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Effect of Seasonality and Processing on Physicochemical Characteristics of Goat and Sheep Milk

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ABSTRACT

The interest toward goat and sheep milk consumption is growing with the scientific evidence for the unique health benefits of milk from non-bovine species. However, it is not fully understood whether goat and sheep milk will have the same processability when subjected to standard dairy processing developed for cow milk. Besides, it is of interest to the dairy industry how seasonality interacts with the processing treatments in affecting the properties of goat and sheep milk. The aim of this research was to understand the interspecies difference in physicochemical characteristics of milk, and the processing-induced changes as influenced by seasonality.

Fresh goat and sheep milk samples were collected from local producers and characterised for their compositional and physicochemical properties over three different seasons. The fresh whole milk (goat and sheep) was subjected to various processing conditions: 75°C/15s with or without homogenisation and 95°C/5 min with homogenisation. The seasonal and processing-induced changes in goat and sheep milk were analysed using conventional methods. The gelation properties of the milk (acid and rennet) were also investigated.

Goat milk was similar to cow milk in major components, but the protein composition was different. It contained a significantly lower amount of α_{s1}-casein. Seasonality showed minimal effect on the composition and physicochemical properties of goat milk due to non-seasonal kidding management. Sheep milk was characterised with a higher content of macronutrients and minerals, and the casein micelle was more mineralised. It had higher buffering capacity and higher viscosity due to the rich amount of buffering components and total solids. The composition and physicochemical properties were broadly same across early and mid-season but changed during late milking season.

Heat treatment (95°C/5 min) increased the size of casein micelles in both types of milk, and the effect was more pronounced for sheep milk (48% versus 24% increase in goat milk). The heat-induced increase in micelles size was much bigger in goat and sheep milk than in cow milk, suggesting different mechanisms of casein micelle modification. After heated at 95°C for 5 min, the denaturation level of whey protein and their association was lower in goat milk than in sheep milk. The goat α-lactalbumin was found more heat stable than the sheep counterpart.

Goat milk formed weak gels when inoculated with rennet or acidified. Homogenisation decreased final storage modulus (G') and the final loss tangent of goat milk rennet gel. Heating (95°C/5 min) increased the final G' of acid gels made from goat milk, but the impact was far less pronounced than that in cow milk. Sheep milk formed much stronger rennet and acid gels compared to goat milk. Heat treatment (95°C/5 min) improved the acid gelation properties of sheep milk

significantly (shorter gelation time, higher gelation pH and increased G' value). However, the extent of improvement was less pronounced in the late season despite the higher protein content.

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List of abbreviations

BC	Buffering capacity
BSA	Bovine serum albumin
CCP	Colloidal calcium phosphate
CLA	Conjugated linoleic acid
CLSM	Confocal laser scanning microscopy
DSC	Differential scanning calorimetry
DTT	Dithiothreitol
FA	Fatty acid
GDL	Glucono-δ-lactone
GMP	Glycomacropeptide
HMF	High melting fraction
IMCU	International milk clotting unit
ISE	Ion-selective electrode
LMF	Low melting fraction
LT	Loss tangent
GdnHCl	Guanidine hydrochloride
MFG	Milk fat globule
MFGM	Milk fat globule membrane
MMF	Medium melting fraction

MUFA	Monounsaturated fatty acid
NPN	Non-protein nitrogen
PUFA	Polyunsaturated fatty acid
RO	Reversed osmosis
RP-HPLC	Reversed-Phase High-performance liquid chromatography
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide electrophoresis
SEM	Scanning electron microscopy
SFA	Saturated fatty acid
SOL	Stage of lactation
SOP	Standard operation procedure
TAG	Triglycerides
TEM	Transmission electron microscopy
UC	Ultracentrifuge
UHT	Ultra-high temperature
v/v	volume/volume
w/v	weight/volume
w/w	weight/weight
WP	Whey protein
α-LA	α-Lactalbumin
β-LG	β-Lactoglobulin

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CHAPTER 1: INTRODUCTION

The global population of goats and sheep has been constantly increasing over the last few decades and is now over 1 billion of each. In 2018, the annual production of fresh goat milk was 18.7 million tons (2.7% of total cow milk production) while that of sheep milk was 10.6 million ton (1.6% of total cow milk production) (FAOSTAT, 2018). There is a global trend of growing demand for non-bovine milk that is projected to increase by 30-50% by 2030 (Pulina et al., 2018).

New Zealand has an estimated number of 66,100 dairy goats (2017) and 22,000 dairy sheep (2019), giving an annual milk production capacity of 51,128 and 1,600 tons, respectively (Hales & Kells, 2020; Smith et al., 2017). Currently, the majority of this milk is being used to manufacture dairy products such as artisan cheese and powdered formula for both infants and adults (Lopez-Lozano et al., 2017; Peterson & Prichard, 2015). New Zealand's dairy goat and sheep industries have high potential with parallel development of the right breeding program, genetic improvement and other technologies (Griffiths, 2014; Solis-Ramirez, 2014).

The unprecedented increase in goat and sheep milk production is due to the perceived health benefits of these milks as well as detrimental effects of bovine milk (Selvaggi et al., 2014b). E.g. bovine milk proteins, α_s- and β-caseins and whey protein β-lactoglobulin have been reported to be the major allergens. The proportion of these casein fractions vary in goat and sheep milks. Goat milk has lower α_{s1}-casein than cow milk while sheep milk lacks A1 β-casein, therefore these milks are considered as an alternative for health-conscious dairy consumers (Downie-Melrose, 2014; El-Agamy, 2007).

In addition, goat milk has been associated with several health benefits, which are desired by consumers and a potential marketing opportunity for manufacturers (Selvaggi et al., 2014b). Smaller fat droplet size and a higher concentration of short to medium-chain fatty acids in goat milk are suggested to ease lipid metabolism (Jenness, 1980; Park, 1994). Other health benefits of goat milk include higher mineral bioavailability (Barrionuevo et al., 2003; Park et al., 1986), a high level of short-medium chain fatty acids (Park et al., 2007) and high oligosaccharide content, which is known for its prebiotic effect (Leong et al., 2019; Martinez-Ferez et al., 2006).

Key health benefits of sheep milk are associated with its fatty acid composition. Sheep milk contains a higher level of conjugated linoleic acid which has anticancer (Lp et al., 1994), anti-obesity (Blankson et al., 2000), antioxidant (Chen et al., 1997), and immunomodulatory (O'Shea et al., 2004) properties. A recent study suggests that consumption of sheep milk, in comparison

with cow milk, may have a positive impact on bone structural integrity (Burrow et al., 2019), protein digestibility and lactose malabsorption (Amber et al., 2020).

In addition, products made of non-bovine milk are considered to be a suitable carrier of probiotics due to their ability to maintain the activity of beneficial microorganisms for longer during the storage. This is due to them having a suitable pH, buffering capacity and high nutrients, which makes them suitable for manufacturing dairy products such as fermented milk, yoghurt, ice-cream, and cheese (Ranadheera et al., 2018).

As outlined above, goat and sheep milk have different compositional and physicochemical beneficial effects on human health. As a statutory requirement, all types of milks intended for human consumption have to be processed which induces physicochemical changes in milk that affect its technological properties. The processing induced changes in goat and sheep milk have not been studied in detail in the past. This thesis characterises the impacts of processing (homogenisation and thermal treatment) on the physicochemical properties of goat and sheep milk sampled across different times during the year to address the knowledge gap.

The relevant literature concerning the compositional aspects of macro-and micronutrients and physicochemical properties of these milks and their variability with respect to the seasons have been reviewed in Chapter 2. The properties of these non-bovine milks have been compared with that of bovine milk wherever applicable. Chapter 3 outlines the major protocols used in this work to systematically characterise the impact of processing on physicochemical and gelation properties of goat and sheep milk in Chapters 4 and 5, respectively. Finally, Chapter 6 discusses the interspecies differences in physicochemical characteristics and technological behaviours and concluding remarks are given on the possible industrial application. Figure 1.1 below provides an outline of thesis chapters.

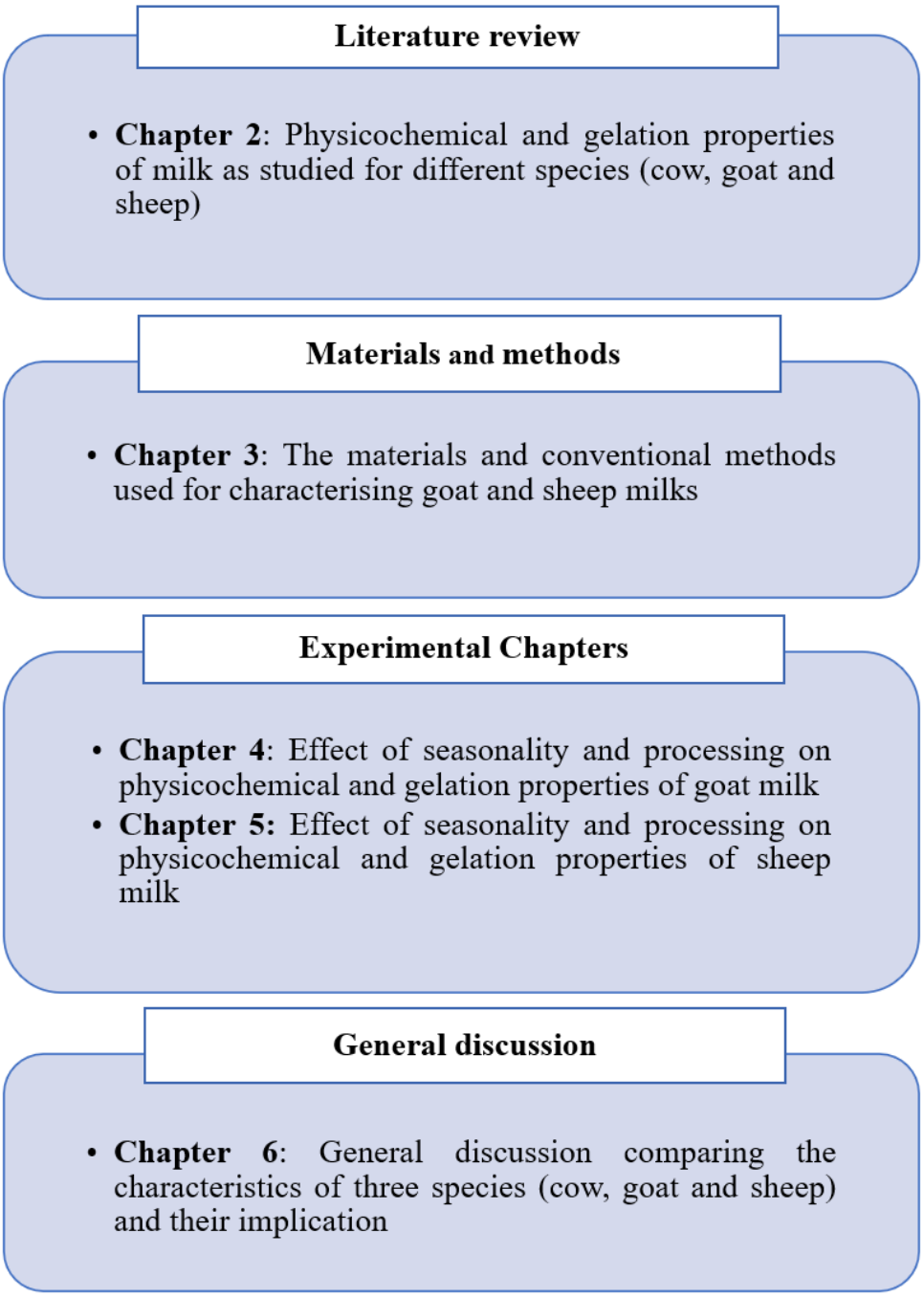


Figure 1. 1 Schematic outline of the thesis

CHAPTER 2: CHARACTERISTICS OF GOAT AND SHEEP MILK

2.1 Composition of goat and sheep milk

2.1.1 Proximate composition

Goat and sheep milk differ from bovine milk in several physicochemical characteristics and exhibits different technological behaviours. The key differences in goat and sheep milk are outlined in Table 2.1.

Table 2.1 Differences in goat and sheep milk compositions and physical properties

Parameters	Goat milk	Sheep milk	Cow milk
Components (%)			
Fat	3.8	7.9	3.6
Protein	3.4	6.2	3.2
Casein	2.4	4.2	2.6
Whey protein (WP)	0.6	1.0	0.6
Non-Protein N	0.4	0.8	0.2
Lactose	4.1	4.9	4.7
Ash	0.8	0.9	0.7
Non-fat solids	8.9	12.0	9.0
Some physical properties			
Density	1.029 -1.039	1.035 - 1.038	1.023 - 1.040
Viscosity (cP)	2.12	2.86 - 3.93	2.0
Freezing point (-°C)	0.540 - 0.573	0.510 - 0560[b]	0.530 - 0.570
pH	6.50 - 6.80	6.51 - 6.85	6.65 - 6.71

Adapted from Park et al. (2007), and Mayer and Fiechter (2012).

The gross compositions of macronutrients and some physicochemical properties of goat milk are similar to that of cow milk, although lactose content is slightly lower in goat milk. Sheep milk has the highest solid contents (fat, protein, and lactose) among the three species (Table 2.1). In addition, the viscosity of sheep milk is higher than goat and cow milk (Jenness & Patton, 1976; Park, 2007) most likely due to the higher solid content. Other physical properties such as density,

freezing point and pH show minor variations but are largely similar for both, the goat and sheep species.

The concentration of milk components has a significant impact on the technological behaviours and rheological characteristics of dairy products. For instance, milk with higher fat (>10%) or total solid contents (>15%) requires increased pasteurisation temperature, 2.8°C higher than the specified heat parameter ("Heat treatment code of practice," 2009) to achieve sufficient pasteurisation effect and inactivation of enzymes (Weihrauch, 1988).

In addition, both the type and the amount of macronutrients and minerals greatly affect the physicochemical and rheological properties of milk. Higher protein content in milk may affect the gelation properties during the manufacturing of products such as yoghurt and cheese (Fox et al., 2015d; Walstra & Jenness, 1984). The impact of the differences in the composition of sheep and goat milk have been discussed in detail in the later (Section 2.2).

2.1.2 Protein composition

The total protein content of bovine milk is 3.2 to 3.6 g/100 g milk, and these proteins have been classified into caseins and whey proteins based on their solubility at pH 4.6 (Fox, 2003). Non-protein nitrogen (NPN) compounds are also found in milk and those include ammonia, urea, creatine, uric acid, and free amino acids (Fox, 2003; Park et al., 2007; Walstra, 1999).

2.1.2.1 Caseins

The major caseins that constitute about 80% of the total proteins in cow milk include α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein, and these are present in the approximate ratio of 4:1:3.5:1.5 (Dalgleish & Corredig, 2012). Goat and sheep milk contain the same casein as cow milk; but differ in their proportions and the genetic variants of individual caseins (Jenness, 1980; Park, 2007).

Table 2.2 compares the fraction of major proteins in goat, sheep and cow milk. Amongst the three types of milk, sheep milk has the highest concentration of total protein and caseins. The net casein content in goat and bovine milk is similar, but these milks differ in the concentration of various casein fractions. Goat and sheep milk contains a significantly lower amount of α_{s1}-casein (Table 2.2) than cow milk, in which α_{s1}-casein accounts for 40% of total casein (Huppertz, 2013). The fraction of α_{s2}-casein is the highest in sheep milk and the lowest in cow milk as compared below.

Table 2.2 Casein fractions in milk from different species

Caseins	Goat [a]	Sheep [b]	Cow [c]
Total protein (g/kg)	37.2	55	32.1
Casein proteins (g/kg)	24	47	24.5
α_{s1}-casein (% of total casein)	5.6	6.6	39.6
α_{s2}-casein (% of total casein)	19.2	22.8	10.3
β-casein (% of total casein)	54.8	61.6	36.9
κ-casein (% of total casein)	20.4	8.9	13
NPN × 6.38 (g/kg)	5.8	-	-

Adapted from [a] Selvaggi et al. (2014b), [b] Selvaggi et al. (2014a), and [c] Dupont et al. (2013).

β-casein is the most abundant protein in goat and sheep milk, whereas α_{s1}-casein is the major protein in cow milk. The proportion of κ-casein is the lowest in sheep milk followed by cow and goat milk (Table 2.2). The difference between the species in their protein fractions affects a number of technological properties and these will be discussed further in this section.

Genetic variants of milk proteins occur due to the substitution or deletion of specific amino acids along the polypeptide chains, causing slight variations in the molecular structure and weight (Recio et al., 1997; Wendorff & Haenlein, 2017). Several genetic variants of both caseins and whey proteins have been reported in goat and sheep (Table 2.3) milks. Their correlation with milk components and technological properties have been reviewed elsewhere in detail (Amigo et al., 2000; Moioli et al., 1998; Trujillo et al., 1998).

