 0.24^{a}	0.001
MUFA (g/100g)	0.92 ± 0.01^{b}	0.62 ± 0.10^{b}	1.56 ± 0.27^{a}	0.001
PUFA (g/100g)	0.13 ± 0.00^{b}	0.18 ± 0.01^{b}	0.43 ± 0.03^{a}	0.001
C14:0 (g/100g)	0.46 ± 0.00^{b}	0.56 ± 0.08^{ab}	0.73 ± 0.08^{a}	0.008
C16:0 (g/100g)	1.05 ± 0.01	0.97 ± 0.11	1.14 ± 0.21	0.384
C18:0 (g/100g)	0.68 ± 0.01^{b}	0.52 ± 0.05^{b}	1.46 ± 0.11^{a}	0.001
C18:1C9 (g/100g)	0.75 ± 0.02^{b}	0.39 ± 0.09^{b}	1.14 ± 0.24^{a}	0.002
pH	6.63 ± 0.26^{a}	6.61 ± 0.05^{a}	6.41 ± 0.03^{b}	0.001

Mean values ± standard deviation. Means within a row that do not share a letter are significantly different. $p<0.05$ is considered significant. Biological replicates for each milk type $n=3$. Abbreviations: SFA=saturated fatty acids, UFA=unsaturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids, SCC=somatic cell count, C14:0=myristic acid, C16:0=palmitic acid, C18:0=stearic acid, C18:1C9=oleic acid.

4.2 Casein, casein number, and whey measurements

Average values of casein content (g/100g of milk) from three different batches of cow, goat, and sheep milk in combination with four rennets are shown in Table 3. The differences between batches within a specific rennet type are evaluated row-wise. The effect of rennets on each milk type is evaluated column-wise. The casein content was calculated by subtracting the whey protein from the total milk protein. Sheep milk, independent of rennet type, showed the highest casein content (p=0.001) compared to cow and goat milk, where the casein was 52% and 80% lower, respectively. No significant effect of rennets within the milk types was observed.

Table 3 . Casein amount in cow, goat, and sheep milks, in combination with rennet types

Rennet type	**Cow milk**	**Goat milk**	**Sheep milk**	**P-value**
	Casein (g/100g)	**Casein (g/100g)**	**Casein (g/100g)**	
Chymosin	2.69 ± 0.04^{b}	2.29 ± 0.11^{b}	4.11 ± 0.54^{a}	0.001
Pepsin	2.70 ± 0.04^{b}	2.30 ± 0.12^{b}	4.10 ± 0.55^{a}	0.001
Lamb	2.68 ± 0.04^{b}	2.26 ± 0.16^{b}	4.07 ± 0.54^{a}	0.001
Kid	2.69 ± 0.03^{b}	2.26 ± 0.11^{b}	4.03 ± 0.56^{a}	0.001
P-value	0.953	0.962	0.998	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. $p<0.05$ is considered significant. Mean values ± standard deviation are based on three biological replicates where each biological replicate was analyzed three times (n=9).

Average values for casein number (%) from three different batches of cow, goat, and sheep milk in combination with four rennet types are shown in Table 4. The differences between milk types within a specific rennet type are evaluated row-wise. The effect of the rennets on each milk type is evaluated column-wise. The effect of milk type on casein number showed that cow milk had the highest casein number whereas there was no difference between sheep and goat milk. In combination with chymosin, cow milk showed 6% and 5% higher casein numbers than goat and sheep milk (p = 0.009). In combination with pepsin, the casein number in cow milk was 5% higher than in goat and sheep milk (p = 0.015). No effects were observed between the milk types when lamb rennet was used. In combination with kid rennet, the casein number in cow milk was 7% higher compared to that of goat and sheep milk (p=0.004). No significant differences between the rennets within each specific milk type were observed.

Table 4. Casein number in cow, goat, and sheep, in combination with rennet types

Rennet type	Cow milk	Goat milk	Sheep milk	P-value
	Casein no. (%)	Casein no. (%)	Casein no. (%)	
Chymosin	74.60 ± 0.53^a	70.32 ± 1.45^b	70.74 ± 1.42^b	0.009
Pepsin	74.70 ± 0.83^a	70.62 ± 1.70^b	70.67 ± 1.36^b	0.015
Lamb	74.24 ± 0.81	69.38 ± 3.09	70.09 ± 1.41	0.051
Kid	74.42 ± 0.39^a	69.20 ± 1.28^b	69.31 ± 1.83^b	0.004
P-Value	0.835	0.781	0.652	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. p<0.05 is considered significant. Mean values ± standard deviation are based on three biological replicates where each biological replicate was analyzed three times (n=9).

Average values for whey protein (g/100g) for the three different batches of cow, goat, and sheep milk in combination with four types of rennet are shown in Table 5. The differences between batches within a specific type of rennet are evaluated row-wise. The effect of rennets on each milk type is evaluated column-wise. The whey protein was obtained after the milk coagulation using the four different rennets. The effect of milk type on whey protein showed that sheep milk had the highest- and cow milk had the lowest levels in general. In combination with chymosin, sheep milk has 43% and 46% higher whey protein than goat and cow milk (p=0.002). In combination with pepsin, sheep milk showed 44% and 47% higher whey protein than goat and cow milk (p=0.002). In combination with lamb rennet, sheep milk had 42% and 46% higher whey protein than goat and cow milk (p=0.002). In combination with kid rennet, sheep milk showed 44% and 49% higher whey amounts than goat and cow milk (p=0.001). No significant effect from the rennets on the milk types was observed.

Table 5. Whey protein in milk types: cow, goat, and sheep, in combination with rennet types

Rennet type	Cow milk	Goat milk	Sheep milk	P-value
	Whey (g/100g)	Whey (g/100g)	Whey (g/100g)	
Chymosin	0.92 ± 0.01^b	0.97 ± 0.03^b	1.71 ± 0.29^a	0.002
Pepsin	0.91 ± 0.03^b	0.96 ± 0.03^b	1.71 ± 0.28^a	0.002
Lamb	0.93 ± 0.03^b	1.00 ± 0.08^b	1.74 ± 0.30^a	0.002
Kid	0.92 ± 0.01^b	1.00 ± 0.02^b	1.79 ± 0.30^a	0.001
P-value	0.794	0.566	0.985	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. p<0.05 is considered significant. Mean values ± standard deviation are based on three biological replicates where each biological replicate was analyzed three times (n=9).

4.3 Curd yield: effects of milk batches and effects of rennets

Average values for curd yield (g/100g of milk) from three different batches of goat milk in combination with four different rennet types are shown in Table 7. The differences between batches within a specific rennet type are evaluated row-wise. The effect of rennets on each milk batch is evaluated column-wise. Cow milk batches two and three showed 10% and 8 % higher curd yield, respectively, in combination with lamb rennet compared to batch one (p=0.001). In combination with kid rennet, batch one showed 7% and 11% lower curd yields than milk batches two and three (p=0.001). Chymosin and pepsin showed no significant effects on the curd yields between the three cow milk batches. Within the milk batches (column-wise), the effects of the rennets observed in batch two showed that the curd yield was 6% higher when lamb rennet was used, compared to both chymosin and pepsin and 8% higher with the kid rennet (p=0.002). No significant differences between any of the four rennets were observed in cow milk batches one and three.