Table 2.3 Genetic variants of goat and sheep milk caseins

Caseins	Goat [a]	Sheep [b]
α_{s1}-casein	A, B_1, B_2, B_3, B_4, C, D, E, F, G, H, I, L, M, N, O_1, O_2	A, B, C, D, E, F
α_{s2}-casein	A, B, C, D, E, F, G, O	A, B, Fast
β-casein	A, A1, B, C, D, E, O, O'	A, B, C
κ-casein	A, B, B', B'', C, C', D, E, F, G, H, I, J, K, L, M	A, B

Adapted from [a] Selvaggi et al. (2014b) and [b] Wendorff and Haenlein (2017).

A. α_{s1}-casein

The bovine α_{s1}-casein has 199 amino acid residues in its primary structure and has a molecular weight of 24 kDa. It contains eight phosphoserine residues in the sequence and is highly calcium-sensitive (Dave & Singh, 2019; Farrell et al., 2004). The α_{s1}-caseins from goat and sheep milk have the same chain length as that from cow milk but differ by several amino acid substitutions. Goat and sheep α_{s1}-caseins have great similarity (97.9%) in the primary structure (Ferranti et al., 1995)

The concentration of α_{s1}-casein strongly impacts the coagulation properties of milk, such as gelation time and gel consistency. The lower α_{s1}- casein content in goat milk results in shorter rennet gelation time and weaker gel formation (Remeuf & Lenoir, 1986) and often explain the difference between goat and cow milk performance in cheese-making (Birkenhäger & Ventimiglia, 2012).

In goat milk, the level of α_{s1} -casein is strongly affected by the occurrence of genetic variants (Grosclaude et al., 1987). The variants A, B, C, H, L and M have been reported to be present when α_{s1}-casein was higher. However, other variants D, F and G were found to be frequently present when the level of α_{s1} -casein in goat milk was lower (Grosclaude et al., 1987; Selvaggi et al., 2014b). Increasing the strong variants of goat α_{s1}-casein in breed selection is suggested to optimise the cheese-making process and enhance the yield (Clark & Sherbon, 2000b; Selvaggi et al., 2014b).

B. α_{s2}-casein

The bovine α_{s2}-casein has 207 amino acid residues and has a molecular weight of 25 kDa. There are 11 phosphoserine residues in the sequence (Huppertz, 2013). The α_{s2}-casein is the most highly phosphorylated among the calcium-sensitive caseins and more hydrophilic than α_{s1}-casein (Martin et al., 2003; Swaisgood, 2003). Goat α_{s2}-casein was characterised with 208 residues (Marletta et al., 2007) which is one amino acid longer than its cow counterpart. On the other hand, sheep α_{s2}-casein has 208 amino acid residues and shares 88% similarity with that from cow milk (Boisnard et al., 1991). The technological significance of α_{s2}-casein has not yet been elucidated probably due to difficulties in separation (Selvaggi et al., 2014a) but it is likely that α_{s2}-casein plays a part in structure formation in products such as cheese and yoghurts.

C. β-casein

Bovine β-casein consist of 209 amino acid residues and it has a molecular weight 24kDa. It has five phosphoserine residues in its primary structure. The degree of phosphorylation in β-casein is less and the sensitivity to calcium is moderate in comparison to α_{s1}- and α_{s2}-caseins (Huppertz, 2013; Wong et al., 1996). β-Casein is the most abundant casein fraction in goat milk and dissociates readily from the casein micelles at lower temperature (Jenness, 1980). The primary structures of goat and sheep β-casein have similar chain lengths with 207 residues (Marletta et al., 2007; Richardson & Mercier, 1979), which is shorter than that of cow β-casein (Farrell et al., 2004).

Genetic variants of β-casein have been suggested to cause differences in the proteolytic cleavage digestibility of these proteins resulting in major health implications. For example, β-casomorphin-7 (BCM7) is a peptide released from the A1 variant of bovine β-casein during gastric digestion, but not from variant A2 (Jinsmaa & Yoshikawa, 1999). The consumption of A1 type milk might be associated with a higher risk of coronary heart disease, type 1 diabetes and symptoms of neurological disorders (McLachlan, 2001; Sun et al., 1999).

Cow milk in which more than 99% of β-casein is A2 variant is referred to as "A2 milk". The other milks from non-bovine species including goat and sheep are considered "A2-like" due to the presence of proline residue at the equivalent position 67 of their β-casein (Woodford, 2011). Therefore, the above-mentioned BCM7 is not produced during gastric digestion (Jung et al., 2017).

D. κ-casein

κ-casein is the smallest of all caseins with a molecular weight of 19 kDa and it consists of 169 amino acid residues. It has three phosphoserine residues in its sequence (Huppertz, 2013). On the other hand, κ-casein in goat and sheep milk was characterised with 171 amino acid residues in its primary structure (Jollès et al., 1974; Mercier et al., 1976). Also, κ-casein is the only casein that occurs in glycosylated form. The glycosylated κ-casein can contain up to 6 glycans attached to Thr residues (Huppertz, 2013).

The κ-casein is present at the casein micelle surface and plays an important role in stabilising the casein micelles. The hydrophilic end consisting of the C-terminal region extends out of the micelle and stabilises the micelle by providing a net negative charge to the micelle surface (Dave & Singh, 2019; Martin et al., 2003).

During cheese manufacture, the κ-casein is involved in enzymatic coagulation of milk. The enzyme rennet cleaves the peptide bond 105-106 (Phe-Met) resulting in a hydrophilic glyco-macro-peptide and para- κ-casein retained in the cheese. Also, its interactions with whey proteins plan a vital role in the development of the desired texture of yoghurts (Fox et al., 2015c).

Structural and physicochemical characteristics of casein micelles

The caseins in milk (cow, goat, and sheep) exist as spherical structures with an average diameter of 200 nm (Fox et al., 2015c). A common structural feature of all caseins: 1) the presence of clusters of hydrophobic amino acids in close proximity; 2) the phosphoserine residues at the N-terminal region allow the formation of a three-dimensional network referred to as micelles.

The core of the casein micelle consists of highly phosphorylated α_{s1}-, α_{s2}- and β-caseins. The phosphate groups on these caseins from these proteins self-associate with calcium phosphate to form nanoclusters that are about 15 nm in size (Dave & Singh, 2019). The nanoclusters are linked through calcium bridging and several nanoclusters associate to form a three-dimensional body of casein micelle. The micelle surface is covered by a layer of κ-caseins which does not participate in the formation of nanoclusters. The negatively charged C-terminal region of κ-casein extends out of the micelle and creates a steric effect that stabilises the micelles against aggregation. The N-terminal region of κ-casein is linked to nanoclusters inside the hydrophobic core (Dalgleish, 2011; Dave & Singh, 2019). Figure 2.1 shows the recent models of casein micelles.

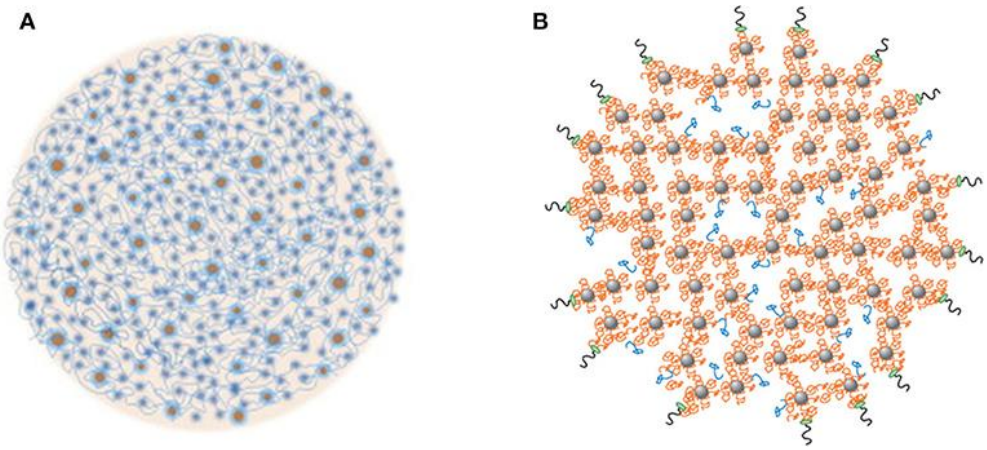


Figure 2.1 Schematic representations of casein micelles structure. (A) the spheres represent the calcium phosphate nanoclusters, and the strands represent the casein network. (B) β- and α_s-caseins (orange strands) are bound to the calcium phosphate nanoclusters (grey spheres), some β-casein (blue) are bound via hydrophobic interactions to other caseins; the κ-casein molecules (black and green) are bound on the surface of the casein micelles. Adapted from Acevedo-Fani et al. (2020).

It is generally assumed that casein micelles are structurally similar in the milk of all other species, although the majority of studies on milk protein have been conducted for bovine milk (Fox et al., 2015c; Whitney, 1998). Nevertheless, some characteristics of caseins micelles in the three species are compared in Table 2.4.

Table 2.4 Comparison of protein characteristics in goat, sheep and cow milk

Characteristics	Goat milk	Sheep milk	Cow milk
Major casein	β-casein	β-casein	α_{s1}-casein
Ratio of caseins (α_{s1}:α_{s2}:β:k)	0.5:2:5.5:2	0.5:2.5:6:1	4:1:3.5:1.5
* Casein micelle size (nm)	199-280	195-220	154-230
Hydration of micelle (g/g MS)	1.77	n/a	1.9
Mineralisation of micelle (g Ca/100g casein)	3.6	3.7	2.9

Compiled from De Kruif et al. (2012); Remeuf and Lenoir (1986), Pierre et al. (1995) and Pirisi et al. (1999). * Measured by dynamic light scattering technique.

Casein micelles show a large variation in their sizes irrespective of the species. Although the range of casein micelle sizes is largely similar in cow, goat or sheep milk, the average size of

casein micelles is in the order of goat > sheep > cow milk (Remeuf & Lenoir, 1986). The larger micelles in goat and sheep milk may be attributed to the higher (25% more) mineral content than that in cow milk caseins (Table 2.4).

Understanding the micelle structure, and their modification by processing conditions is important for solving technological problems in the dairy industry (Fox et al., 2015c). Some examples of micrographic images of casein micelles produced by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are shown in Figure 2.2. The image produced by TEM shows a raspberry-like appearance, which may be suggesting substructures of casein micelle (Dalgleish et al., 2004). The TEM image of cow milk (Figure.2.2B) shows the micelles in roughly spherical forms with different sizes.

A **B**

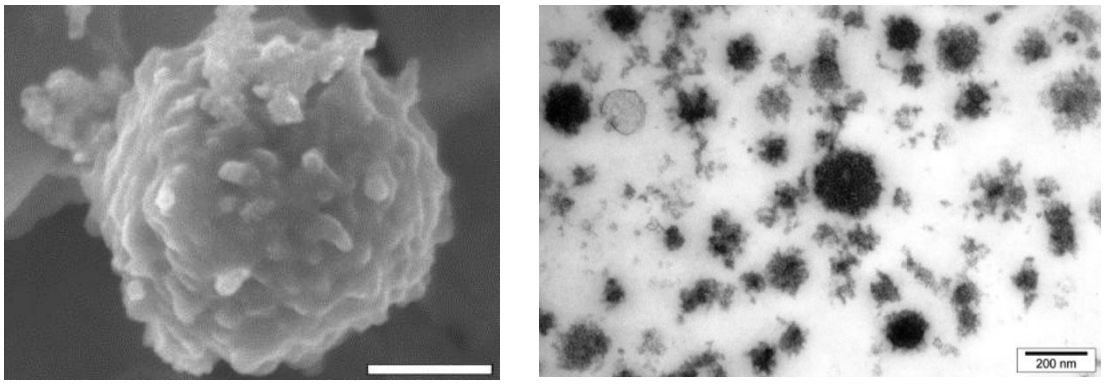


Figure 2.2 Micrographs of casein micelles. (A) TEM image of casein micelles in cow milk. Reproduced with permission from Dalgleish et al. (2004). (B) SEM image of individual casein. Reproduced with permission from Mittal et al. (2015). The scale bar in image A is 100 nm while that in image B is 200 nm.

2.1.2.2 Whey proteins

The proteins remaining in the serum after precipitation of the caseins at pH 4.6 are classified as whey (serum) proteins, and unlike caseins have a well-defined structure (Dave & Singh, 2019). Whey proteins in bovine milk constitute up to 20% of the total proteins in milk and consist of α-Lactalbumins (α-LA), β-Lactoglobulins (β-LG), bovine serum albumin (BSA) and a small proportion of immunoglobulins (Fox, 2003; Whitney, 1998).

As shown in Table 2.5, the total amount of whey protein (WP) and the proportions of α-LA and β-LG are similar in goat and cow milk. In contrast, sheep milk has the highest amount of WP as the total protein concentration is higher and also differs in the ratio of α-LA and β-LG from cow and goat milk.

Table 2.5 Whey protein contents in goat, sheep and cow milk

Whey proteins	Goat	Sheep	Cow
Whey proteins (g/L)	3.7-7.0	10.2-11.0	5.5-7.0
α-LA	0.7-2.3	1.0-1.9	1.2-1.3
β-LG	1.5-5.0	6.5-8.5	3.2-3.3
Minor whey proteins (g/L)			
BSA	-	0.4-0.6	0.3-0.4
Lactoferrin	0.02-0.2	0.8	0.02-0.5
Immunoglobulins (Ig)	0.1-0.5	0.7	0.5-1.0

Adapted from Claeys et al. (2014) and Park et al. (2007).

β-Lactoglobulin (18kDa) is a major whey protein in cow, goat and sheep milk representing more than 50% of total whey proteins (Table 2.5). Its structure has been extensively characterised using different techniques and is a compact globular structure with a hollow hydrophobic cavity in its core. It is rich in sulphur-containing amino acids with two cysteines and one free thiol group (Fox, 2003). Like its bovine homologue, goat and sheep milk β-LG consists of 162 amino acid residues and shows great similarities with several substitutions in their amino acid sequences (Fox et al., 2015c). Different genetic variants have been identified for goat and sheep milk β-LG (Table 2.3). Some genetic variants have been reported to affect milk yield, total protein content, denaturation heat of β-LG and rheological properties, although the study results are contradictory in the literature (Amigo et al., 2000; Selvaggi et al., 2014a).

The heat denaturation of whey proteins, primarily β-LG and their interactions with caseins is of significant technological importance especially in the manufacturing of yoghurts where the parameters such as texture and water-binding capacities are impacted positively (Anema, 2020; Dave & Singh, 2019). In comparison, the heat-induced denaturation of whey proteins and their interactions with caseins is not desirable during the manufacture of cheese and the concentration of milk in evaporators since this leads to fouling of thermal processing equipment (Dave & Singh, 2019). In addition, β-LG facilitates the irreversible denaturation of α-LA by forming disulphide bonds (Li et al., 2019).

α-Lactalbumin (α-LA, 14 kDa) represents about 20% of the whey proteins in cow, goat and sheep milk (Table 2.5). Like most ruminants, goat, sheep and cow α-LA consists of 123 amino acid residues and has a molecular weight of 14 kDa. (Brew, 2003). It is structurally a small protein that is rich in essential amino acids and sulphur but contains no phosphorus (Fox et al., 2015c; Heine et al., 1991). α-LA is an integral part of lactose synthetase, the enzyme which takes a role of a catalyser in the biosynthesis of lactose; thus the concentration of lactose is strongly related to the level of α-LA (Fox, 2003).

2.1.3 Lipids

In cow milk, lipids represent 3.5-5.2% of the total milk composition (Singh & Gallier, 2017) and consist of a mixture of triglycerides with fatty acid chain lengths ranging from C_4 to C_{18} (Table 2.5). From a nutritional perspective, milk lipids are the most valuable constituent in milk as they provide a good source of energy for one's diet and also impart distinct sensorial characteristics to the dairy products such as butter and cheese (MacGibbon & Taylor, 2006). For instance, the "goaty" flavour in goat milk is attributed to its higher concentrations of caproic ($C_{6:0}$), caprylic ($C_{8:0}$), and capric ($C_{10:0}$) acids (Park et al., 2006).

2.1.3.1 Milk fat globule structure

The neutral lipids that form the bulk of the lipids in milk are contained in spherical lipid droplets called milk fat globules. The milk fat globules are polydisperse droplets with diameters ranging from 0.1-15 μm (Singh & Gallier, 2017). The average fat globule size in goat and sheep milk is 3.49 μm and 3.30 μm, respectively, which is smaller than that in cow milk 4.55 μm, and hence these milks typically do not require homogenisation for liquid milk production (Park et al., 2006). The smaller fat globule size in goat milk is beneficial for efficient lipid metabolism due to the larger interaction surface (Park, 1994). The creaming ability of chilled goat milk is relatively poor because goat milk lacks agglutinin which is a substance that adsorbs to fat globules and causes aggregation, whereas the opposite is a concern in cow milk (Jennes & Parkash, 1971).

The structure of milk fat globules consists of a hydrophobic core mainly consisting of triacylglycerols and an interfacial layer called milk fat globule membrane (MFGM) (Acevedo-Fani et al., 2020; Singh & Gallier, 2017). The MFGM acts as a natural emulsifier and provides uniform dispersion of fat globules in the aqueous phase of milk (MacGibbon & Taylor, 2006; McPherson & Kitchen, 1983). It is a complex material consisting of proteins, phospholipids,

glycoproteins, triglycerides, cholesterol, enzymes and other minor components. Figure 2.3 illustrates the structure of milk fat globule and the arrangement of membrane materials.

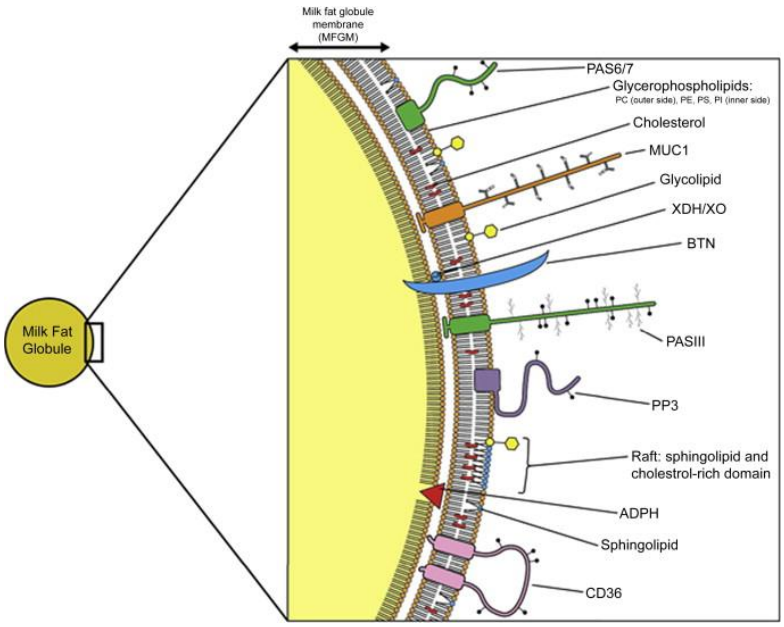


Figure 2.3 Distribution of the MFGM constituents. Reproduced with permission from Gallier et al. (2014).

The MFGM is closely involved in the natural behaviours of fat globules (e.g. aggregation and creaming) in milk, and their structure and physical properties are greatly affected by mechanical and thermal treatments (McPherson & Kitchen, 1983; Rudd, 2013). Due to its prominent impact on the stability of fat globules during processing, cow MFGM has been extensively studied and reviewed (El-Loly, 2011; Mather & Keenan, 1975; McPherson & Kitchen, 1983; Singh & Gallier, 2017).

2.1.3.2 Triglycerides of goat and sheep milk lipids

TAG form the major component of lipids in bovine milk constituting up to 98%, while the minor lipids include diacylglycerols, monoacylglycerols, free fatty acids, phospholipids, and sterols (Jenness, 1988; MacGibbon & Taylor, 2006). Bovine milk contains more than 400 fatty acids (FA) (Jenness, 1988), which vary in the chain lengths, degree of saturation (number of double bonds and their locations) and *cis-trans* isomerisation (Mulder & Walstra, 1974)). These variables of fatty acids and their mode of combination in triglyceride molecules are associated with the melting and crystallisation behaviour of the fat (Mulder & Walstra, 1974; Park et al., 2007). In

ruminant milk, saturated fatty acids (SFA) account for 70% of the total FA, and palmitic acid is the most abundant among the SFA group (Table 2.6).

Table 2.6 Quantification of common fatty acids in goat, sheep and cow milk

Fatty acids (%)	**Notation**	*** Melting point (°C)**	**Goat**	**Sheep**	**Cow**
Butyric	$C_{4:0}$	-7.9	2.03	2.57	2.87
Caproic	$C_{6:0}$	-3.9	2.78	1.87	2.01
Caprylic	$C_{8:0}$	16.3	2.92	1.87	1.39
Capric	$C_{10:0}$	31.3	9.59	6.63	3.03
Lauric	$C_{12:0}$	44.0	4.52	3.99	3.64
Myristic	$C_{14:0}$	54.0	9.83	10.17	10.92
Palmitic	$C_{16:0}$	62.9	24.64	25.1	28.7
Stearic	$C_{18:0}$	69.6	8.87	8.85	11.23
Oleic	$C_{18:1}$ *cis*-9	13.4	18.65	20.18	22.36
Linoleic	$C_{18:2}$ *cis*-9, *cis*-12	-5.0	2.25	2.32	2.57
Conjugated Linoleic	$C_{18:2}$ *cis*-9, *trans*-11		0.45	0.76	0.57
α-Linolenic	$C_{18:3}$ *cis*-9, *cis*-12, *cis*-15	-11.0	0.77	0.92	0.5
Total SFA			68.79	64.23	68.72
Total MUFA			24.48	29.75	27.4
Total PUFA			3.7	4.82	4.05
Total Omega-6			1.78	2.97	2.83
Total Omega-3			0.44	1.31	0.56

Adapted from Markiewicz-Keszycka et al. (2013) and * Fox et al. (2015b).

Goat and sheep milk have a higher level of short to medium-chain fatty acids such as caproic ($C_{6:0}$), caprylic ($C_{8:0}$), capric ($C_{10:0}$), and lauric acid ($C_{12:0}$). Dairy products made of goat and sheep milk have a distinct flavour which is attributed to these fatty acids (Park et al., 2007). In goat milk, medium-chain fatty acids (C_4-C_{12}) account for approximately 20% of the total fatty acids. The ester linkages of these chain fatty acids can be more readily hydrolysed by lipases than those of longer chain fatty acids in the gut and may contribute to easier digestion of milk fat (Jenness, 1980; Park et al., 2007).

Sheep milk is significantly higher in the total amount of conjugated linoleic acid (CLA) than cow and goat milk. CLA is a generic term for all positional and geometric isomers of linoleic acid ($C_{18:2}$) containing two double bonds, and *cis*-9, *trans*-11 is the major isomer that represents about 75–90% of total CLA in milk fat (Tsiplakou et al., 2006). The growing interest in a higher content of CLA is linked to the reported effects on human health (e.g. anticarcinogenic and immunomodulatory effects) and modifying the fatty acid profile by feed management (Park et al., 2007).

Fatty acids in milk originate from two major sources. Long-chain fatty acids, predominantly oleic acid (C_{18}) are derived from the diet, whereas short- and medium-chain fatty acids are synthesised de novo in the mammary glands (Palmquist, 2006). Fatty acids in milk can be manipulated by altering the dietary fat in the feed of the dairy animals. The increased stearic acid in the oil feed increases the amount of $C_{18:1}$ in milk due to stearoyl-CoA desaturase enzyme action during lipid metabolism (Palmquist et al., 1993).

2.1.3.3 Melting characteristics of goat and sheep milk fat

Milk fat is a mixture of various triglycerides that melt at the temperature range between -40°C and 40°C. At ambient temperature, some part of milk fat is in a liquid state, while some of it is in solid form (Mulder & Walstra, 1974). The milk TAGs can be classified based on their melting points as under (Deffense, 1993).

- Low-melting fraction (LMF) with a melting point < 10°C,
- Medium-melting fraction (MMF) with a melting point between 10-20°C
- High-melting fraction (HMF) with melting point > 20°C.

The proportion of these fat groups can vary with the fatty acid composition. For example, LMF is mainly contributed by short-chain fatty acids and unsaturated long-chain fatty acids (Lopez, 2020). A higher amount of unsaturated fatty acids in milk resulted in softer and more spreadable butter (Bobe et al., 2003).

The melting properties of milk fat are largely determined by the FA composition, which can be influenced by the animal diet. For example, a higher intake of grain (fermentable starch) increases the total protein content in milk but depresses the fat concentration. With a lowering amount of fat, the concentration of short-chain FAs in milk decreases (Palmquist et al., 1993). Besides, other factors such as the breed of the animal (genetics) and SOL also affect the fatty acid composition and hence melting behaviour of milk triglycerides (Larsen et al., 2014; Palmquist et al., 1993).

The variation between different species in melting behaviour mainly arises from the higher molecular weight TAGs. Cow and sheep milk fat contain a higher concentration of C_{50}-C_{54} TAG, while goat milk fat has a higher content of C_{40}-C_{44} TAG (Park et al., 2007; Smiddy et al., 2012). However, the melting properties of milk from non-bovine species has not characterised well, and relevant reports are sparse in the literature.

The melting properties of milk fat determine the texture of dairy products with higher fat content. A higher amount of unsaturated fatty acids in milk fat, which typically is reported during summer, results in softer butter with better spreadability (Heck et al., 2009; Larsen et al., 2014). Moreover, the sensorial properties and overall acceptability of the product is related to the melting characteristic of fat in it. Especially, MMF mainly contributed by short-chain or unsaturated FAs have a significant impact on the sensorial properties (Lopez, 2020).

2.1.4 Minor components

In addition to macro constituents, milk contains hundreds of minor components which mainly consist of minerals and vitamins. These are only small fractions of milk; however, have a significant impact on the nutritional and technological properties (Fox et al., 2015e).

2.1.4.1 Vitamins

Milk provides necessary vitamins to the young animal and supports their growth; thus, milk and dairy products are good sources of micronutrients in the human diet (Fox et al., 2015g). Vitamins are categorised into two groups based on their solubility in fat and water and the contents of these vitamins in milk from different species are compared in Table 2.7.

Table 2.7 Vitamin contents (per 100g) of goat and sheep milk in comparison to cow milk

Vitamins	Goat	Sheep	Cow
Fat-soluble			
Vitamin A (mg)	0.04	0.08	0.04
Beta carotene (mg)	-	-	0.02
Vitamin D (μg)	0.06	0.18	0.08
Vitamin E (mg)	0.04	0.11	0.11
Water-soluble			
Thiamin (mg)	0.05	0.08	0.04
Riboflavin (mg)	0.14	0.35	0.17
Niacin (PP) (mg)	0.2	0.42	0.09
Pantothenic acid (mg)	0.31	0.41	0.34
Pyridoxin (mg)	0.05	0.08	0.04
Biotin (μg)	2	n/d	2
Folic acid (μg)	1	5	5.3
Vitamin B_{12} (μg)	0.06	0.71	0.35
Vitamin C (mg)	1.3	5	1

Adapted from Raynal-Ljutovac et al. (2008).

Goat milk is considerably lower in folic acid and Vitamin B_{12}, compared to cow milk (Jenness, 1980). Sheep milk contains most of these vitamins (Table 2.7) in a higher amount than cow and goat milk. Goat and sheep milks have whiter colour because of the absence of β-carotene, which are all converted into vitamin A. In contrast, cow milk is yellowish, which indicates the presence of β-carotene (Park, 2007; Raynal-Ljutovac et al., 2008).

2.1.4.2 Minerals

The mineral content in bovine milk is approximately 8-9 g/L and the major minerals present in bovine milk are calcium, magnesium, sodium and potassium and these exist as their phosphate, chloride, citrate, sulphate and carbonate salts (Gaucheron, 2005). A comparison of major minerals ion milk from different species (cow, goat and sheep) is presented in Table 2.8. Some salts (Cl^-, Na^+, K^+) are primarily dissolved in the soluble phase of milk, while some of them (Ca^{2+}, Mg^{2+}, inorganic phosphorus) are distributed between the serum and colloidal phase being associated with casein micelle (Fox et al., 2015f; Gaucheron, 2005).

A significant part of calcium and phosphate are found in the micelles as an integral part of the structure and play an important role in maintaining the stability of casein micelles. The calcium binds to the phosphate groups on the caseins (α_{s1}-, α_{s2}- and β-caseins) from multiple nanoclusters and creates the CCP bridges between them preventing precipitation (Dave & Singh, 2019; Gaucheron, 2005).

Table 2.8 Mineral contents (per 100g) of goat and sheep milk in comparison to cow milk

Minerals	Goat	Sheep	Cow
Ca (mg)	134	193	122
P (mg)	121	158	119
Mg (mg)	16	18	12
K (mg)	181	136	152
Na (mg)	41	44	58
Cl (mg)	150	160	100
S (mg)	28	29	32
Fe (mg)	0.07	0.08	0.08
Cu (mg)	0.05	0.04	0.06
Mn (mg)	0.032	0.007	0.02
Zn (mg)	0.56	0.57	0.53
I (mg)	0.022	0.02	0.021
Se (μg)	1.33	1	0.96
Al (mg)	NA	0.05–0.18	NA

Adapted from Park et al. (2007).

Calcium, phosphorous and magnesium are perhaps the most important of the minerals present in milk and are mainly responsible for the technological and nutritional properties. Other elements such as iron, copper, iodine and zinc are found in trace quantities and are of importance in human nutrition (Fox et al., 2015f).

As compared in Table 2.8, goat milk had a higher amount of calcium, phosphorus, magnesium and potassium than cow milk but it was lower in sodium content. On the other hand, the levels of trace minerals in both goat and cow milk are mostly similar. Sheep milk is the richest in most of the major minerals with few exceptions. Sodium and potassium levels in sheep milk are lower than those in cow milk. In comparison with goat milk, sheep milk has lower amount of potassium and manganese (4-5 times lower).

The mineral content and the composition of minerals in milk have a strong impact on technological properties such as heat stability and coagulation. The heat stability of milk is determined by the soluble salt balance ratio (SBR) expressed in equation 2.1 below (Donnelly & Horne, 1986).

$$SBR = \frac{(Ca + Mg)}{(Phosphate + citrate)}$$

Eq.2.1

The addition of calcium salt to milk improves the rennet gelation properties (shorter clotting time and firmer gel) possibly by changing the Ca^{2+} activity, the concentration of CCP and proportion of caseins in the micelle (Lucey & Fox, 1993; Udabage et al., 2001). The increasing level of Ca^{2+} and CCP decreases the electrostatic repulsion between the renneted micelles and increases the rate of coagulation (Udabage et al., 2001). On the other hand, loss of CCP causes disintegration of micelles and increases the soluble proteins resulting in poor gelation characteristics (Dave & Singh, 2019).

2.1.5 Bioactive constituents

Bioactive constituents are regarded as components that affect physiological and biochemical functions (Schrezenmeir et al., 2000). Milk contains bioactive components in the form of lipids, carbohydrates (lactose and oligosaccharides), enzymes, proteins (casein and WP) and peptides. Those components have a significant impact on immunological and gastrointestinal development and prevent against pathogens and illnesses (Park, 2009). For the purpose of this work bioactive constituents were not considered in the scope of this review and the information on milk-derived bioactive peptides is available in excellent reviews by Park (2009) and Recio et al. (2009).

2.1.6 Seasonal variation

The composition of milk is greatly affected by seasonal variation. Table 2.9 shows the variation in cow milk yield, gross components and some minerals across the milking seasons in New Zealand and Ireland, where the milk is produced mostly from the spring-calving herd. The seasonal variation in milk characteristics can be minimised by year-round calving, which would result in consistent milk supply and processability throughout the year (Auldist et al., 1998; Phelan et al., 1982).

In seasonal calving system, variation in milk composition reflects the stage of lactation (SOL) and nutritional status. The SOL is associated with physiological changes in animal, e.g. decline in secretion cell numbers, while nutritional status is related to the pasture quality changing over the seasons (Auldist et al., 1998; Lucey, 1996). In contrast, the variation in milk composition mainly results from the changing diet in seasonal calving system, which is commonly adopted by European countries (Heck et al., 2009). The significance of the seasonal variation is considered in detail in chapter 4 and 5.

The global goat and sheep milk production is still small in comparison to cow milk, and only recently have gained attention for research on seasonal variation in milk composition. However, most of the major components in goat and sheep milk appeared to vary during the lactation following the typical seasonal pattern observed for cow milk components (Bhosale et al., 2009; Park & Chukwu, 1988; Pavić et al., 2002).

Table 2.9 General seasonal trends in milk composition and yield

Parameter	Early	Mid	Late
Milk yield	↑↑↑	↑↑	↑
Fat	↑↑	↑↑	↑↑↑
Protein	↑↑	↑↑	↑↑↑
Lactose	↑↑	↑↑	↑
Calcium	↑	↑	↑
Inorganic P	↑↑↑	↑↑	↑
Magnesium	↑	↑	↑
Sodium	↑	↑	↑↑
Chloride	↑	↑	↑↑
Potassium	↑↑	↑↑	↑

Compiled from Auldist et al. (2000), Auldist et al. (1998), Lucey (1996), O'Brien et al. (1999) and Phelan et al. (1982).

2.2 Physicochemical properties of goat and sheep milk

2.2.1 pH

The acidity of a dilute solution (milk) is expressed as pH and approximated by the negative logarithm of the hydrogen ion concentration (Fox et al., 2015d).

$$pH = - \log_{10} [H^+] \quad \text{(Eq.2.1)}$$

At 25°C, the pH of bovine milk ranges between 6.5 and 6.7. The pH value of milk is highly related to the temperature because of the temperature dependence of the solubility of calcium phosphate (Fox et al., 2015d).