Table 6. Curd yield (g/100g milk) from three batches of cow's milk in combination with rennet types

Rennet Type	**Curd yield (g/100g)**			**P-value**
	Cow milk 1	**Cow milk 2**	**Cow milk 3**	
Chymosin	44.61 ± 7.29	44.96 ± 3.37^{2}	47.39 ± 2.64	0.185
Pepsin	44.51 ± 5.50	44.74 ± 2.66^{2}	46.52 ± 3.29	0.264
Lamb	42.87 ± 4.55^{b}	47.71 ± 2.42^{a1}	46.56 ± 3.19^{a}	0.001
Kid	40.70 ± 5.09^{b}	43.88 ± 3.27^{a2}	45.71 ± 2.72^{a}	0.001
P-value	0.146	0.002	0.413	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Mean values ± standard deviation. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. p<0.05 is considered significant. Each value is the average of 6 technical replicates (n=6).

Average values for curd yield (g/100g of milk) from three different batches of goat milk in combination with four different rennet types are shown in Table 7. The differences between batches within a specific rennet type are evaluated row-wise. The effect of rennets on each milk batch is evaluated column-wise. In combination with lamb rennet, goat milk batch three exhibited 8% and 13% higher curd yield than batches one and two, respectively (p=0.004). In combination with kid rennet, milk batch three had an 18% and 27% higher curd yield than milk batches one and two (p=0.001). Chymosin and pepsin showed no significant effects on the curd yield between the three goat milk batches. Within goat milk batches, the effect of rennets showed that the curd yield in batch one was 13% higher when lamb rennet was used than chymosin and pepsin and 31% higher than kid rennet (p=0.001). The curd yield in batch two when kid rennet was used was 24%, 27%, and 35% lower than for pepsin, chymosin, and lamb rennet (p=0.001). There were no significant differences between chymosin and pepsin in batches one and two. When lamb rennet was used in batch three, the curd yield was 22%, 23%, and 26% higher compared to goat, pepsin, and chymosin rennet (p=0.001). Batch three showed no significant differences between chymosin, pepsin, and kid rennet.

Table 7. Curd yield (g/100g milk) from three batches of goat's milk in combination with rennet types

Rennet type	**Curd yield (g/100g)**			**P-value**
	Goat milk 1	**Goat milk 2**	**Goat milk 3**	
Chymosin	33.82 ± 7.16^{2}	33.09 ± 4.18^{2}	31.39 ± 5.57^{2}	0.448
Pepsin	33.59 ± 5.72^{2}	31.79 ± 5.65^{2}	32.58 ± 8.76^{2}	0.878
Lamb	38.92 ± 2.84^{b1}	37.00 ± 1.31^{b1}	42.42 ± 2.71^{a1}	0.004
Kid	26.98 ± 1.92^{b3}	24.02 ± 0.84^{b3}	32.92 ± 14.23^{a2}	0.001
P-value	0.001	0.001	0.001	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Mean values ± standard deviation. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. p<0.05 is considered significant. Each value is the average of 6 technical replicates (n=6).

Average values for curd yield (g/100g of milk) from three different batches of sheep milk in combination with four different rennet types are shown in Table 8. The differences between batches within each specific rennet type are evaluated row-wise. The effect of rennets on each of the milk batches is evaluated column-wise. In combination with chymosin, sheep milk batch two showed 23% and 30% higher curd yield than batches three and one (p=0.001). In combination with pepsin, batch two had a 16% and 24% higher curd yield than batches three and one (p=0.001). In combination with lamb rennet, milk batches two and one showed a 6% and 9% lower curd yield than batch three. In combination with kid rennet, the milk from batch one showed 28% and 29% lower curd yield than in batches three and two, respectively (p=0.001). Within sheep milk batches, the effects from rennets showed that the curd yield in batch one was 16%, 20%, and 32% lower when kid rennet was used compared to pepsin, chymosin, and lamb rennet (p=0.001). There were no significant differences between chymosin and pepsin in batch one. When using chymosin, the curd yield in batch two was 12%, 16%, and 21% higher than when pepsin, lamb, and kid rennet were used (p=0.001). No significant differences were observed between chymosin and pepsin or between lamb and kid rennet in batch two. When using lamb rennet, the curd yield in batch three was 14% higher than when chymosin and kid rennet were used and 18% higher than pepsin (p=0.001). Batch three showed no significant differences in curd yield when chymosin, pepsin, and kid rennet were used.

Table 8. Curd yield (g/100g milk) from three batches of sheep's milk in combination with rennet types

Rennet type	**Curd yield (g/100g)**			**P-value**
	Sheep milk 1	**Sheep milk 2**	**Sheep milk 3**	
Chymosin	42.51 ± 2.94^{b2*}	60.73 ± 9.69^{a1}	46.66 ± 2.11^{b2}	0.001
Pepsin	40.48 ± 2.12^{b2*}	53.46 ± 9.27^{a1}	44.86 ± 2.14^{b2}	0.001
Lamb	49.54 ± 1.08^{b1*}	51.04 ± 3.78^{b2}	54.58 ± 1.77^{a1}	0.001
Kid	33.89 ± 6.38^{b3*}	47.82 ± 12.83^{a2}	46.90 ± 12.87^{a2}	0.001
P-value	0.001	0.001	0.001	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Mean values ± standard deviation. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. $p<0.05$ is considered significant. Each value is the average of 6 technical replicates ($n=6$, except for $n^{*}=4$).

4.4 Curd yield: effects of milk types and effects of rennets

Average values of curd yield (g/100g of milk) from three different batches of cow, goat, and sheep milk in combination with four rennets are shown in Table 9. The differences between batches within a specific rennet type are evaluated row-wise. The effect of rennets on each milk type is evaluated column-wise. In combination with chymosin, the curd yield from the sheep milk was 9% and 35% higher than from cow and goat milk (p=0.001). In combination with pepsin, goat milk showed a 28% and 30% lower curd yield than cow and sheep milk (p=0.001). Together with lamb rennet, sheep milk exhibited a 12% and 24% higher curd yield than cow and goat milk (p=0.001). Together with kid rennet, goat milk had a 37% lower curd yield than cow and sheep milk (p=0.001). Within the milk types, the curd yield was 5% higher in cow milk when chymosin and lamb rennet were used compared to kid rennet (p=0.021). There was no significant difference between chymosin, pepsin, and lamb rennet and between pepsin and kid rennet in cow milk. When using kid rennet, the curd yield in goat milk was 15% and 29% lower than when using chymosin and lamb rennet (p=0.001). There were no significant differences between chymosin and pepsin in goat milk. The curd yield in sheep milk was 10% and 17% higher when lamb rennet was used compared to pepsin and kid rennet (p=0.001). No significant differences in curd yield were observed in sheep milk between chymosin and pepsin, chymosin and lamb rennet, and between pepsin and kid rennet.