2.2.2 Buffering capacity

Buffering capacity (BC) is the ability to resist changes in pH upon the addition of acid or base. Buffering capacity of milk is correlated to buffering constituents: proteins, inorganic phosphate and dissolved CO_2 (Park, 1992; Salaün et al., 2005). A higher protein content leads to an increased buffering capacity arising from acidic amino acids in proteins of milk: Caseins and whey proteins. The buffering capacity of milk is maximum at pH 5.0 on acidification due to free inorganic and organic phosphates released with solubilisation of CCP (Salaün et al., 2005).

The effectiveness of a buffer is expressed as its buffering index, and the evaluation procedure involves adding acid or base solution and tracking the change in pH value (Fox et al., 2015d; Salaün et al., 2005). Differential ratio dB/dpH was first introduced by Van Slyke (1922) to express the buffer effect.

$$\frac{dB}{dpH} = \frac{(volume\ of\ acid\ or\ base\ added)\times(normality\ of\ acid\ or\ base)}{(volume\ of\ sample)\times(pH\ change\ produced)}$$

(Eq. 2.2)

The rate of pH change in goat milk upon acidification is more rapid than that in cow and sheep milk due to the lower amount of casein, as well as lower phosphorylation level of β-casein in goat milk β-casein (Salaün et al., 2005). The goat milk BC can also vary depending on the breed. Nubian goat milk contains a higher level of total nitrogen and phosphate, thus it exhibits higher BC than cow and Alpine goat milk (Park, 1992). On the other hand, buffering capacity of sheep milk is higher, with its high protein, higher mineral and dissolved CO_2 contents (as cited in Salaün et al., 2005; Wendorff & Haenlein, 2017).

2.2.3 Ionic calcium

Calcium in milk is distributed between the aqueous and colloidal phase (casein micelles). About one-third of calcium is in soluble form, including ionic calcium. The ionic calcium accounts for approximately 10% of the total calcium in milk with a normal pH level. The Ca^{2+} concentration has been investigated in relation to colloidal stability, coagulation properties, heat stability, and fouling upon heat treatment (Lewis, 2011; Lin et al., 2006; Zamberlin et al., 2012).

The early methods quantified the calcium ion concentration for bovine milk between 2.5-3.4 mM, which is higher than those reported by protocols that use ion-selective electrodes (ISE). In a later study, ionic calcium level in cow milk varied between 1.43 and 2.50 mM throughout the lactation periods (Lin et al., 2006). Silanikove et al. (2003) measured the free calcium ion contents in goat and sheep milk with ISE. The ranges were 1.7-3.7 and 2.0-4.3 mM for goat and sheep milks, respectively. The interrelationship between ionic calcium concentration and pH has also been known. Ionic calcium concentration increases with a reduction in milk pH (Lewis, 2011).

Ionic calcium greatly affects the stability of casein and their susceptibility to aggregation, especially the non-enzymatic stage of rennet coagulation. It has been reported that a higher Ca^{2+} concentration hastens the rennet coagulation. The free calcium ions may neutralise the negatively charged residues on the casein and promote faster aggregation of renneted micelles (Lucey &

Fox, 1993; Tsioulpas et al., 2007; Udabage et al., 2001). The free calcium ions also play important role in controlling the heat stability of milk. Decreasing the ionic calcium activity with calcium chelators increases the heat stability of milk (de Kort et al., 2012; Singh, 2004).

2.2.4 Ethanol stability

The alcohol test is a practical means of determining the susceptibility of milk to coagulation by heat. The test can be done for bovine milk by mixing the milk sample with an equal amount of ethyl alcohol with a standard concentration of 72% (v/v) (Guo et al., 1998). The ethanol-induced reaction is an isoelectric precipitation process, and the isoelectric point increases with higher alcohol concentration (Horne, 2016).

Goat milk has considerably lower ethanol stability than cow milk due to the differences in composition. The ratio of Na to K in goat milk is lower than in cow milk and this could partially contribute to the lower ethanol stability (Guo et al., 1998). Goat milk has considerably poorer heat stability than cow milk, which might be attributed to the higher level of ionic calcium and lower micellar solvation. (Park, 2007; Remeuf, 1992). Lin et al. (2006) found that milk with a higher level of ionic calcium had lower ethanol stability (Figure 2.4). On the other hand, ethanol stability of sheep milk and the establishment of the standard test is scarce in the literature.

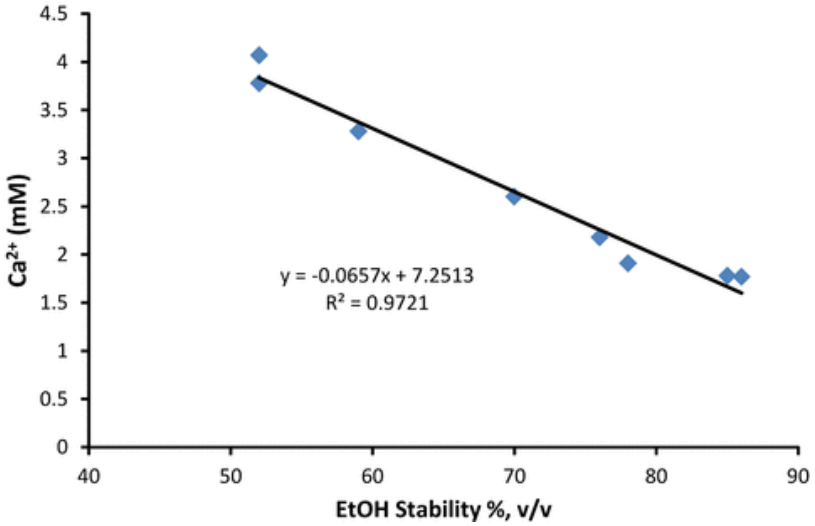


Figure 2.4 Correlation of free ionic calcium levels with ethanol stability of milk collected in early lactation with natural pH. Reproduced with permission from Horne (2016).

2.2.5 Viscosity

Jenness and Patton (1976) described viscosity as "the physical property of the fluid to resist its flow or pour. It depends on internal friction within a liquid and the relationship between kinetic motion and free surface". The viscosity of milk is mainly governed by the milk particles, casein protein and milk fat globules. Other factors such as milk pH, temperature, thermal history and processing condition also have an influence on the viscosity (Fox et al., 2015d). Increasing milk pH results in a slight increase in the milk viscosity, probably because of micellar swelling. However, a sharp decrease in milk pH increases the viscosity due to the protein aggregation. The milk viscosity can also increase with ageing due to the changes in ionic equilibria (Fox et al., 2015d; Walstra, 1999).

The viscosity of cow milk (without fat globule clustering) ranges from 1.79 cP to 2.13 cP at 20°C (Spreer, 1998). The average viscosity of sheep milk has been reported higher (2.48 cP) than those of goat (2.12 cP) and cow milks and most likely due to the higher fat and protein contents (Park, 2007).

2.2.6 Seasonal variation

Most physicochemical characteristics of milk are related to the compositional properties of milk. As discussed earlier, a number of properties of milk e.g. buffering capacity, ethanol stability and viscosity are highly dependent on the composition of milk solids. Therefore, any changes in milk composition such as seasonal variation greatly impact the physicochemical properties of the milk.

2.3 The impact of homogenisation and heat treatment on physicochemical properties

2.3.1 Homogenisation

Homogenisation (20-25 MPa and 65-70°C) is a process designed to break native milk fat globules (1-10 µm) into smaller ones (1 µm or less). During homogenization, fluid milk is forced through a narrow gap in the homogeniser, increasing the proportion of smaller fat globules (Bylund, 2003; Mulder & Walstra, 1974). Figure 2.5 below shows the schematic representation of the homogenization process. Typically, two-stage homogenisation is used for dairy processing, and the second step (4-5MPa) is required to disrupt the clusters of reduced size fat globules that may have formed after the first step of homogenisation (Hardham et al., 2000).

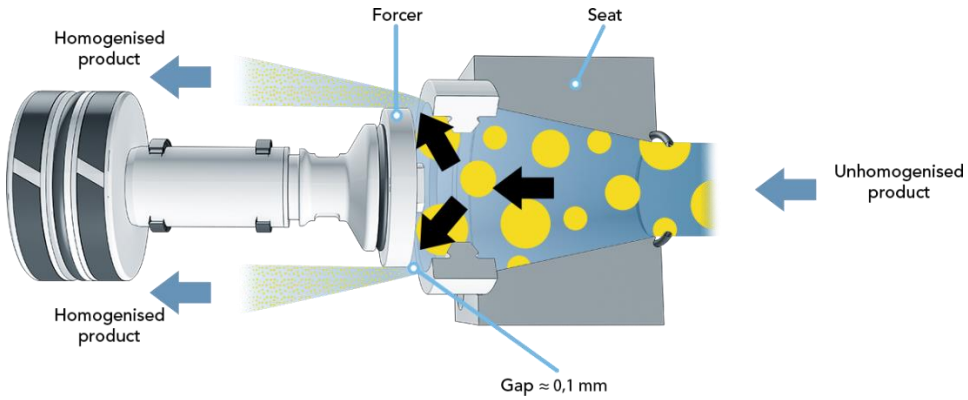


Figure 2.5 An illustration of homogeniser (Bylund, 2003)

After the homogenisation, the fat droplets become smaller in size, but the surface area is increased by 5-6-fold. The original membrane materials are not enough to stabilise the newly created small droplets; thus, those droplets are covered by the adsorption of proteins from the milk plasma (Bylund, 2003). This protein surface coverage leads to the increased volume fraction of fat and increases the viscosity (Mulder & Walstra, 1974). But the increase is partially compensated by the decrease in the volume fractions of casein and whey proteins because some are adsorbed to

the fat globule interface. Therefore, the effect of homogenisation on the milk viscosity can be low (Fox et al., 2015d).

Homogenization of milk prevents creaming in milk and improves the stability and consistency of fermented products (Bylund, 2003). Homogenization is also a technological requirement when making recombined milk by blending anhydrous milk fat with milk powder. Some other advantages of homogenised milk are whiter colour which is more appetising and enhanced flavour and mouthfeel (Bylund, 2003).

2.3.2 Thermal treatment

Heat treatment is a standard unit operation to inactivate pathogenic microorganisms and ensure microbiological safety in milk used for manufacturing different types of dairy products (Bylund, 2003). The inactivation effect can be achieved by the combination of temperature and time. The most common treatment is pasteurisation, and it can be applied in a batch or continuous system. In low-temperature and long-time pasteurisation (LTLT), the milk is heated in open vats at 63°C for 30 min. In contrast, high-temperature short time (HTST) pasteurisation uses the temperature range 72-75°C and holding time of 15-20s in a continuous processor ("Heat treatment code of practice," 2009).

The physicochemical changes brought about in milk are strongly dependent on temperature and the discussion here is largely restricted to temperatures above 65°C. The heat-induced changes in milk include denaturation and aggregation of WPs, decrease in soluble calcium concentration and increase in micelle size and viscosity. Heat treatment also improves the shelf-life during which the milk retains its chemical, physical, and microbiological stability. Ultra-high temperature (135-150°C/2-5s) is used in combination with aseptic packaging to produce long-life milk with a negligible survival risk of microorganisms including bacterial spores (Bylund, 2003).

Heating milk at 65°C or above causes an irreversible structural change (unfolding) in whey proteins, which is known as denaturation. The degree of heat treatment impacts the level of denaturation of whey proteins and their disulfide-mediated interactions with caseins. These in turn are responsible for a number of physicochemical and technological properties of processed milks. The principal part of denatured WPs associate with κ-casein to form a whey protein/κ-casein complex, while a small part of whey proteins aggregate with each other via thiol/disulphate exchange and hydrophobic bonding (Dave & Singh, 2019; Donato & Guyomarc'H, 2009). The schematic in Figure 2.6 below demonstrates the proposed mechanism of heat-induced changes as studied for cow milk.

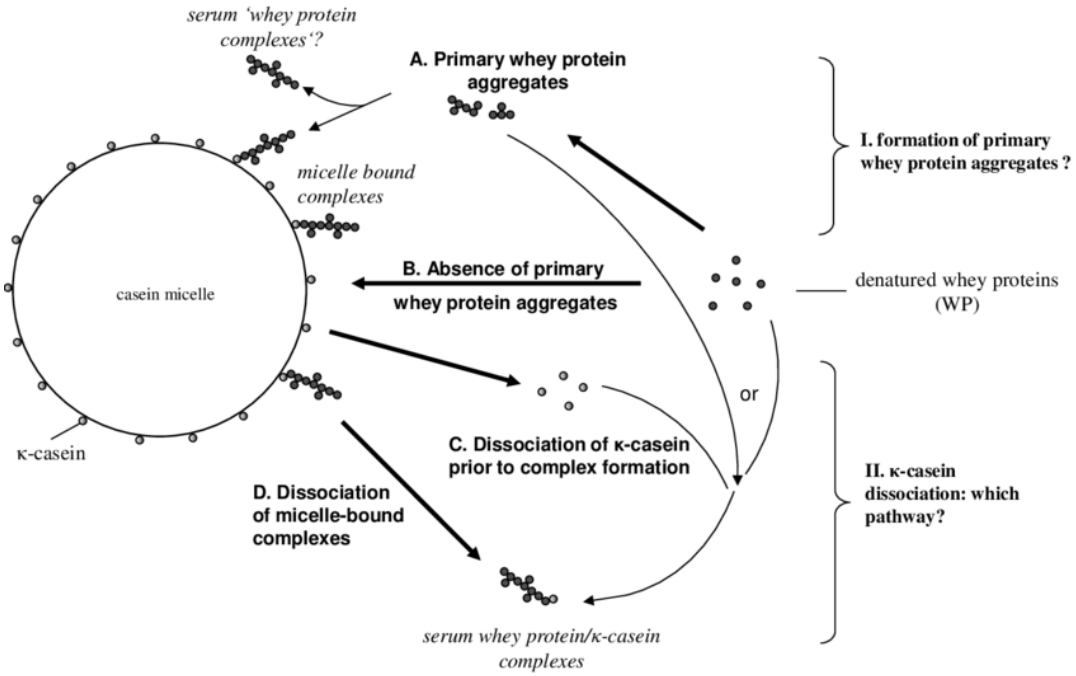


Figure 2.6 Schematic representation of the currently proposed pathways of formation of the heat-induced whey protein/κ-casein complexes in heated skim milk. Reproduced with permission from Donato and Guyomarc'H (2009).

Heating can change the salt equilibria in milk and their interaction with casein micelles (Raynal & Remeuf, 1998). The solubility of calcium phosphate decreases upon heating, resulting in a partial transfer to the colloidal phase and association with the casein micelles. This mechanism explains the decrease in soluble calcium concentration on heat treatment. With the precipitation of calcium phosphate, ionic calcium content also decreases, and such change is irreversible after severe heating (de la Fuente, 1998; Fox et al., 2015f).

The heat-induced denaturation and interactions of whey proteins with casein micelles bring about an increase in casein micelle size. This further leads to an increase in milk viscosity. The increased viscosity of heated milk is attributed to the increase in volume fraction and the aggregation of casein micelles (Anema et al., 2004b; Raynal & Remeuf, 1998). Jeurnink and De Kruif (1993) concluded that the unfolding of β-LG is the principal reason for the increase in milk viscosity upon heating, as no change in viscosity was observed in the absence of whey protein. However, the effect of heating on viscosity is dependent on the initial pH of the milk. A slight change in milk pH from 6.7 to 6.5 resulted in a higher level of denatured WP associated with caseins, thereby increases casein micelle size and viscosity (Anema et al., 2004b).

In addition, when milk is subjected to a high-temperature heat treatment, Maillard reaction (non-enzymatic browning) occurs between lactose and lysine residues on the milk proteins. This reaction results in several changes in the milk including development of brownish colour, the formation of flavour compounds and loss of nutrition due to the involvement of lysine in the reaction (Van Boekel, 1998).

2.4 Gelation properties of goat and sheep milk

2.4.1 Acid gelation

During the manufacturing of products such as yoghurt, the milk undergoes gradual acidification due to fermentation of lactose by lactic acid-producing bacterial cultures to lactic acid. Gluconо-delta-lactone (GDL) is a commonly used chemical acidifier for imitating acid gelation of milk. The hydrolysis of GDL to gluconic acid results in gradual pH reduction in milk similar to that during yoghurt fermentation and induces gel formation (Lucey & Singh, 1997). At pH 4.6, the casein micelles lose their integrity as a result of neutralised surface charge and solubilised CCP, and aggregate to form a three-dimensional gel network (Heertje et al., 1985; Lucey & Singh, 1997). Nevertheless, acid gel formation is more complicated than the aggregation of casein micelles and it involves rearrangement of caseins and milk salt system, particularly the distribution of CCP (Heertje et al., 1985).

Goat milk has poor gelation properties compared to cow milk. The G' value, which is an indication of gel strength, has been consistently reported to be lower for acid gels made from goat milk (Miocinovic et al., 2016; Nguyen et al., 2018; Roy et al., 2020b; Vlahopoulou & Bell, 1995). The proportion of caseins, particularly lower level of α_{s1}-casein in goat milk and different characteristics of casein micelles might be the cause of such poor texture of goat milk acid gel or yoghurt (Park et al., 2007). In contrast, sheep milk acid gels exhibited denser and firmer structure than cow and goat milk most likely due to the higher protein and total solid contents (Nguyen et al., 2018; Roy et al., 2020b). Domagała (2009) reported the highest gel hardness and adhesiveness (sheep > cow > goat), as well as the lowest susceptibility to syneresis for sheep milk yoghurt.

Nguyen et al. (2018) compared the microstructure of yoghurt systems made of reconstituted cow, goat and sheep skim milk. As shown in figure 2.7, the gel structure of goat yoghurt is more porous and likely to have fewer crosslinks between the protein aggregates compared to those of cow and sheep yoghurt (Nguyen et al., 2018).

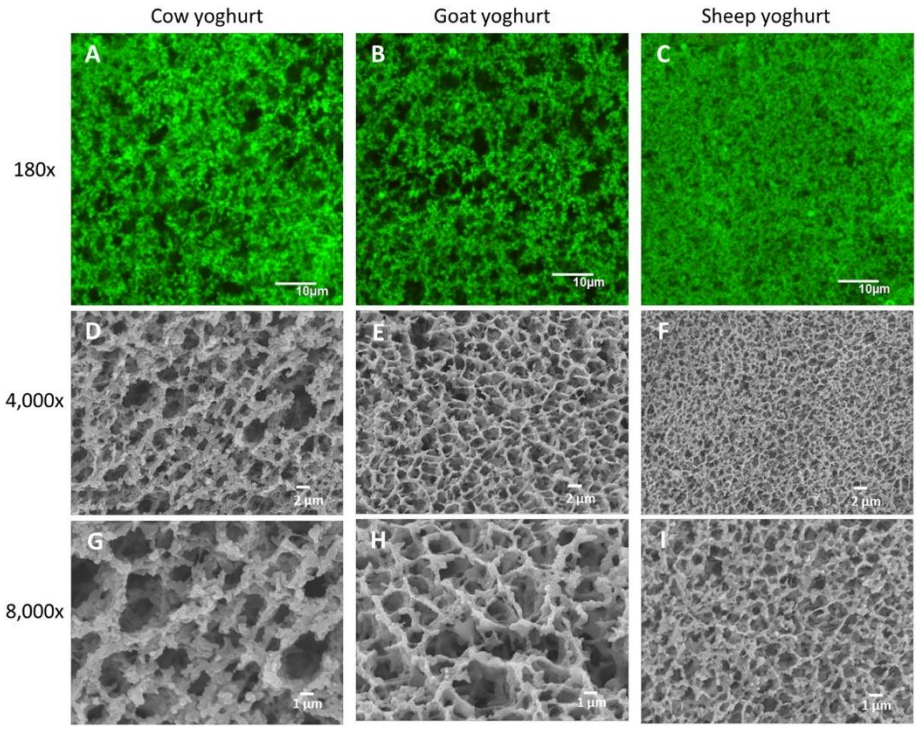


Figure 2.7 The microstructure of cow, goat and sheep yoghurts observed using confocal laser scanning microscopy (CLSM) (A–C) and cryo-SEM (D–I). In CLSM images, protein stained by Fast Green FCF appears as green and non-fluorescent areas (dark areas) correspond to the serum pores. Reproduced with permission from Nguyen et al., (2018).

The gelation characteristics of the GDL gels can provide useful information about the acid gelation process. The gelation time and final storage modulus (G') are the main rheological measurements defining gelation behaviour. These factors are presumably affected by milk composition, primarily casein contents, and physicochemical properties. The effect of heat treatment on gelation properties of milk have been extensively studied and will be discussed later in this chapter (Section 2.4.4).

2.4.2 Enzymatic gelation

Enzymatic gelation of milk by rennet is one of the key characteristics of milk proteins caseins and is of importance in cheese manufacture. During the cheese manufacturing, the enzymatic coagulation of milk occurs in two stages: Proteolysis of κ-casein by rennet and aggregation of destabilised micelles. Firstly, the enzyme rennet (chymosin) acts on the sensitive bond Phe-Met (at the position 105-106) of κ-casein and releases para-κ-casein (residues 1-105) and macro-peptides (106-169). As a result of this proteolytic cleavage, the micelle stability is reduced due to the removal of the hydrophilic glycomacropeptide (GMP) fraction which diffuses into the

aqueous phase. (Fox et al., 2015a; Whitney, 1998). In the secondary phase of enzymatic coagulation, the calcium-sensitive α_{s1}-, α_{s2}- and β- caseins interact with each other via calcium bridging in the presence of calcium ions and aggregate to form a three-dimensional gel matrix (Dave & Singh, 2019). The second phase of gelation is temperature dependent with the improved gelation characteristics at higher temperatures than at low temperatures (< 4°C). Other factors influencing rennet gelation characteristics include fat and protein levels, casein content, total calcium concentration, ionic calcium content, casein micelle size and milk pH (Guinee et al., 1997; Remeuf & Lenoir, 1986; Storry et al., 1983) Also, processing parameters such as heating intensity, homogenisation pressure and coagulation temperature affect the rennet coagulation properties(Bencini, 2002; Guinee et al., 1997; Robson & Dalgleish, 1984).

Goat milk is known to produce weaker curd because of the lower casein content, particularly α_{s1}-casein (Remeuf, 1992). The lower content of α_{s1}-casein is associated with a lower level of TS, protein, and casein, consequently, poorer gel formation (Ambrosoli et al., 1988; Clark & Sherbon, 2000a). Renneting properties of goat milk are also influenced by the goat breed linked to protein composition, whereas the effect of the breed is minimal for cow and sheep milk (Pazzola et al., 2018). A marked difference in coagulation properties has been observed between different goat breeds despite similar compositions (Ambrosoli et al., 1988; Pazzola et al., 2018).

On the other hand, sheep milk has a higher level of total solids, protein and mineral than goat and cow milk. These compositional differences result in a shorter rennet coagulation time and a more consistent curd structure, which is favourable in cheesemaking (Grandison, 1986; Hilali et al., 2011; Jandal, 1996). Moreover, sheep milk is sensitive to rennet due to its higher β/α_s-casein ratio; therefore, coagulation of sheep milk proceeds faster than that of cow milk (Selvaggi et al., 2014a).

2.4.3 Seasonal variation in gelation properties

As reviewed in this chapter, seasonal variation in milk composition has been well studied, especially for cow milk. However, the reports on seasonal changes in gelation properties of milk (cow, goat and sheep) are scarce in the literature.

Lucey et al. (1992) compared rennet gelation properties of cow milk obtained during mid-and late lactation and reported longer coagulation time and weaker gel firmness for the late-lactation milk. Similarly, Li et al. (2020) reported that late-season cow milk had inferior acid gelation properties (longer gelation time and lower gel firmness) compared to the milk from early and mid-season, despite the standardised fat and protein contents for all seasons. The authors attributed the different acid gelation behaviour of late-season milk to the physicochemical properties, rather than the protein content.

On the other hand, the seasonal impact on the gelation properties of goat milk has not been extensively studied. In a recent study by Inglingstad et al. (2014), rennet gelation properties of goat milk were found to be influenced by the season, irrespective of SOL. Goat milk from late season had a longer gelation time and poorer gel firmness compared with the milk from early season. The gel firmness was positively correlated with individual casein contents rather than total casein or protein contents (Inglingstad et al., 2014).

In sheep milk, most of the physicochemical properties varied significantly throughout the lactation, and so did the rennet gelation properties. Late lactation sheep milk had longer coagulation but slightly improved gel firmness. The longer gelation time results from the decreased firming rate, which might be related to the increased micelle size and a reduced proportion of diffusible calcium (Pellegrini et al., 1997). Similarly, Abilleira et al. (2010) reported that rennet clotting time and curd firmness of sheep milk continuously increased throughout the seasons with changing feed management. In contrast, Nájera et al. (2009) reported consistent rennet clotting time for sheep milk regardless of changing diet regime and advancing lactation. They also found that the curd firmness enhanced during late lactation compared to that of early lactation.

2.4.4 Processing impact on gelation properties

Heat treatment is an important processing step in the dairy industry and is critical for a number of reasons e.g. food safety, technological requirement or for improving functionality. The heating of milk greatly impacts its gelation properties under acid or enzyme. For this reason, the effect of heating on acid gelation properties has been studied to a great extent (Anema et al., 2004a; Lucey et al., 1999; Lucey & Singh, 1997; Lucey et al., 1998).

As discussed in Section 2.3.2, the heating of milk greatly modulates the interactions of heat-denatured whey proteins with caseins. This in turn has an impact on the protein-protein interactions during gelation. Preheating milk (85-95°C/10-15min) favours yoghurt making with earlier onset of gelation and firmer gel network. The structure of acid gels could be improved because of the increasing number of proteins in the gel network. Moreover, such gels show reduced syneresis due to the high-water binding capacity of denatured WPs participating in the gel network (Dave & Singh, 2019; Donato & Guyomarc'H, 2009).

However, intensive heat treatment impairs the rennet gelation properties resulting in longer gelation time and weaker gel strength. Because, the denatured WP associated with κ-casein delays the first stage of rennet coagulation by blocking the enzyme that is supposed to attack the reactive site (Phe105 - Met106) of κ-casein (Dave & Singh, 2019; Guinee et al., 1997; Waungana et al.,

1996). Also, such interaction results in poorly formed curd with porous structure due to the sterically impeded casein aggregation (Singh & Waungana, 2001). As shown in figure 2.8, rennet gelation time of cow milk increases and gel strength decreases with intensifying heat treatment. Moreover, ultra-high temperature (140°C) resulted in non-coagulation.

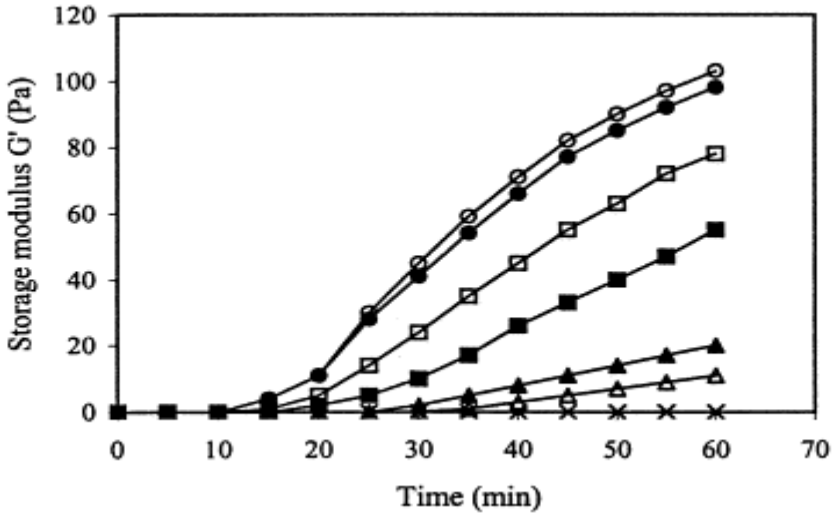


Figure 2.8 The effects of temperature for a holding time of 4 s on the storage modulus (G′) as a function of time after addition of rennet in skim milk. Heating temperatures: unheated milk (○), 80, (•), 90 (□), 100 (■), 110 (▲), 120 (▵), 130 (+) or 140°C (×). All samples were renneted at pH of 6.5 at 32°C. Reproduced with permission from Singh and Waungana (2001).

On the other hand, rennet gelation properties of goat milk are not affected by heat treatment. Heating cow milk at 85°C for 30 min increased the rennet clotting time of cow milk, but the same heating condition did not cause a significant change in clotting time of goat milk (Montilla et al., 1995). The difference in rennet gelation behaviours between cow and goat milk could be attributable to their micelle characteristics and individual casein components (Montilla et al., 1995). In contrast, heating sheep milk at 80°C or above decreased the gel strength of rennet-gels. The heat-induced increase in micelle size was more pronounced in sheep milk than in cow and goat milk. It is possible that the larger micelles in heated sheep milk may cause a decrease in rennet gel strength, as these two variables are negatively correlated (Raynal & Remeuf, 1998).

Sheep milk exhibits superior rheological properties in yoghurt production. Because higher protein content in sheep milk results in compact protein matrix with a large number of crosslinks

(Balthazar et al., 2017; Nguyen et al., 2018). However, there is not much published work investigating the effect of heating on the gelation properties of sheep milk in particular.

Also, the extent of WP and κ-casein complex formation upon heating is pH-dependent in goat milk. At pH below 6.8, a reduced interaction between WP and κ-casein was observed, and this could be the possible explanation for unaffected rennet coagulation time by heat treatment (Anema & Stanley, 1998). Pesic et al. (2016) proposed a different heating effect on the formation and distribution of WP and κ-casein complex in goat milk (Figure 2.9).

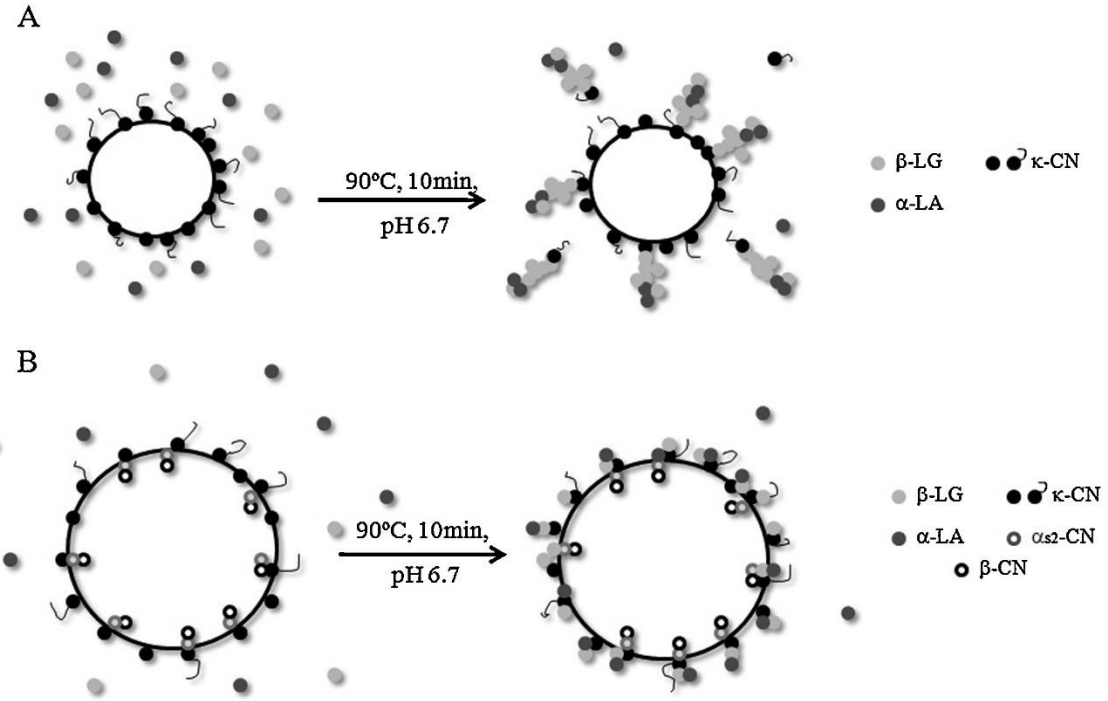


Figure 2.9 A schematic representation of the interactions between casein micelles and denatured whey proteins occurred in bovine (A) and caprine (B) milk after heat treatment at 90 °C for 10 min and pH 6.71. Reproduced with permission from Pesic et al. (2016).

2.5 Summary of the literature

From the above discussion, it is clear that there are compositional differences between goat and sheep milk themselves, and between these and cow milk. Given that the composition of milk greatly affects the physicochemical properties, the changing milk composition over the seasons may also result in seasonal variation in the physicochemical characteristics.

The current knowledge of seasonal variation in milk properties has been gained primarily from cow milk due to its commercial significance. Goat and sheep milks have been well characterised for their compositional properties, but the processing-induced changes and the technological

properties have not been extensively studied. Understanding the seasonal variation in goat and sheep milk characteristics and processing-induced changes is important for the manufacturers to address the challenges associated with seasonal milk production and achieve optimum processability.

The objectives of this study are:

1. To investigate the seasonal variation in physicochemical characteristics of New Zealand's goat and sheep milk
2. To investigate the effect of seasonal changes in milk composition on physicochemical characteristics of processed milk
3. To gain mechanistic insights behind different technological properties of goat and sheep milk in comparison with cow milk

Based on the literature summary, it can be hypothesized that processing will affect goat and sheep milk differently and hence impact their physicochemical and technological properties.