Table 9. Curd yield (g/100g milk) in cow, goat, and sheep milks, in combination with rennet types

Rennet type	**Curd yield (g/100g)**			**P-value**
	Cow milk	**Goat milk**	**Sheep milk**	
Chymosin	45.65 ± 4.95^{b1}	32.77 ± 5.75^{c2}	$50.25 \pm 9.87^{a12*}$	0.001
Pepsin	45.26 ± 4.03^{a12}	32.65 ± 6.77^{b2}	$46.49 \pm 7.80^{a23*}$	0.001
Lamb	45.72 ± 4.02^{b1}	39.45 ± 3.26^{c1}	$51.81 \pm 3.27^{a1*}$	0.001
Kid	43.43 ± 4.29^{a2}	27.98 ± 8.96^{b3}	$43.22 \pm 12.72^{a3*}$	0.001
P-value	0.021	0.001	0.001	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. p<0.05 is considered significant. Mean values ± standard deviation are based on three biological replicates where each biological replicate was analyzed six times (n=18 except for n=16).*

4.5 Rheological measurements

4.5.1 Coagulation time: effects of milk batches and effects of rennets

Average values of coagulation time (seconds) for the three different batches of cow milk in combination with four different rennet types are shown in Table 10. The differences between batches within a specific rennet type are evaluated row-wise. The effect of rennets on each milk batch is evaluated column-wise. Cow milk batch one showed 13% longer coagulation time when chymosin was used compared to cow milk batch two (p=0.044). In combination with pepsin, cow milk batch one showed 11% longer coagulation time than batch two (p=0.017). Using chymosin and pepsin showed no significant difference in coagulation time between batches one and three and between batches two and three. In combination with lamb rennet, batch two had 12% and 13% shorter coagulation time than batches three and one, respectively (p=0.015). No effects were observed between cow milk batches in combination with kid rennet. Within the cow milk batches, the effect of lamb rennet on coagulation time showed that batch one was 9%, 25%, and 78% longer than with chymosin, pepsin, and kid rennet (p=0.001). When using kid rennet, the coagulation time in batch two was 64%, 70%, and 72% shorter than when pepsin, chymosin, and lamb rennet were used (p=0.001). There was no significant difference between chymosin and lamb rennet in batch two. When using lamb rennet, the coagulation time in batch three was 30% and 75% longer compared to pepsin and kid rennet (p=0.001). There was no significant difference between chymosin and lamb rennet in batch three.

Table 10. Coagulation time (seconds) from three batches of cow's milk in combination with rennet types

Rennet type	Coagulation time (sec)			P-value
	Cow milk 1	Cow milk 2	Cow milk 3	
Chymosin	426.75 ± 14.97^{a2}	373.25 ± 17.31^{b1}	415.00 ± 39.80^{ab1}	0.044
Pepsin	354.00 ± 20.90^{a3}	316.50 ± 13.18^{b2}	325.25 ± 9.00^{ab2}	0.017
Lamb	470.00 ± 11.40^{a1}	406.50 ± 25.40^{b1}	464.30 ± 37.10^{a1}	0.015
Kid	101.75 ± 6.60^{4}	113.75 ± 8.54^{3}	113.80 ± 23.60^{3}	0.457
P-value	0.001	0.001	0.001	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. p<0.05 is considered significant. Mean values ± standard deviation are based on 4 technical replicates for each milk batch (n=4).

Average values for coagulation time (seconds) from three different batches of goat milk in combination with four different rennet types are shown in Table 11. The differences between batches within a specific rennet type are evaluated row-wise. The effect of rennets on each milk batch is evaluated column-wise. The effect of the goat milk batches on coagulation time when chymosin was used showed that batch three was 29% longer than batch two (p=0.032). In combination with pepsin, batch three was 26% and 33% longer than batches one and two (p=0.001). In combination with lamb rennet, batch three indicated a 36% and 41% longer time than batches one and two (p=0.001). In combination with kid rennet, batch three showed a 59% and 73% longer coagulation time than batches one and two (p=0.001). Within goat milk batches, lamb rennet's effect on coagulation time showed that batch one was 43%, 49%, and 76% longer than with chymosin, pepsin, and kid rennet (p=0.001). When using kid rennet, the coagulation time in batch two was 66%, 69%, and 83% shorter than when pepsin, chymosin, and lamb rennet were used (p=0.001). There were no significant differences between chymosin and pepsin in batches one and two. The coagulation time in batch three was 54%, 56%, and 63% longer when lamb rennet was used compared to chymosin, pepsin, and kid rennet (p=0.001). Batch three showed no significant differences between chymosin, pepsin, and kid rennet.

Table 11. Coagulation time (seconds) from three batches of goat's milk in combination with rennet types

Rennet type	Coagulation time (sec)			P-value
	Goat milk 1	Goat milk 2	Goat milk 3	
Chymosin	192.75 ± 8.22^{ab2}	171.00 ± 5.16^{b2}	240.80 ± 53.30^{a2}	0.032
Pepsin	172.50 ± 4.65^{b2}	155.75 ± 1.89^{b2}	234.00 ± 24.20^{a2}	0.001
Lamb	336.50 ± 12.18^{b1}	311.50 ± 27.90^{b1}	527.00 ± 29.00^{a1}	0.001
Kid	79.80 ± 58.20^{b3}	52.50 ± 4.51^{b3}	194.75 ± 9.18^{a2}	0.001
P-value	0.001	0.001	0.001	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. p<0.05 is considered significant. Mean values ± standard deviation are based on 4 technical replicates for each milk batch (n=4).

Average values for coagulation time (seconds) from three different batches of sheep milk in combination with four different rennet types are shown in Table 12. The differences between batches within a specific rennet type are evaluated row-wise. The effect of rennets on each milk batch is evaluated column-wise. The coagulation time in batch three, when chymosin was used, showed a 22% and 23% longer coagulation time compared to batches one and two (p=0.004). In combination with lamb rennet, milk batch three showed a 33% and 36% longer coagulation time than batches one and two (p=0.001). There were no observed effects between the three milk batches when pepsin and kid rennet were used. Within the sheep milk batches, kid rennet's effect on coagulation time showed that batch one was 42%, 44%, and 54% shorter than pepsin, chymosin, and lamb rennet (p=0.001). When using kid rennet, the coagulation time in batch two was 50%, 51%, and 58% shorter when chymosin, pepsin, and lamb rennet were used (p=0.001). There were no significant differences between chymosin, pepsin, and lamb rennet in batches one and two. When using lamb rennet, the coagulation time in batch three was 29%, 39%, and 68% longer compared to chymosin, pepsin, and kid rennet (p=0.001). There was no significant difference between chymosin and pepsin rennet in batch three.

Table 12. Coagulation time (seconds) from three batches of sheep's milk in combination with rennet types

Rennet type	**Coagulation time (sec)**			**P-value**
	Sheep milk 1	**Sheep milk 2**	**Sheep milk 3**	
Chymosin	148.67 ± 5.51^{b1*}	147.75 ± 5.85^{b1}	190.80 ± 21.00^{a2}	0.004
Pepsin	142.70 ± 20.80^{1*}	148.50 ± 10.15^{1}	164.75 ± 5.68^{2}	0.107
Lamb	179.67 ± 11.02^{b1*}	172.50 ± 5.92^{b1}	269.00 ± 12.03^{a1}	0.001
Kid	82.67 ± 17.04^{2*}	73.20 ± 31.70^{2}	87.00 ± 20.90^{3}	0.733
P-value	0.001	0.001	0.001	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. p<0.05 is considered significant. Mean values ± standard deviation are based on 4 technical replicates for each milk batch (n=4, except n=3).*

4.5.2 Coagulation time: effects of milk types and effects of rennets

Average values for coagulation time (seconds) from three different batches of cow, goat, and sheep milk in combination with four rennets are shown in Table 13. The differences between batches within a specific rennet type are evaluated row-wise.

The effect of rennets on each milk type is evaluated column-wise. The effect of milk type on coagulation time showed that cow milk with chymosin had 50% and 60% longer coagulation time than goat and sheep milk ($p=0.001$). In combination with pepsin rennet, cow milk displayed 44% and 54% longer coagulation times than goat and sheep milk ($p=0.001$). Sheep milk with lamb rennet exhibited 46% and 53% shorter coagulation times than goat and cow milk ($p=0.001$). There was no significant difference between cow and goat milk when lamb rennet was used. There were no observed significant differences between kid rennet and the milk types. Within the milk types, lamb rennet's effect on coagulation time in cow milk was 9%, 26%, and 54% longer than chymosin, pepsin, and kid rennet ($p=0.001$). The coagulation time in goat milk when lamb rennet was used was 48%, 52%, and 72% longer than that of chymosin, pepsin, and kid rennet ($p=0.001$). When using kid rennet, the coagulation time in sheep milk was 47%, 51%, and 61% shorter than when pepsin, chymosin, and lamb rennet were used ($p=0.001$). There were no significant differences between chymosin and pepsin in both goat and sheep milk.

Table 13. Coagulation time (seconds) in cow, goat, and sheep milks combined with rennet types

Rennet type	**Coagulation time (sec)**			**P-value**
	Cow milk	**Goat milk**	**Sheep milk**	
Chymosin	405.00 ± 33.90^{a2}	201.50 ± 41.60^{b2}	$163.64 \pm 24.72^{c2*}$	0.001
Pepsin	331.92 ± 21.65^{a3}	187.40 ± 37.40^{b2}	$152.82 \pm 14.91^{c2*}$	0.001
Lamb	446.90 ± 38.50^{a1}	391.70 ± 102.90^{a1}	$209.50 \pm 48.10^{b1*}$	0.001
Kid	109.75 ± 14.78^{4}	109.00 ± 71.40^{3}	$80.79 \pm 23.05^{3*}$	0.228
P-value	0.001	0.001	0.001	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. $p<0.05$ is considered significant. Mean values ± standard deviation are based on three biological replicates where each biological replicate was analyzed four times ($n=12$ except for $n^*=9$).

4.5.3 Gel firmness: effects of milk batches and effects of rennets

Average values for gel firmness (in Pascal) from three different batches of cow milk in combination with four different rennet types are shown in Table 14. The differences between batches within a specific rennet type are evaluated row-wise. The effect of rennets on each milk batch is evaluated column-wise. In combination with lamb rennet, cow milk batch two showed 11% and 12% higher gel firmness than batches one and three ($p=0.005$). Using chymosin, pepsin, and kid rennet resulted in no significant differences between the three batches of cow milk. Within cow milk batches, the effects from rennets showed that the gel firmness in batch one was 35% and 45%, higher when kid rennet was used compared to pepsin and lamb rennet (p=0.001). The gel firmness in batch three when kid rennet was used was 35% and 47% higher than pepsin and lamb rennet (p=0.001). There were no significant differences between the gel firmness when chymosin and lamb rennet were used in batches one and three. No significant differences were observed between any of the rennets in batch two of cow milk.

Table 14. Gel firmness (Pascal) from three batches of cow's milk in combination with rennet types

Rennet type	**Gel firmness (Pa)**			**P-value**
	Cow milk 1	**Cow milk 2**	**Cow milk 3**	
Chymosin	97.18 ± 5.05^{3}	93.11 ± 4.75	93.22 ± 7.49^{3}	0.560
Pepsin	110.72 ± 6.16^{2}	104.94 ± 6.53	114.29 ± 6.72^{2}	0.175
Lamb	93.76 ± 1.81^{b3}	105.84 ± 6.97^{a}	93.10 ± 3.09^{b3}	0.005
Kid	170.79 ± 8.04^{1}	131.70 ± 39.80	178.25 ± 9.35^{1}	0.048^{*}
P-value	0.001	0.110	0.001	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Mean values ± standard deviation. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. $p<0.05$ is considered significant. Technical replicates for each milk batch n=4. **No significant difference between groups, suggesting that the effect size (the magnitude of the difference) is small, having no practical impact.*

Average values for gel firmness (in Pascal) from three different batches of goat milk in combination with four different rennet types are shown in Table 15. The differences between batches within a specific rennet type are evaluated row-wise. The effect of rennets on each milk batch is evaluated column-wise. When chymosin was used, the gel firmness showed that batch three had a 32% and 35% lower gel firmness than batches two and one (p=0.001). In combination with pepsin, batch three had a 26% and 33% lower gel firmness than batches two and one (p=0.001). In combination with lamb rennet, batch three showed a 53% lower gel firmness compared to batches one and two (p=0.001). There were no observed significant differences in gel firmness between the kid rennet and the three milk batches. Within the goat milk batches, the effects from rennets showed that the gel firmness in batch one was 48% higher when chymosin and pepsin rennet were used compared to kid rennet (p=0.003). There were no significant differences between chymosin, pepsin, and lamb rennet and between lamb and kid rennet in batch one. When using kid rennet, the gel firmness in batch two was 47%, 51%, and 53% lower compared to lamb, pepsin, and chymosin rennet (p=0.001). Batch two showed no significant differences between chymosin, pepsin, and lamb rennet. When using lamb rennet, the gel firmness in batch three was 39%, 41%, and 51% lower than when chymosin, pepsin, and kid rennet were used (p=0.001). There were no significant differences between gel firmness when chymosin and pepsin were used in batch three.

Table 15. Gel firmness (Pascal) from three batches of goat's milk in combination with rennet types

Rennet type	**Gel firmness (Pa)**			**P-value**
	Goat milk 1	**Goat milk 2**	**Goat milk 3**	
Chymosin	137.94 ± 4.49^{a1}	131.51 ± 15.92^{a1}	89.56 ± 3.64^{b2}	0.001
Pepsin	138.87 ± 0.94^{a1}	126.13 ± 16.00^{a1}	92.97 ± 3.48^{b2}	0.001
Lamb	116.81 ± 5.16^{a12}	116.69 ± 4.66^{a1}	54.39 ± 7.71^{b3}	0.001
Kid	71.30 ± 43.10^{2}	61.38 ± 7.67^{2}	110.72 ± 3.05^{1}	0.050
P-value	0.003	0.001	0.001	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Mean values ± standard deviation. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. p<0.05 is considered significant. Technical replicates for each milk batch n=4.