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

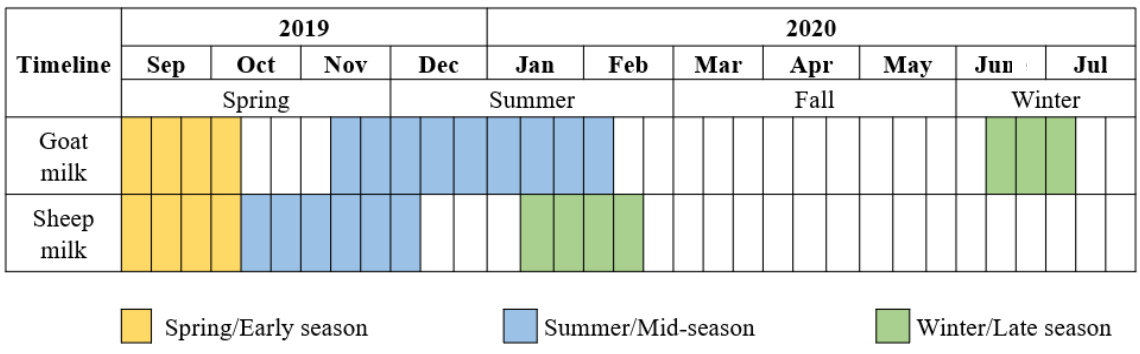
The chemicals and reagents were supplied by Sigma Aldrich Ltd. (St. Louis, MO, USA) and the water used for making the solutions and cream washing were purified by Milli-Q apparatus (Millipore Corp., Bedford, MA, USA).

Fresh goat milk was supplied by local cheese manufacturer Cilantro Cheese Ltd (Waikato, New Zealand) for three different seasons: spring, summer and winter. Goat milk is produced year-round by mixing a spring-kidding herd and an autumn kidding herd. As such, goat milk sampled in this study will not have a synchronised variation pattern with the progressing stages of lactation. The supplier is chosen to represent the common farm management practice in the Waikato region.

Fresh sheep milk was supplied by Spring Sheep Milk Co and Maui Milk Co., Ltd, both based in Waikato region (New Zealand) and mixed at a 1:1 ratio to make the samples more representative of commercial sheep milk. Unlike goat milk, sheep milk was produced by spring-lambing herds only as is commonly adopted in New Zealand. Sheep milk was produced from mid-August 2019 to mid-February 2020. The sampling periods for sheep milk also corresponded to the stages of lactation. Early-, mid-and late-seasons corresponded to the stage of are defined as 0-60, 60-130 and 130-180 days in milk, respectively.

Table 3.1 shows the specified time periods for the milk samplings. For both goat and sheep milk, the samples were collected on at least three different occasions in each season. For example, mid-season goat milk was sampled between mid-November and early February.

Table 3.1 Milk reception plan



3.2 Methods

3.2.1 Sample preparation

Aliquots of fresh whole milk samples were stored at -20ºC for compositional analysis. Another portion was preserved with sodium azide (0.02% wt/wt) and skimmed by centrifugation (Thermo Scientific Multifuge X3R; 3,000g/15min at 10°C). The fat layer was collected for milk fat analysis (MFGM protein and melting characterisation), and the skim milk was ultracentrifuged (Sorval WX Ultra 100, Thermo Scientific; 63,000g/60min) at 20°C to separate the serum part. The collected serum was filtered (Amicon® Ultra-15, 10 kDa) and stored at -20°C for determination of mineral distribution in milk. Figure 3.1 demonstrates the scheme for sample preparation and processing. The prepared samples were stored at -80°C until required for the analysis.

3.2.1.1 Processing

The remaining whole milk samples were processed within 48 hours in Massey University FoodPilot plant (Palmerston North, New Zealand). Goat and sheep milk samples were treated under different processing conditions (unhomogenised and pasteurised at 75°C for 15s, homogenised and pasteurised at 75°C for 15s, and homogenised and heated at 95°C for 5min).

These heat treatments have been selected in relevance to industrial dairy processing which mainly involves a continuous heat treatment system. For example, heating milk at 95°C with for 5 min is a standard thermal treatment used for yoghurt manufacturing. The UHT (Ultra-high temperature) has not been considered for processing goat and sheep milk due to the lower heat stability of both types of milk.

The milk was homogenised using a two-stage homogeniser (20 MPa in the first stage and 5 MPa in the second stage at 65°C) and heated at specific temperature conditions using an indirect UHT system. A preheated water bath (95°C) was used to complete the holding process for the milk heated at 95°C in the UHT. Both degrees of heat treatments were followed by immediate cooling (20°C) using ice water. Processed milk samples were analysed for physicochemical characteristics and rheological properties within 72 hours.

3.2.1.2 Serum sample preparation

The milk serum samples (Figure 3.1) were obtained from raw (R), pasteurised (P), and heated (H) skim milk using an ultracentrifuge (63,000g/60min, 20°C) and acid precipitation method described in Vasbinder and de Kruif (2003). The serum separated by ultracentrifugation (UC)

contains native whey proteins and soluble aggregates of denatured whey proteins, whereas the serum obtained by acid precipitation contains only native whey proteins as all denatured whey proteins precipitate (Vasbinder & de Kruif, 2003).

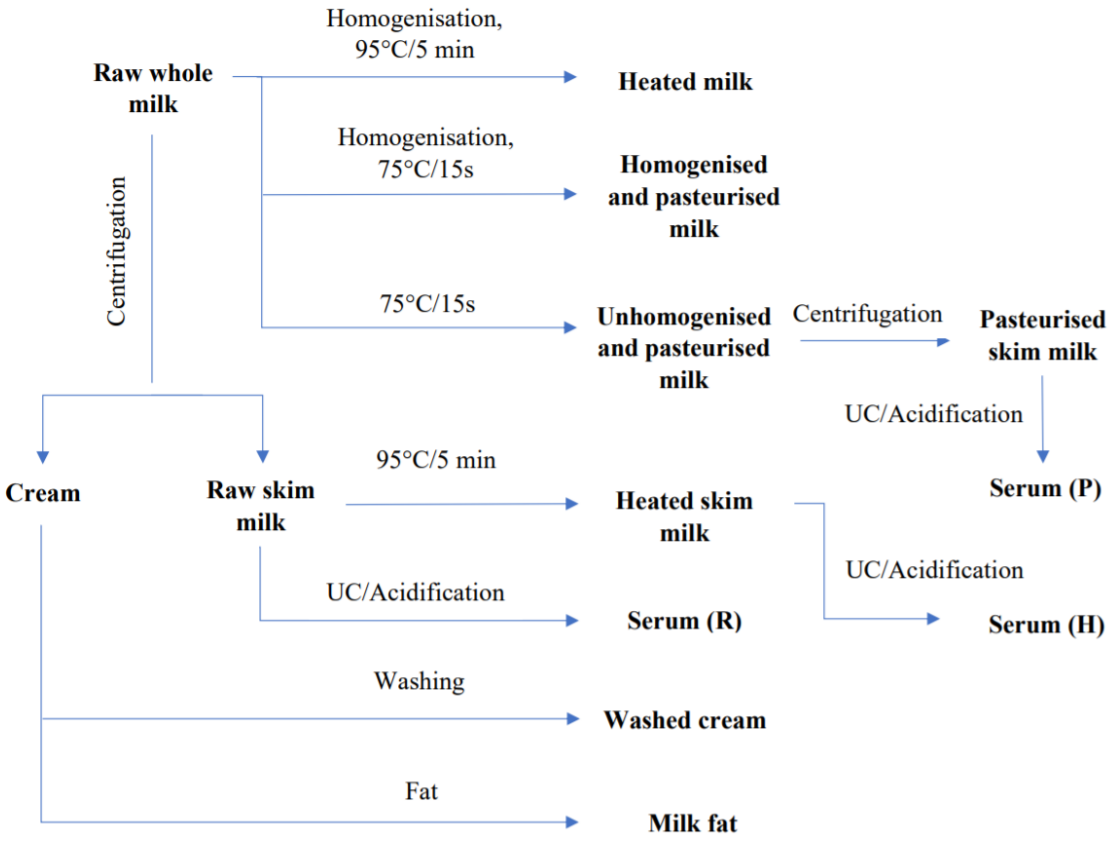


Figure 3.1 Sample preparation scheme for characterising goat and sheep milk and investigate the impact of processing

3.2.2 Compositional analysis

Fat, crude protein, lactose and total solid contents in goat and sheep milk were measured by MilkoScan FT1 (Foss Electric, Denmark). Calcium, magnesium, potassium, sodium and phosphorus concentrations were determined by inductively coupled plasma optical emission spectrometry. The concentration of copper, iodine, selenium and zinc were measured by inductively coupled plasma-mass spectrometry. Chloride content was determined by potentiometric titration (AOAC 971.27) in Hill Laboratories (Hamilton, New Zealand).

3.2.3 Protein characterisation

3.2.3.1 SDS-PAGE

The protein composition of goat and sheep milk and their MFGM proteins have been identified by SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis). This is the most common technique to characterise individual protein and determine their molecular weight.

SDS (sodium dodecyl sulphate), binds to the protein molecules mainly through hydrophobic interactions and makes them negatively charged. When an electric current is applied, SDS-bound proteins migrate through the gel matrix with a porous structure toward the positively charged electrode. The small protein molecules mobilise quicker than the proteins with larger molecular mass because of the sieving effect of the gel (Tremblay et al., 2003).

Acrylamide gels were hand-casted in Mini-PROTEAN II cell (Bio-Rad Laboratories, Richmond, USA) using the stock solutions listed in table 3.2. The resolving and stacking gel solutions contained 16% and 4% acrylamide, respectively.

Table 3.2 Recipes for resolving and stacking gels

Solutions	Resolving gels (16%)	Stacking gels (4%)
30% Acrylamide/bis	5.3 ml	0.65 ml
0.5M Tris-HCl buffer, pH6.8	-	1.25 ml
1.5M Tris-HCl buffer, pH8.8	2.5 ml	-
Milli-Q water	2.02 ml	3.05 ml
10% SDS	100 µl	50 µl
10% Ammonium Persulphate	50 µl	25 µl
TEMED	5 µl	5 µl

The raw skim milk samples were diluted with sample buffer (Table 3.3) to standardise the protein concentration as 1 mg per ml. Then the mixtures were heated at 95°C for 5 min using a water bath.

The washed cream was diluted (1:2 w/w) in a sample buffer solution containing 5 % β-mercaptoethanol to dissociate the MFGM proteins and heated as described by Ye et al. (2002). The heated samples were cooled and centrifuged using MiniSpin® plus centrifuge (2500g/30 min), and the sub-natant was taken for SDS-PAGE analysis.

Table 3.3 Recipe for sample buffer used for diluting raw skim milk

Components	Amount
Milli-Q water	25 ml
10% SDS	5 ml
Glycerol	2.5 ml
0.5 M Tris-HCl, pH 6.8	3.125 ml
0.1 % Bromophenol Blue	625 µl

The loading amount was 15 µl for diluted skim milk samples and 10 µl for cream samples and protein standard (Precision Plus Protein™ Unstained, 161-0363; Bio-Rad). The gel electrophoresis was performed at a constant voltage (150mV) for approximately 1 hour until the bromophenol dye reached near the bottom of the gel. The gels were removed carefully under RO water flow and stained in a solution containing 0.3% Coomassie Brilliant Blue R-250 (w/v), 10% glacial acetic acid (v/v) and 20% isopropanol (v/v) for 45 min. Then the gels were de-stained in a clear solution containing 10% glacial acetic acid (v/v) and 10% isopropanol (v/v). Gel-Doc XR+ system (Bio-Rad) with Image Lab software (ver 5.2.1) was used for scanning the gels and producing the image.

3.2.3.2 RP-HPLC

RP- HPLC (Reversed-Phase High-Performance Liquid chromatography) is a widely used technique to separate and quantify individual proteins in milk. The separation is based on hydrophobic interaction between stationary phase made of silica with non-polar alkyl groups (C4, C8 or C18) and the solutes (protein molecules) in aqueous solution (milk) (Dupont et al., 2018; Tremblay et al., 2003).

The skim milk and serum samples (Figure 3.1) obtained from raw, pasteurised and heated skim milks were mixed with the same amount of solution A (Table 3.4) containing 0.1 M Bis-Tris buffer, 6 M Guanidine hydrochloride (Gdn-HCl), 5.37mM sodium citrate, and 19.5 mM dithiothreitol (DTT) as described by Bobe et al. (1998). The samples were allowed to sit at room temperature for incubation and then centrifuged using MiniSpin® plus centrifuge (14,100g/5 min). The mixture of sample and buffer was diluted four times in solution B containing 4.5M Gdn-HCl. The diluted samples were filtered by syringe filter (0.2µm) and transferred into HPLC vials.

Table 3.4 Recipes for the solutions used for sample preparation (per 100mL)

Solution	A	B
Bis-Tris	2.1 g	-
Gdn-HCl	57.3g	43,0g
Sodium Citrate	0.157g	-
DTT	0.3g	-
The solvent used to make up the volume	Milli-Q water	Solvent A
Adjusted pH	7.0	2.0

The HPLC system (Shimadzu Corporation, Japan) consisted of degassing unit (DGU-20A5R), pumping unit (LC-20AD), autosampler (SIL-20A HT), wavelength absorbance detector (SPD-20AV) and column oven (CTO-20AC). Lab solution software (ver 5.97) was used for controlling the system and post-run data analysis. Reversed-phase C18 column (Aeris Widepore 3.6µm XB-C18 RP; Phenomenex, Torrance, CA) was used to separate the major proteins in the samples. Solution A (Acetonitrile, water, and trifluoroacetic acid at a ratio of 100:900:1 (v/v/v)) and B (Acetonitrile, water, and trifluoroacetic acid at a ratio of 900:100:1 (v/v/v)) were used as mobile phases for the separation.

The initial concentration of mobile phase (solvent B) was selected at 27% in the gradient program and changed throughout the separation gradient as modified from Bobe et al., (1998). With the injection of the sample (20µl), a gradient was generated by continuously increasing the concentration of solvent B to 32% (2 min), 45.6% (29 min), and to 50.2% (1 min). Then this concentration was maintained for 2 min before being brought to the initial condition in the next 2 min. The column was equilibrated for the injection of next sample (9 min). The total run time was 45 min, and the flow rate was 0.6mL/min.

The fractions of six major proteins in raw goat and sheep milk were calculated from the area under chromatogram peaks for the corresponding proteins and expressed in percentage.

$$\text{Fraction of individual protein (\%)} = \frac{\text{Peak area for individual protein}}{\text{Peak area for all major proteins}} \times 100 \qquad \text{(Eq.3.1)}$$

The proportions of serum WP and micelle-bound WP in heated milk were calculated from the peak areas for serum (R, P and H) obtained by UC as below:

$$\text{Serum WP (\%)} = \frac{\text{Peak area for WP in heated serum}}{\text{Peak area for WP in raw serum}} \times 100 \quad \text{(Eq.3.2)}$$

$$\text{Micelle-bound WP (\%)} = 100\% - \text{Serum WP (\%)} \quad \text{(Eq.3.3)}$$

Proportions of denatured and native WP in heated milk were calculated from the peak areas for serum (R, P and H) obtained by acid precipitation method as below:

$$\text{Native WP (\%)} = \frac{\text{Peak area for WP in heated serum}}{\text{Peak area for raw serum}} \times 100 \quad \text{(Eq.3.4)}$$

$$\text{Denatured WP (\%)} = 100 - \text{Native WP (\%)} \quad \text{(Eq.3.5)}$$

The difference between serum WP and native WP is expressed as aggregated WP in heated milk as follow:

$$\text{Serum aggregates (\%)} = \text{Serum WP (\%)} - \text{Native WP (\%)} \quad \text{(Eq.3.6)}$$

The negative values calculated from the equation above (Eq.3.6) were replaced with zero as those have possibly resulted from the minor errors in selecting the peak areas on the chromatogram.

3.2.4 Physicochemical properties

3.2.4.1 Fat globule size

The size distribution of fat globules in raw and processed whole milk was measured by laser diffraction technique (also known as static light scattering) using Malvern MasterSizer 2000 (Malvern Instruments Ltd, Malvern, UK). In this technique, monochromatic laser light is scattered by fat globules, and the scattering pattern is received by detectors positioned at different angles. The received signals are then converted into particle size distribution using theoretical models (Truong et al., 2016).

The milk samples were diluted (1:1 v/v) in a 2% SDS solution containing 50mM ethylenediaminetetraacetic acid (pH 6.7) to dissociate the casein micelles and other aggregates as described in Ye at al., (2002). The mixture was then added to the dispersant (RO water) gradually within the laser obscuration range of 8-10%. The measurement was performed in triplicate. The average volume moment means (D [4,3]) were used to express the diameter of MFG.

3.2.4.2 Casein micelle size

The casein micelle size was measured in triplicate by Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK) using a scattering angle of 173° and temperature of 25ºC.

Raw, pasteurised and heated skim milk samples were diluted 5 and 50 times for goat and sheep milk, respectively, in calcium imidazole buffering solution (pH 7.0) as described in Anema (1997) to obtain an attenuation level of 6 for the measurement. The diluted samples were passed through 0.45μm polyvinylidene fluoride syringe filters to remove the interfering large particles.

3.2.4.3 Ethanol stability

Raw skim milk samples were mixed with an equal amount of ethanol with different concentrations (42.5-55%, increasing by 2.5% intervals) and the highest ethanol concentration that did not cause instant coagulation was determined and recorded as the ethanol stability.

3.2.4.4 pH and Buffering capacity

Raw and processed whole milk pH were measured by HI-2202 edge®blu pH meter (Hanna Instruments, Woonsocket, RI) with daily calibration at room temperature.