Average values for gel firmness (in Pascal) from three different batches of sheep milk in combination with four different rennet types are shown in Table 16. The differences between batches within a specific rennet type are evaluated row-wise. The effect of rennets on each milk batch is evaluated column-wise. The gel firmness on sheep milk batches, when chymosin was used showed that batch two had a 55% and 19% higher gel firmness than batches one and three (p=0.001). When pepsin was used, batch two displayed 57% and 24% higher gel firmness compared to batches one and three (p=0.001). The gel firmness in batch two was 59% and 27% higher than in batches one and three when lamb rennet was used (p=0.001). There were no significant differences in gel firmness between the milk batches when the kid rennet was used. Within sheep milk batch one, there were no significant differences between the rennets. Within sheep milk batch two, the effects from rennets showed that the gel firmness was 34% and 40% lower when kid rennet was used compared to chymosin and pepsin rennet (p=0.005). There were no significant differences between chymosin, pepsin, and lamb rennet and between kid and lamb rennet in batch two. The gel firmness was 39%, 48%, and 49% lower when kid rennet was used, compared to chymosin, pepsin, and lamb rennet in batch three (p=0.001). Batch three showed no significant difference between chymosin, pepsin, and lamb rennet.

Table 16. Gel firmness (Pascal) from three batches of sheep's milk in combination with rennet types

Rennet type	**Gel firmness (Pa)**			**P-value**
	Sheep milk 1	**Sheep milk 2**	**Sheep milk 3**	
Chymosin	345.07 ± 7.11^{c*}	760.51 ± 12.46^{a1}	616.20 ± 12.90^{b1}	0.001
Pepsin	361.91 ± 7.93^{c*}	839.40 ± 100.20^{a1}	635.69 ± 16.56^{b1}	0.001
Lamb	298.96 ± 7.20^{c*}	720.80 ± 37.60^{a12}	524.69 ± 15.97^{b1}	0.001
Kid	270.10 ± 160.50*	504.10 ± 184.00^{2}	322.30 ± 112.20^{2}	0.160
P-value	0.519	0.005	0.001	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Mean values ± standard deviation. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. p<0.05 is considered significant. Technical replicates for each milk batch n=4,except for n=3.*

4.5.4 Gel firmness: effects of milk types and effects of rennets

Average values for gel firmness (in Pascal) from three different batches of cow, goat, and sheep milk in combination with four different rennet types are shown in Table 17. The differences between batches within a specific rennet type are evaluated row-wise. The effect of rennets on each milk type is evaluated column-wise. The gel firmness when chymosin was used in sheep milk showed 84% and 80% higher gel firmness than in cow and goat milk (p=0.001). When pepsin was used, sheep milk displayed 83% and 81% higher gel firmness than cow and goat milk (p=0.001). In combination with lamb rennet, sheep milk exhibited an 82% higher gel firmness than cow and goat milk (p=0.001). In combination with kid rennet, goat milk showed 49% and 78% lower gel firmness when compared to cow milk and sheep milk (p=0.001). Within the milk types, the effects of rennets showed that gel firmness when kid rennet was used in cow milk was 31%, 39%, and 41% higher compared to pepsin, lamb, and chymosin rennet (p=0.001). There were no significant differences between chymosin, pepsin, and lamb rennet in cow milk. Kid rennet with goat milk had a 32% lower gel firmness than chymosin and pepsin (p=0.002). The gel firmness was 37% and 41% lower in sheep milk when kid rennet was used compared to chymosin and pepsin rennet (p=0.010). There were no significant differences between chymosin, pepsin, and lamb rennet and between lamb and kid rennet in goat and sheep milk.

Table 17. Gel firmness (Pascal) in cow, goat, and sheep milk, combined with rennet types

Rennet type	Gel firmness (Pa)			P-value
	Cow milk	Goat milk	Sheep milk	
Chymosin	94.50 ± 5.68^{b2}	119.67 ± 24.09^{b1}	$594.70 \pm 173.20^{a1*}$	0.001
Pepsin	109.98 ± 7.10^{b2}	119.32 ± 21.94^{b1}	$635.10 \pm 205.40^{a1*}$	0.001
Lamb	97.57 ± 7.36^{b2}	95.96 ± 31.18^{b12}	$534.50 \pm 176.30^{a12*}$	0.001
Kid	160.24 ± 30.47^{b1}	81.14 ± 31.93^{b2}	$374.20 \pm 173.70^{a2*}$	0.001
P-value	0.001	0.002	0.010	

Differences between milk types within rennets are evaluated row-wise. The effect of rennets on milk types are evaluated column-wise. Means within a row that do not share a letter are significantly different. Means within a column that do not share a number are significantly different. p<0.05 is considered significant. Mean values ± standard deviation are based on three biological replicates where each biological replicate was analyzed four times (n=12,except for $n^{*}=9$).

5. Discussion

5.1 Milk gross composition

The aim of this study was to evaluate conventional rennets with rennets from Swedish ruminants, kids, and lambs in combination with species-specific milk from Swedish dairy farms. In this study, mini cheese production, rheological measurements (coagulation time and gel firmness), and gross composition were statistically evaluated and compiled.

Components in milk, such as fat, protein, lactose, and fatty acids, can affect everything from curd yield to coagulation and textural properties (Harris et al. 1998). Evaluating the ruminant's milk gross composition is necessary and beneficial because the raw milk components influence the production process and the finished cheese's characteristics (Priyashantha et al. 2021). Concerning the gross composition of the milk types, the fat and protein content, pH, and density agreed with the study of Park et al. (2007). However, the lactose content in cow milk in this study deviated from Park et al. (2007). A potential cause of the goat milk's lower lactose levels in this study could be its relatively high SCC. Higher SCCs are connected to reduced biosynthesis associated with lower lactose and more protein breakdown (Bobbo et al. 2016). The sheep milk's SCC had the highest value at 1034 ± 1402 $x10^3$cells/ml. However, the large standard deviation indicates variation between batches. This can probably be due to individual animals contributing to the milk collections during these three occasions. The limit for SCCs for sheep and goat milk is 450,000 cells/ml (Commission Regulation (EC) 853/2004 2004).

5.2 Casein, casein number, and whey protein

High casein concentrations benefit curd yield and thus contribute to efficient cheese production. Lower casein content makes cheese production less favorable, reducing the amount of cheese produced (Högberg et al. 2016). The results of this study showed that sheep milk had the highest average casein content and the highest curd yield, while goat milk had the lowest casein content and the lowest curd yield (see Tables 3 and 9). It can be concluded that casein content affected the curd yield, which was in agreement with Högberg et al. (2016). The whey protein content in sheep milk was the highest in this study, and the curd made from this milk had the highest gel firmness (see Tables 4 and 17). This was in agreement with Warncke et al. (2022), who showed that higher amounts of whey proteins can increase the firmness of the gel.

5.3 Curd yield

Milk batches and milk types within rennet types

Protein, fat, and calcium are essential components in cheese yield, yet caseins are considered vital in cheese production. A consequence of high amounts of fat is that the curd is less likely to contract, resulting in higher yields. The proteins in milk are primarily made up of caseins. Low protein amounts indicate low casein levels, which lead to decreased yields (Walstra et al. 2006). It was observed in this research that the highest curd yield was connected to the highest fat amount in cow milk in batches two and three with lamb rennet, and low protein content resulted in the lowest curd yield in batch one using kid rennet (see Tables 6 and A1). This was also observed in sheep milk batches, where batch two with chymosin and the highest fat content was associated with the highest curd yield. Batch one with kid rennet had a lower total protein, corresponding with the lowest curd yield, as shown in Tables 8 and A3. Regarding all three milk types (Table 9), the highest fat amount was associated with high curd yield, and the lowest protein amount was related to the lowest curd yields (see Table 1). The effects of milk's high fat and low protein contents in this study agree with the conclusions of Walstra et al. (2006). As the fat and protein levels were continuously related to curd yield in this study, these factors could be considered the key determinants of yield outcomes. This is in agreement with Mona et al. (2011), a study that showed that milk composition is related to cheese yield, especially fat and protein quantities. However, goat milk in batch two with lamb rennet contradicts this study. The highest curd yield was in batch three, even though batch two had the highest fat and casein content (see Table 7). One factor to consider could be that the SCC for batch two was high at 1219 x10^3cells/ml, compared to batch three at 595 x10^3cells/ml (Table A2). A higher amount of SCC decreases the caseins, which lowers the cheese yield (McSweeney 2007). Consequently, the deviating quality of the goat milk in batch two may have influenced its lower curd yield despite its high-fat values.

Rennets within milk batches and milk types
The amount of fat and protein in all three types of milk- cow, goat, and sheep- and the enzymatic properties of the rennet influenced the curd yields in this study. Different rennets have different milk clotting proteolytic activity ratios between, e.g., chymosin and pepsin, which can impact yields (McSweeney 2007). Kid rennet with goat milk resulted in the lowest curd yield among all milk types and within most individual milk batches. This might suggest that the proteolytic enzymatic properties of the kid rennet and the milk's low fat and protein levels lead to the least favorable curd yields.

5.4 Coagulation time

Milk batches and milk types within rennet types
Coagulation speed is influenced by the pH in milk, where lower pH levels can result in faster coagulation, and higher pH values can slow coagulation (Nájera et al. 2003). Regarding the coagulation time with significant differences, goat milk batch three had the longest CT and the highest pH values. Goat milk batch two was associated with the shortest CT and the lowest pH values (see Tables 11 and A2). Coagulation time is faster at a lower pH, while a higher pH can result in slower coagulation (McSweeney 2007). This was observed in this study, where the sheep milk batch three with lamb rennet had the longest CT and the highest pH (see Tables 12 and A3). Comparing the three milk types -cow, goat, and sheep-, the longest coagulation time was observed in cow and goat milk with lamb rennet, 446 and 391 seconds, respectively, with the highest average pH of 6.63 and 6.61. This is to be compared with sheep milk, which had the shortest CT of 209 seconds and the lowest milk pH of 6.41 (See Tables 13 and 2). As the coagulation time can be linked to the pH levels in this study, it can be considered one of the main influencing factors, which is supported by the findings of McSweeney (2007) and Nájera et al. (2003). It is important to note that other factors, such as the amount of protein, specifically casein and fat, can also be associated with the coagulation rate.

Rennets within milk batches and milk types
The research results revealed that lamb rennet had the longest coagulation times across all milk types, while kid rennet had the shortest coagulation times, which was only significant when used in goat milk batches. These results could offer insights into the characteristics of the innovative goat and lamb rennets, although more analysis is needed to make these distinctions.

5.5 Gel firmness

Milk batches and milk types within rennet types and rennets within milk batches and milk types

When measuring firmness, the amount of casein can influence rheological properties. The proteins in the cheese can affect and create the cheese texture. The content of caseins can also determine its internal structure (Park 2007). Higher gel firmness (GF) values can also be associated with higher casein percentages. When curd firmness is increased, fewer caseins are lost in the serum (Mona et al. 2011). Batch three of goat milk with pepsin rennet showed lower GF results and lower casein amounts, while batch one with pepsin rennet had the highest GF and the highest casein amounts. Goat milk batch three with lamb rennet had the lowest overall GF, which corresponded with the lowest amount of casein (see Tables 15 and A2). This study regularly connected the highest and lowest casein amounts with the highest and lowest gel firmness, which agrees with both Park (2007) and Mona et al. (2011) studies. Conversely, the cow milk batches were not in agreement with these authors as there was no association between the amount of casein and GF. However, this research might, in general, draw a connection between the coagulation time and the gel firmness. Coagulation time is strongly associated with gel firmness, indicating that it is an important determinant (Johansson et al. 2015). Rapid coagulation causes the curds to form rapidly, which makes the curd firmer and the gel stronger (Malchiodi et al. 2014). Although the amounts of casein can influence gel textures, the amounts of fat can also contribute to its strength. The higher amounts of saturated fatty acids might have impacted the final cheese product as these fatty acids in milk can be associated with a firmer cheese texture (Bonanno et al. 2016). In this study, it was observed that sheep milk had the highest levels of saturated fat, which was associated with the highest gel firmness (see Tables 17 and A3). It is important to note that factors influencing the fatty acid variables in milk are based on the feed and can be changed (Jensen 2002). It is known that the amounts of fat and protein can affect the gel firmness (Mateo et al. 2009). Also, sheep milk's whey protein content (g/100g) was the highest in this study, and higher amounts of whey proteins can increase firmness (Warncke et al. 2022). The factors that affected the gel firmness in this study were the pH, amounts of casein and whey, coagulation time, and amounts of saturated fatty acids.

6. Conclusion

The results of this study showed that both the composition of the milk, i.e., its fat and protein content, and pH, as well as the enzymatic properties of the innovative rennets, influenced the curd yield, coagulation time, and gel firmness. Milk pH values impacted the coagulation times. While the impacts from goat and lamb rennet in all milk types showed strong regularities in coagulation times, more research needs to be done. Gel firmness was associated with the casein and whey protein content, the coagulation time, and the levels of saturated fatty acids. The milk and rennet combinations observed in this study that could be considered least favorable in cheese production were goat milk with kid rennet due to its low curd yield. Sheep milk with pepsin resulted in a high gel firmness, which might be unwanted in the manufacture of a specific cheese. The data from this research could contribute to certain optimization of the production of Swedish artisanal cheeses if the innovative rennets were commercially available.