Buffering capacity of raw and processed whole milk was determined by acid titration as described by Park et al. (1991) in duplicate. 1 ml of 0.5M hydrochloric acid was added to 25 ml of milk sample under stirring, and this process is repeated until the pH value drops below 2.0. The pH was measured by Eutech pH 700 ph/mV meter (Thermo Scientific, USA) and recorded following the addition of each 1ml of HCl. Then the buffering capacity was determined by the Van Slyke equation.

$$\mathrm{dB/dpH} = \frac{\text{mL acid added} \times \text{Normality of acid}}{\text{The volume of milk} \times \text{pH change}} \qquad \text{(Eq.3.7)}$$

3.2.4.5 Ionic calcium concentration

Ionic calcium concentration was determined in raw and processed whole milk samples using Orion 9720BNWP calcium selective electrode (Thermo Scientific, USA) and Eutech pH 700 ph/mV meter (Thermo Scientific, USA). A calibration curve was produced using calcium chloride standard solutions (1.0, 2.0, 3.0, 4.0, and 5.0 mM) with ionic strength adjusted to 80m*M* as described in Li et al. (2019). The milk samples and $CaCl_2$ solutions were allowed to sit in the

same water bath for at least one hour to provide a minimum temperature range (20 ± 1°C) for the measurements.

The electrode was dipped in the milk sample until the mV value was stabilised. The ionic calcium concentration was calculated from the recorded mV values using the linear equation for the calibration curve obtained.

3.2.4.6 Viscosity

The viscosities of raw and processed whole milk samples were measured by AR-G2 magnetic bearing rheometer (TA Instruments, Crawley, West Sussex, UK) with Peltier concentric cylinder geometries that include a standard cup (r = 15mm) and DIN rotor (r = 14mm, h = 44mm). The milk sample (20ml) was poured into the rheometer cup, and the measurement was carried out in a continuous flow procedure at 20°C. The shear rate was increased continuously from 10^{-2} to 10^{3} s^{-1} over 3 min. The measurement of each sample was duplicated with two sub-measurements. The average was taken from the stable values obtained at a shear rate range of 10-10^{2} s^{-1}.

3.2.4.7 Thermal analysis of fat

Thermal behaviour of goat and sheep milk fat were analysed in duplicate by differential scanning calorimetry (DSC) using TA Instruments DSC Q2000 (TA Instruments, New Castle, DE) equipped with Advantage Software (version 5.5.24). DSC technique measures the difference in heat flow rate (W/g) between a sample and reference material as a function of time and temperature when both materials are subjected to a controlled temperature program (Wright, 1984).

The milk fat was extracted using a method modified from Hara and Radin (1978). 1.5g of washed cream (Figure 3.1) was mixed with 24mL of organic solvents mixture (hexane and isopropanol at 3:2 v/v), and 12 mL of sodium sulfate solution (prepared from 1 g of the anhydrous salt and 15 ml of water). The top phase of the mixture containing the milk fat was transferred into a glass tube, and solvents were evaporated under a nitrogen stream. The extracted fat was stored at -20ºC until required for the thermal analysis.

The frozen fat samples were melted using a warm water bath to achieve homogenous sampling. Approximately 10µg of samples were weighed into T_{zero} aluminium hermetic pan and sealed. The DSC machine was equilibrated at 20°C, and the sample pan was placed with an empty reference pan on the platforms inside the furnace. The temperature was raised to 65ºC at a rate of 20ºC/min and held for 15 min. Then it was decreased to -40ºC at a rate of 10ºC/min, and the temperature

condition was maintained for 10 min. As a final step, the temperature was increased back to 60ºC at a rate of 10ºC/min.

The data were processed and analysed using TA Universal Analysis 2000 software (version. 4.5A). A baseline is drawn from - 40°C to 60°C to cover the whole melting range and the area between the melting curve, and the baseline was integrated. The milk fat was grouped as the low-melting fraction (LMF, < 5°C), the medium-melting fraction (MMF, 5-20°C), and the high-melting fraction (HMF, > 20°C) depending on their melting range. Each fraction was calculated using the equations below.

LMF = 100% - %SFC (5°C) (Eq.3.8)

MMF = 100% - (LMF + HMF) (Eq.3.9)

HMF = %SFC (20°C) (Eq.3.10)

3.2.5 Gelation properties

Gelation properties of raw and processed whole milk were measured by AR-G2 magnetic bearing rheometer (TA Instruments, Crawley, West Sussex, UK) with Peltier concentric cylinder geometries that include a standard cup (r = 15mm) and DIN rotor (r = 14mm, h = 44mm). Rheology Advantage Instrument Control AR software (version 5.8.2) was used for operating the instrument.

Storage modulus (G') is a parameter that defines the ability of the material to store energy or the elasticity of material in rheology. In contrast, loss modulus (G″) is the measurement of a viscous property of the material or defined as the ability of the material to lose energy. The loss tangent **(tan δ)** is the ratio of the viscous to the elastic behaviour (G″/G').

3.2.5.1 Rennet gelation

Rennet gelation was induced by chymosin (HANNILASE® XP 1050 NB; Christian Hansen A/S, Horsholm, Denmark), and the gelation test was carried out using low-amplitude oscillation procedure (0.1 Hz frequency and 0.01 applied strain) at 32ºC for 40 min as described by Glantz et al. (2011). The milk pH was adjusted to pH 6.5 using 0.1M HCl solution and prewarmed to 32ºC in a water bath. The warm milk was inoculated at 38 international milk

clotting unit (IMCU)/L and stirred for 2 min before transferred into the rheometer cup and starting the test.

3.2.5.2 Acid gelation

The milk samples were acidified by glucono-δ-lactone (GDL), and their gelation properties were measured by an 8-hour low-amplitude oscillation test as modified from Anema et al. (2004a) and Li et al. (2020).

The concentrations of GDL used for goat and sheep milk were selected as 1.8% (w/v) and 2.4% (w/v), respectively, to achieve the approximate pH of 4.2 after 8 hours of acidification. 100 ml of prewarmed milk (30°C) was mixed with GDL powder and stirred for 2 min using a magnetic stirrer.

Then 20 ml of acidified milk was transferred into the rheometer cup for oscillation test (0.1 Hz frequency and 0.01 applied strain). The remaining sample was transferred into a jacketed beaker connected with a water bath preset at 30°C, and the pH of acidified milk was recorded simultaneously at 1 min interval by HI-2202 edge®blu pH meter (Hanna Instruments, Woonsocket, RI).

3.2.5.3 Data processing

The time difference between the inoculation or acidification of the milk sample and the beginning of the test was recorded to determine the actual gelation time and other corresponding properties (final G′, gelation pH, and loss tangent). Rheology Advantage Data Analysis software (version 5.7.0) was used for analysing the data file. Gelation time was defined as the point at which the storage modulus (G′) turn greater than 1.0 Pa.

3.2.6 Statistical analysis

Minitab software (version 19.1.1) was used to analyse the results statistically. One-way ANOVA (95% confidence level) with Tukey post hoc test was used to determine the effect of seasonality or processing as a single factor. A two-way ANOVA test was used to determine the interaction effect of both factors on physicochemical and gelation properties. The correlation between variables (n = 9) was determined by the Pearson correlation test.

CHAPTER 4: EFFECT OF SEASONALITY AND PROCESSING ON GOAT MILK COMPOSITION AND CHARACTERISTICS

4.1 Introduction

Goat milk composition and their physicochemical properties have been extensively studied and reviewed in the literature (Clark & Mora García, 2017; Jenness, 1980; Park, 2007; Park et al., 2007). Milk (cow, goat and sheep) composition can be influenced by several factors such as animal breed, farming system and climate, which differ from region to region. However, a comprehensive investigation assessing the impact of seasonal variation in composition, and physicochemical and gelation properties of New Zealand goat milk has not yet been considered.

Understanding the impact of seasonality and processing conditions on goat milk characteristics is essential for improving the consistency in product quality and optimisation of industrial processing. This chapter evaluates goat milk characteristics determined during spring, summer and winter. Physicochemical and gelation properties of raw and processed goat milk have been investigated for each season to understand how processing is influenced by seasonality.

4.2 Results and discussion

4.2.1 Seasonal variation in goat milk composition

Seasonal variation in proximate composition of raw goat milk is summarised in Table 4.1. The average protein and fat contents in the goat milk were 3.2% and 3.8%, respectively, and these percentages were within the range reported for goat milk compositions previously (Jenness, 1980; Mayer & Fiechter, 2012; Park et al., 2007). However, goat milk from some indigenous breeds (Indian and Ethiopian) had a much higher concentration of fat and protein (Agnihotri & Rajkumar, 2007; Mestawet et al., 2012).

Table 4.1 Seasonal composition of raw goat milk

Components	Season	Concentration (%)
Fat	Spring	3.87 ± 0.24^{a}
	Summer	3.33 ± 0.03^{b}
	Winter	4.16 ± 0.09^{a}
Protein	Spring	3.21 ± 0.06^{ab}
	Summer	3.11 ± 0.02^{b}
	Winter	3.25 ± 0.06^{a}
Lactose	Spring	4.43 ± 0.02
	Summer	4.34 ± 0.06
	Winter	4.42 ± 0.00
Total solids	Spring	12.05 ± 0.30^{a}
	Summer	11.26 ± 0.03^{b}
	Winter	12.40 ± 0.01^{a}

Mean values (Mean ± SD) with different superscripts within the column differ significantly ($P < 0.05$).

Both fat and protein contents in goat milk increased during winter compared to those in summer. Similarly, fat and crude protein in non-seasonal goat milk decreased from spring (March) toward summer (May - June) and increased during late autumn (late October) in Europe (Mayer & Fiechter, 2012). Cow milk showed a similar trend in these components, having the minimum values in summer and the peak values in winter in non-seasonal calving system (Heck et al., 2009).

In contrast, the lactose concentration remained constant throughout the seasons (Table 4.1). Lactose concentration in cow milk is influenced by both seasonality and SOL and usually decreases toward the end of lactation (Auldist et al., 1998; Li et al., 2019; Phelan et al., 1982). A similar decrease in lactose concentration has also been observed in late-lactation goat milk (Barłowska et al., 2013). In the present study, the insignificant seasonal variation in lactose concentration of goat milk was likely the result of the non-seasonal kidding.

Table 4.2 shows the seasonal variation in the fraction of four caseins and the two principal whey proteins in goat milk. The proportion of α_s-, β- and κ-caseins were found within the same range as that reported previously in the literature. The current findings confirm that β-casein is the most abundant protein in goat milk; however, the proportion of α_{s1}-casein was found to be higher (~14% of total casein) than the average proportions reported previously (Park et al., 2006; Pesic

et al., 2012; Tamime et al., 2011). On the other hand, Ha et al. (2014) quantified a much higher proportion of α_{s1}-casein in New Zealand goat milk. The differences in quantifications may have been caused by genetic variants of goat milk protein, which could result in a varying amount of α_{s1}-casein in goat milk (Park et al., 2006).

Table 4.2 Fraction of major proteins in goat milk

Major proteins	Season	Percentage of total protein (%)
Total casein	Spring	80.8 ± 0.6
	Summer	80.9 ± 0.2
	Winter	79.8 ± 0.7
κ-casein	Spring	16.4 ± 0.2^{b}
	Summer	17.4 ± 0.1^{a}
	Winter	15.9 ± 0.2^{c}
α_{s2}-casein	Spring	8.6 ± 0.6
	Summer	9.0 ± 0.4
	Winter	9.0 ± 0.2
α_{s1}-casein	Spring	11.1 ±0.2
	Summer	11.1 ± 0.4
	Winter	10.6 ± 0.1
β-casein	Spring	44.7 ± 0.4
	Summer	43.5 ± 0.3
	Winter	44.3 ± 1.0
Total WP	Spring	19.2 ± 0.6
	Summer	19.1 ± 0.2
	Winter	20.2 ± 0.7
α-LA	Spring	8.5 ± 0.3
	Summer	8.3 ± 0.6
	Winter	8.2 ± 0.5
β-LG	Spring	10.7 ± 0.6^{b}
	Summer	10.7 ± 0.5^{b}
	Winter	12.0 ± 0.3^{a}

Mean values (Mean ± SD) with different superscripts within the column differ significantly ($P < 0.05$).

The proportion of κ-casein was the lowest in winter milk ($P < 0.05$), while the fraction of other proteins did not change significantly over the seasons. The percentage of β-LG in total protein increased in winter milk. Nonetheless, the proportions of total casein and WP remained consistent throughout the seasons (Table 4.2). The seasonal fluctuation of κ-casein might have been caused by insignificant changes in other protein fractions.

Seasonal variations in the concentration of goat milk minerals are shown in Table 4.3. Significant amounts of major minerals in goat milk; calcium, phosphorus, magnesium, potassium, sodium and chloride; were found, and other minerals such as copper and zinc were detected at trace levels only.

The average calcium and phosphorus contents in goat milk were 114 mg and 100 mg (per 100 g milk), respectively. The concentration of calcium and phosphorus increased significantly in winter milk, yet the amount of both minerals showed a small degree of variation (CV 4.7% and 2.8%, respectively). Mayer and Fiechter (2012) reported that calcium and phosphorus contents in goat milk were higher in winter than in summer, similar to the seasonal trend for the protein concentration. In contrast, magnesium level showed no seasonal variation.

With regards to soluble minerals, the levels of potassium and chloride increased during summer (Table 4.3). Similar trends for these minerals have also been observed by Mayer and Fiechter (2012). However, sodium level was unaffected by seasonality, which is attributable to the non-seasonal kidding practice for dairy goats. The summer milk was also different from the spring and winter milks in the concentrations of trace minerals. Copper and zinc levels decreased, and iodine level increased in goat milk during summer ($P < 0.05$).

Table 4.3 Mineral components of raw goat milk

Minerals	Season	Total amount
Calcium (g/100g)	Spring	0.113 ± 0.005^{b}
	Summer	0.110 ± 0.003^{b}
	Winter	0.120 ± 0.002^{a}
Phosphorus (g/100g)	Spring	0.101 ± 0.002^{ab}
	Summer	0.098 ± 0.002^{b}
	Winter	0.103 ± 0.001^{a}
Magnesium (g/100g)	Spring	0.014 ± 0.001
	Summer	0.014 ± 0.001
	Winter	0.014 ± 0.000
Potassium (g/100g)	Spring	0.200 ± 0.000^{b}
	Summer	0.213 ± 0.006^{a}
	Winter	0.196 ± 0.001^{b}
Sodium (g/100g)	Spring	0.037 ± 0.001
	Summer	0.036 ± 0.002
	Winter	0.036 ± 0.002
Chloride (g/100g)	Spring	0.159 ± 0.006^{b}
	Summer	0.174 ± 0.001^{a}
	Winter	0.159 ± 0.003^{b}
Copper (mg/kg)	Spring	0.107 ± 0.006^{a}
	Summer	0.081 ± 0.017^{b}
	Winter	0.122 ± 0.009^{a}
Iodine(mg/kg)	Spring	0.253 ± 0.029^{ab}
	Summer	0.293 ± 0.035^{a}
	Winter	0.205 ± 0.039^{b}
Selenium(mg/kg)	Spring	0.032 ± 0.003
	Summer	0.026 ± 0.003
	Winter	0.029 ± 0.004
Zinc(mg/kg)	Spring	3.600 ± 0.173^{a}
	Summer	3.267 ± 0.058^{b}
	Winter	3.867 ± 0.153^{a}

The mean values (Mean ± SD) with different superscript within the column differ significantly ($P < 0.05$).

The ratio of calcium and phosphorus to total protein was calculated and presented in Table 4.4. The Ca/P ratio in goat milk was 1.14 with no seasonal variation. The average amount of calcium and phosphorus per 1g protein was 35.7 mg and 31.4 mg, respectively and did not vary significantly.

Table 4.4 Seasonal variation in the ratio of some goat milk constituents

Ratio	Season	Value (Mean ± SD)
Ca/P	Spring	1.12 ± 0.02
	Summer	1.12 ± 0.03
	Winter	1.17 ± 0.03
Ca/Protein(mg/g)	Spring	35.0 ± 1.8
	Summer	35.2 ± 1.2
	Winter	36.9 ± 0.2
P/Protein(mg/g)	Spring	31.2 ± 1.0
	Summer	31.4 ± 0.8
	Winter	31.7 ± 0.9

Figure 4.1 shows the seasonal variation in mineral fractions of goat milk. About 30% of total calcium and 50% of total inorganic phosphorus was found in the soluble phase with no seasonal fluctuation ($P > 0.05$). The fraction of soluble magnesium was about 70% and remained consistent throughout the seasons.

The partition of soluble Ca (29.7%), P (49.7%), and Mg (69.2%) in goat milk were found to be closer to those reported for goat and cow milk (Holt et al., 1984; Keogh et al., 1982). However, O'connor and Fox (1977), reported lower concentrations of soluble Ca and P in cow milk by determining the concentration of these minerals in rennet whey. These authors attributed this to a possible shift in salt balance that may have been caused by pH adjustment before rennet coagulation. Nevertheless, the later studies used methods based on membrane ultrafiltration.