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Popular science summary

Are you interested in the methods of making cheese and the role that artisanal production of animal rennets has in preserving traditional cheesemaking in Sweden? Most of us have eaten cheese in flavors ranging from ordinary to spectacular. Cheese is unique to the country in which it is produced, the animal from which it is made, and even the animal rennet used. Each type of animal's milk has a unique flavor and texture. All over the world, countries offer a unique taste of their regions in their cheeses. A small group of artisanal cheesemakers, rennet producers, dairy farmers, meat processors, and university professors in Sweden have begun to offer the taste of Sweden through cheese by producing their animal rennet (kid and lamb).

Why should we still produce cheese with animal rennet when so many other alternatives exist? Well, it is a natural method of coagulating milk, and it has been used for thousands of years and is a traditional ingredient in many kinds of cheese. Animal rennet contributes to a unique flavor that cannot be reproduced with other types of rennet. It also uses animal parts that would otherwise be discarded, thus supporting traditional cheesemaking and sustainability.

During this study, mini cheeses were produced from rennet made from Swedish lambs and kids. The tests included determining how much curd yield could be obtained from one type of milk using different types of rennet. The milk composition was analyzed, which involved finding the amounts of fat, protein, lactose, pH, and some major fatty acids to understand its influence on cheese production. Other tests included determining the curd's firmness, i.e., the texture of the curd, and the time required for the cheese to curdle or coagulate. It was concluded that sheep's milk had the highest content of protein and fat, contributing to higher yields. Other results concluded that the pH levels and the casein and fat content could have impacted the coagulation times in this study. Evaluating the coagulation time suggested that the lamb's rennet may have caused a slower coagulation time, whereas the goat's rennet accelerated it. Curd firmness was affected by the contents of casein, whey protein, and fat, and was related to coagulation times.

Acknowledgments

First, I would like to thank Monika Johansson, my supervisor, who was patient and kind and who helped me with every question I had during the laboratory exercises and the entire writing process. I would also like to thank Anna Karin Gidlund, Carl Johan Granqvist, and Cecilia Ebbesson for providing the milk for the project. Thank you also to Anders Olsson for manufacturing the innovative rennets and Sveriges Gårdsmejerister for their general support. I want to dedicate this to my Mom and Dad, Marie and Juan Treviño, who are not here to see this day. You both always emphasized how important an education was, and it resonated with me when it was time to change careers midway through my life. I would also like to thank my Mother-in-law, Maj-Britt Bengtsson, who passed away around the same time as my Mother. Thank you for your hours at the kitchen table and always helping me with my Swedish homework. Sadly, you are not here either to celebrate my academic accomplishment, but you helped me to reach this point. I would like to thank my children, Liam and Zoe, for enduring the sacrifice of having a Mom who mostly had to stay home and study while life was happening. I love you both to the moon and back! Remember to work hard for your dreams, never give up, and always show up for yourself. Lastly, my husband, Tomas, you are my rock and hero. I am lucky to have you. Thank you for the support and the late-night laboratory pickups from Stockholm to Uppsala and back to Stockholm. I could not have done this without your support. I love you.

The project was financially supported by the Swedish Board of Agriculture and the European Agricultural Fund for Rural Development (Journal. Nr. 2019-170-1).

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Appendix 1: Milk gross composition

The milk gross composition of the three batches of cow milk, goat milk, and sheep milk are shown in A1, A2, and A3, respectively.

Table A 1. The gross milk composition of the three cow milk batches in combination with the four rennet types with casein, casein number, and whey protein

	Cow milk 1 **(n=1)**				**Cow milk 2** **(n=1)**				**Cow milk 3** **(n=1)**			
Casein (g/100)	**CH** 2.71	**P** 2.73	**L** 2.71	**K** 2.70	**CH** 2.72	**P** 2.71	**L** 2.70	**K** 2.71	**CH** 2.65	**P** 2.65	**L** 2.63	**K** 2.65
Casein no. (%)	**CH** 75.07	**P** 75.62	**L** 75.07	**K** 74.79	**CH** 74.73	**P** 74.45	**L** 74.18	**K** 74.45	**CH** 74.02	**P** 74.02	**L** 73.46	**K** 74.02
Whey protein	**CH** 0.90	**P** 0.88	**L** 0.90	**K** 0.91	**CH** 0.92	**P** 0.93	**L** 0.94	**K** 0.93	**CH** 0.93	**P** 0.93	**L** 0.95	**K** 0.93
Protein (g/100g)	3.61				3.64				3.58			
Fat (g/100g)	4.13				4.19				4.13			
SFA (g/100g)	2.74				2.77				2.74			
UFA (g/100g)	1.24				1.25				1.23			
MUFA (g/100g)	0.92				0.94				0.91			
PUFA (g/100g)	0.13				0.13				0.13			
C14:0 (g/100g)	0.46				0.46				0.46			
C16:0 (g/100g)	1.05				1.07				1.05			
C18:0 (g/100g)	0.68				0.69				0.68			
C18:1c9 (g/100g)	0.75				0.77				0.74			
SSC(x10^{3}cells/ml)	105				101				106			
pH	6.60				6.65				6.64			
Lactose (%)	4.75				4.77				4.76			
Solids (%)	13.29				13.37				13.26			

Mean values ± standard deviation. Technical replicates n=3. Abbreviations: SFA=saturated fatty acids, UFA=unsaturated fatty acids, MUFA=monounsaturated fatty acids, PUFA= polyunsaturated fatty acids, and SCC=somatic cell count.C14:0=myristic acid, C16:0=palmitic acid, C18:0=stearic acid, C18:1C9=oleic acid, CH= chymosin rennet, P= pepsin rennet, L= lamb rennet, K= kid rennet.

Table A 2. The gross milk composition of the three goat milk batches in combination with the four rennet types with casein, casein number, and whey protein

	Goat milk 1 (n=1)				**Goat milk 2 (n=1)**				**Goat milk 3 (n=1)**			
Casein (g/100)	**CH** 2.39	**P** 2.41	**L** 2.39	**K** 2.36	**CH** 2.32	**P** 2.33	**L** 2.32	**K** 2.27	**CH** 2.17	**P** 2.17	**L** 2.08	**K** 2.14
Casein no. (%)	**CH** 70.92	**P** 71.51	**L** 70.92	**K** 70.03	**CH** 71.38	**P** 71.70	**L** 71.38	**K** 69.85	**CH** 68.67	**P** 68.67	**L** 65.82	**K** 67.72
Whey protein	**CH** 0.98	**P** 0.96	**L** 0.98	**K** 1.01	**CH** 0.93	**P** 0.92	**L** 0.93	**K** 0.98	**CH** 0.99	**P** 0.99	**L** 1.08	**K** 1.02
Protein (g/100g)	3.37				3.25				3.16			
Fat (g/100g)	3.54				3.90				3.88			
SFA (g/100g)	2.46				2.7				2.9			
UFA (g/100g)	0.97				1.1				0.88			
MUFA (g/100g)	0.61				0.72				0.52			
PUFA (g/100g)	0.17				0.19				0.17			
C14:0 (g/100g)	0.48				0.56				0.64			
C16:0 (g/100g)	0.86				0.99				1.07			
C18:0 (g/100g)	0.57				0.52				0.48			
C18:1c9 (g/100g)	0.38				0.48				0.3			
SSC(x10^3cells/ml)	579				1219				595			
pH	6.62				6.56				6.66			
Lactose (%)	4.22				4.15				4.18			
Solids (%)	11.90				12.12				12.15			
Density (g/ml)	1.028				1.028				1.028			

Mean values ± standard deviation. Technical replicates n=3. Abbreviations: SFA=saturated fatty acids, UFA=unsaturated fatty acids, MUFA=monounsaturated fatty acids, PUFA= polyunsaturated fatty acids, and SCC=somatic cell count.C14:0=myristic acid, C16:0=palmitic acid, C18:0=stearic acid, C18:1C9=oleic acid, CH= chymosin rennet, P= pepsin rennet, L= lamb rennet, K= kid rennet.

Table A 3. The gross milk composition of the three sheep milk batches in combination with the four rennet types with casein, casein number, and whey protein

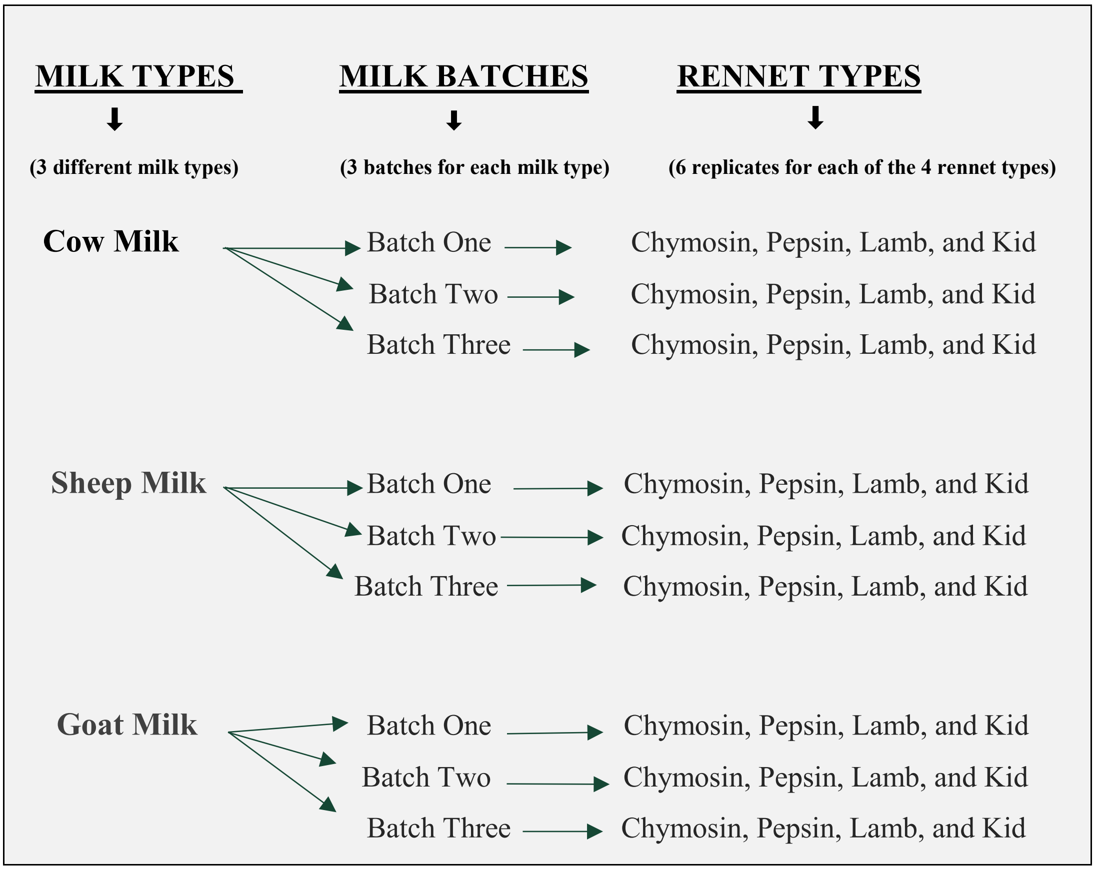
	Sheep milk 1 (n=1)				**Sheep milk 2 (n=1)**				**Sheep milk 3 (n=1)**			
Casein (g/100g)	**CH** 3.49	**P** 3.48	**L** 3.45	**K** 3.39	**CH** 4.48	**P** 4.48	**L** 4.45	**K** 4.45	**CH** 4.36	**P** 4.36	**L** 4.32	**K** 4.25
Casein no. (%)	**CH** 71.66	**P** 71.46	**L** 70.84	**K** 69.61	**CH** 71.45	**P** 71.45	**L** 70.97	**K** 70.97	**CH** 69.10	**P** 69.10	**L** 68.46	**K** 67.35
Whey protein	**CH** 1.38	**P** 1.39	**L** 1.42	**K** 1.48	**CH** 1.79	**P** 1.79	**L** 1.82	**K** 1.82	**CH** 1.95	**P** 1.95	**L** 1.99	**K** 2.06
Protein (g/100g)	4.87				6.27				6.31			
Fat (g/100g)	6.20				7.30				6.49			
SFA (g/100g)	4.22				4.64				4.10			
UFA (g/100g)	1.93				2.41				2.18			
MUFA (g/100g)	1.28				1.82				1.59			
PUFA (g/100g)	0.47				0.41				0.41			
C14:0 (g/100g)	0.82				0.70				0.66			
C16:0 (g/100g)	0.93				1.35				1.14			
C18:0 (g/100g)	1.38				1.58				1.42			
C18:1c9 (g/100g)	0.89				1.37				1.16			
SSC(x10^{3}cells/ml)	402				61				2642			
pH	6.38				6.41				6.44			
Lactose (%)	3.99				4.51				4.40			
Solids (%)	16.04				18.69				17.79			
Density (g/ml)	1.027				1.031				1.031			

Mean values ± standard deviation. Technical replicates n=3. Abbreviations: SFA=saturated fatty acids, UFA=unsaturated fatty acids, MUFA=monounsaturated fatty acids, PUFA= polyunsaturated fatty acids, and SCC=somatic cell count.C14:0=myristic acid, C16:0=palmitic acid, C18:0=stearic acid, C18:1C9=oleic acid, CH= chymosin rennet, P= pepsin rennet, L= lamb rennet, K= kid rennet.

Appendix 2: Project design

The project design for the mini cheese production procedures for the milk types, the batches, the rennet types, and the replicates are shown in Figure A1.

Figure A 1. Project design with milk types, the number of milk batches, the rennet types, and replicates processed during the mini cheese procedure in this study



Note: *For the rheological properties, the project design was in Figure 1 with the difference that four replicates for each of the four rennet types were analyzed.*

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