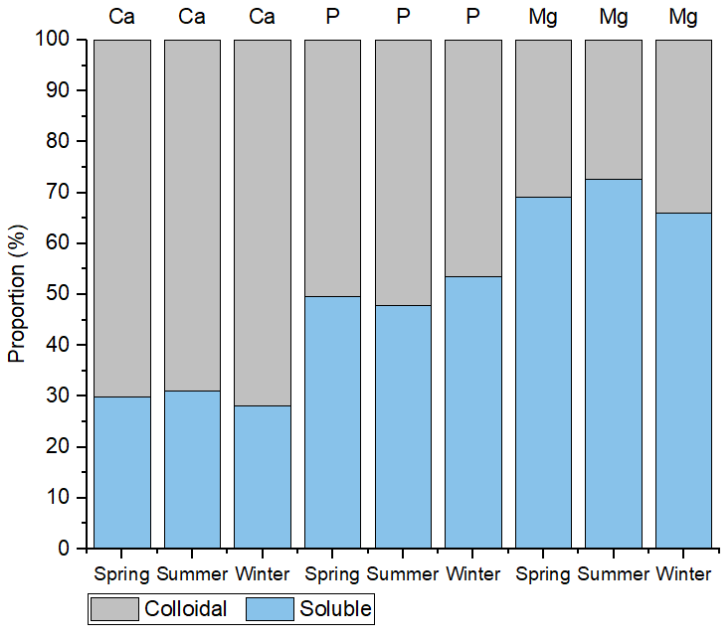


Figure 4.1 Distribution of minerals in goat milk across different seasons

Amongst all the samples analysed, the concentration of soluble Ca, P and Mg were largely similar, indicating minimal seasonal variation in the concentrations of soluble Ca, P and Mg in goat milk (Table 4.5). In comparison, for cow milk, the minerals in colloidal phase have been reported to increase, which is associated with an increased amount of total minerals (Ca and Mg) during the late lactation (Keogh et al., 1982). The consistent seasonal patterns for goat milk salt balance in the current study might be an indication of the minimal lactational impact on New Zealand goat milk.

4.2.2 Effect of seasonality and processing on goat milk characteristics

4.2.2.1 Characterisation of goat milk fat

DSC thermograms of milk fat obtained from different season goat milk are shown in Figure 4.2. The melting curve for spring milk had a shallow peak compared to the curves for the other two seasons. The curves for summer and winter milk fat showed major peaks at around 15°C and 10°C, respectively. The overall heat-flow pattern at temperatures > 20°C was similar for all seasons' milk fat samples.

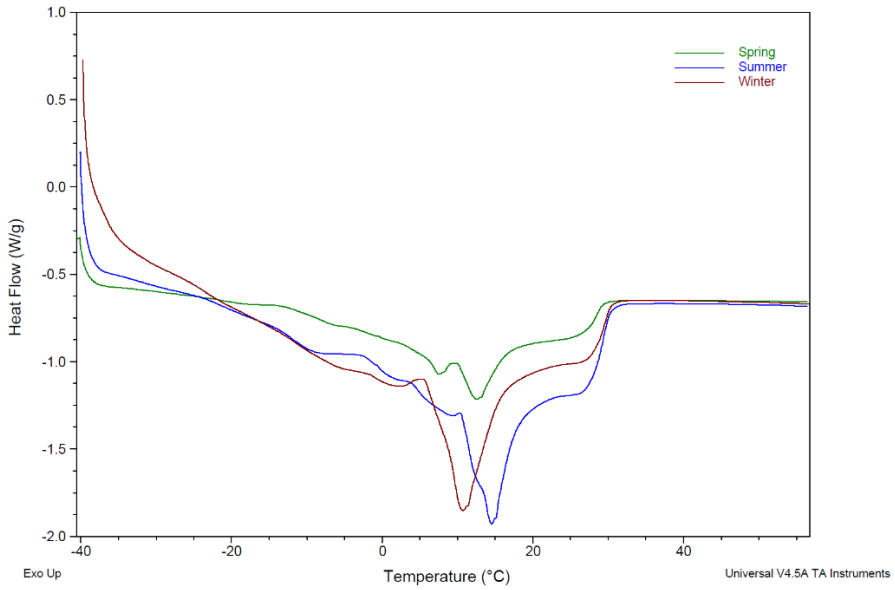


Figure 4.2 DSC melting curves for goat milk fat from different seasons

Table 4.5 presents the seasonal variation in the melting behaviour of goat milk fat. The proportions of fat in LMF, MMF and HMF were calculated using the equations 3.8 – 3.10 (See section 3.2.4.7, Chapter 3). In winter milk, fat in LMF increased while MMF decreased compared to the fractions in spring and summer.

Table 4.5 Seasonal variation in melting behaviour of goat milk fat

Melting range	Season	Fraction (%)
LMF (< 5°C)	Spring	54.1 ± 0.6[b]
	Summer	54.1 ± 0.5[b]
	Winter	59.9 ± 0.7[a]
	Average	56.1 ± 0.3
MMF (5-20°C)	Spring	30.6 ± 1.4[a]
	Summer	30.0 ± 0.9[a]
	Winter	25.2 ± 0.8[b]
	Average	28.6 ± 2.8
HMF (> 20°C)	Spring	15.3 ± 0.9
	Summer	15.8 ± 0.7
	Winter	14.9 ± 0.3
	Average	15.3 ± 0.7

Mean values (Mean ± SD) with different superscripts within the column differ significantly ($P < 0.05$).

The seasonal variation in the thermal properties of milk fat mainly arises from changing fatty acid composition over the seasons. Although, FA composition has not been considered in the scope of this study. Typically, LMF is largely contributed by saturated short-chain FAs and unsaturated long-chain FAs, whereas HMF consists of TAGs with saturated long-chain fatty acids (Lopez, 2020). In New Zealand cow milk, the percentages of the liquid fat at various temperatures (-20-30°C) was negatively correlated with the percentage of long-chain fatty acids (C_{14}-C_{19}) and positively correlated with the sum of short-chain (C_4-C_8) and unsaturated fatty acids (Norris et al., 1973). The elevated LMF in winter goat milk fat might arise from a shift in the feed composition over the seasons, which influences the *de novo* synthesis of FAs (Heck et al., 2009).

Figure 4.3 shows the SDS-PAGE gel image for MFGM proteins in unprocessed spring and summer goat milk. The major proteins were identified as MUC 1 (250 kDa), Xanthine oxidase (XO) (116 kDa), Butyrophilin (67 kDa) and PAS 6/7 (48 kDa). There was no significant difference between spring and summer milk in the proportions of the major MFGM proteins.

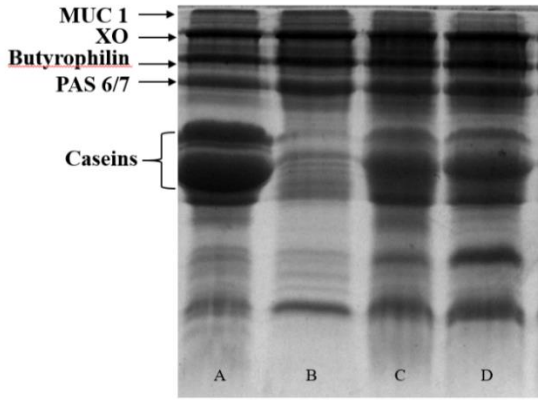


Figure 4.3 Identification of major MFGM proteins in goat milk from spring (A-B), and summer (C-D)

4.2.2.2 Physicochemical properties

Table 4.6 shows the seasonal and processing effects on the physicochemical properties of goat milk. Overall, the impact of seasonality on the physicochemical properties was minimal, but processing affected all properties except for milk pH and buffering capacity.

Table 4.6 Effect of seasonality and processing conditions on physicochemical characteristics of goat milk

Physicochemical properties	Season	R	P	HP	H	* Season × Process interaction
pH	Spring	6.65	6.68	6.68	6.65	NS
	Summer	6.68	6.68	6.73	6.69	
	Winter	6.69	6.69	6.69	6.69	
	SEM	0.01	0.01	0.01	0.02	
Buffering capacity (dB/dpH)	Spring	0.025	0.023	0.021	0.023	NS
	Summer	0.020	0.019	0.019	0.020	
	Winter	0.023	0.023	0.022	0.023	
	SEM	0.001	0.001	0.001	0.001	
Ionic calcium (mM)	Spring	3.02	2.92	2.80	2.55	NS
	Summer	3.26^{a}	3.20^{a}	3.06^{ab}	2.82^{b}	
	Winter	3.27^{a}	3.20^{ab}	3.02^{bc}	2.84^{c}	
	SEM	0.07	0.07	0.08	0.08	
Ethanol stability (%)	Spring	51.7^{A}	ND	ND	ND	ND
	Summer	46.7^{B}	ND	ND	ND	
	Winter	47.5^{B}	ND	ND	ND	
	SEM	0.8	-	-	-	
Casein micelle size (nm)	Spring	209.5^{b}	226.7^{aA}	n/a	239.6^{a}	$P < 0.05$
	Summer	218.6^{ab}	213.8^{bAB}	n/a	237.0^{a}	
	Winter	207.2^{b}	208.3^{bB}	n/a	257.7^{a}	
	SEM	2.94	3.3	-	5.46	
Fat globule size (D [4,3])	Spring	4.00^{a}	4.48^{a}	0.67^{b}	0.65^{b}	NS
	Summer	3.82^{a}	3.99^{a}	0.72^{b}	0.71^{b}	
	Winter	4.17^{a}	4.15^{a}	0.72^{b}	0.60^{b}	
	SEM	0.07	0.15	0.02	0.03	
Viscosity (cP)	Spring	3.71	3.66	3.65^{A}	3.80	NS
	Summer	3.73^{a}	3.59^{bc}	3.52^{cB}	3.63^{b}	
	Winter	3.81^{b}	3.63^{d}	3.71^{cA}	3.91^{a}	
	SEM	0.04	0.02	0.03	0.04	

R - Raw; P - Pasteurised (75°C/15s) without homogenisation; HP - Homogenised and pasteurised (75°C/15s); H - Heated (95°C/5 min) with homogenisation; NS - Not significant; ND - Not determined; SEM – Standard error of mean.

^{abcd} Mean values with different lowercase superscripts within the same row differ significantly ($P < 0.05$).

^{AB} Mean values with different uppercase superscripts within the same column differ significantly ($P < 0.05$).

* Analysed by two-way ANOVA test.

The average pH of raw goat milk was measured at 6.67, unaffected by either seasonality or processing. The buffering capacity of raw goat milk was 0.023 and remained consistent regardless of seasonality and processing types. The milk BC is mainly influenced by the concentration of buffering constituents such as protein and phosphate (Salaün et al., 2005). In the current study, variations in the total protein content (CV 2.4%) and phosphate (CV 2.8%) were relatively small. This might be the reason for consistent BC across the seasons. The average amount of ionic calcium in raw goat milk was determined as 3.02 mM in spring and then increased to 3.26 mM during summer and winter, although the increase was not significant ($P = 0.302$).

The ethanol stability of raw goat milk was 51.7% during spring and decreased to 47.1% across summer and winter ($P < 0.05$). Lin et al. (2006) found that lower ethanol stability was associated with higher ionic calcium concentration. Thus, the decreased ethanol stability during summer and winter may have resulted from the increased ionic calcium concentrations ($P = 0.064$, $r = -0.639$).

The effect of heat treatment on ionic calcium concentration was significant in summer and winter. Heating goat milk at 95°C for 5 min resulted in a 15-16% reduction in ionic calcium concentration as compared to that of raw milk (Table 4.7). The same effect of heat treatment on ionic calcium level in milk has been well reported. When milk is heated, the solubility of calcium phosphate decreases, and this might lead to the change in ionic calcium concentration (De La Fuente et al., 1999; Lewis, 2011; On-Nom et al., 2010). Li et al. (2019) also observed a similar extent of reduction in ionic calcium content after cow milk was heated at 90°C for 6 min.

The average casein micelle size in raw goat milk was measured as 211.8 nm and was significantly affected by the interaction of seasonality and processing (Table 4.6). No seasonal variation in micelle size was observed for raw and heated milk (95°C/5 min). But the micelle size in pasteurised milk was smaller in winter than in spring. Pasteurisation (75°C/ 15s) did not consistently modify the micelle size in goat milk. However, heating milk at 95°C for 5 min increased the micelle size across all seasons and showed a more pronounced effect during winter (24% increase, $P < 0.05$).

Similarly, Raynal and Remeuf (1998) and Hovjecki et al. (2020) reported that pasteurisation (75°C/30s) did not modify the micelle size in goat milk. But a higher degree of heat treatments (90°C/1 min and 95°C/5 min) increased the micelle size to a similar extent observed in this study. The association of denatured β-LG with casein micelles is presumably the main reason for increased casein micelle size in heated milk. The more severe the heat treatment, the bigger the micelle size was measured in cow milk (Anema & Li, 2003).

On the other hand, the average fat globule size in raw goat milk was measured as 4.00 μm and was unaffected by seasonality. Homogenisation decreased the average diameter of MFG 5-7 times, as normally expected. Heat treatment showed no significant impact on the MFG size (Table 4.6).

The viscosity of raw goat milk was measured to be 3.75 cP as an average and showed no significant variation throughout the year ($P > 0.05$). Overall, seasonality showed no consistent effect on the viscosity of goat milk. As shown in Table 4.6, pasteurisation decreased the viscosity of raw milk in summer and winter, but the same effect was not observed in spring milk. The viscosity of heated milk (H) increased by up to 5% compared to that of pasteurised milk (HP), although the increase was not significant in spring ($P < 0.05$).

The increased viscosity of heated milk can be explained by the subsequent increase in volume fraction resulting from the heat-induced change in casein micelle size (Anema & Li, 2003). Jeurnink and De Kruif (1993) demonstrated that the denaturation of β-LG and their association with casein micelles is largely responsible for the increased viscosity of heated milk. On the other hand, the effect of homogenisation on the viscosity was not significant. It only resulted in a 2% increase ($P < 0.05$) in winter milk viscosity.

4.2.2.3 Heat-induced changes

Different levels of whey protein denaturation in processed goat milk were presented in Figure 4.4. Pasteurisation did not result in a considerable level of WP denaturation, but heating milk at 95°C for 5 min resulted in a much higher level of WP denaturation. The denaturation level of total whey proteins in pasteurised (75°C/15s) and heated (95°C/5 min) milk were up to 13% and 96%, respectively.

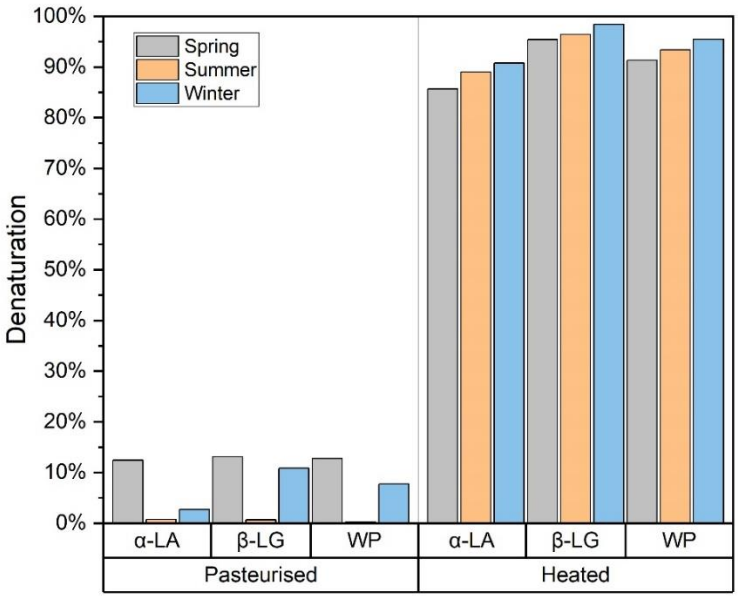


Figure 4.4 Denaturation level of whey proteins after heat treatment determined by HPLC

Table 4.7 shows the seasonal variation in the level of whey protein denaturation in pasteurised (75°C/15s) and heated (95°C/5 min) goat milk. The denaturation level of total WP in heated milk was significantly higher in winter than in spring.

Table 4.7 Effect of heat treatments on denaturation level (%) of whey proteins in goat milk

Heat treatments	Season	α-LA	β-LG	Total WP
75°C/15s	Spring	12.4 ± 5.5^{a}	13.1 ± 8.9	12.8 ± 7.2^{a}
	Summer	0.7 ± 1.1^{b}	0.7 ± 1.2	0.3 ±0.8^{b}
	Winter	2.7 ± 1.1^{b}	10.8 ± 2.0	7.7 ± 1.5^{ab}
	Average	5.3 ± 6.1	8.2 ± 7.3	6.9 ± 6.6
95°C/5 min	Spring	85.7 ± 2.3^{b}	95.4 ± 1.0^{b}	91.3 ± 1.6^{b}
	Summer	89.0 ± 1.6^{ab}	96.4 ± 0.2^{b}	93.3 ± 0.9^{ab}
	Winter	90.7 ± 1.2^{a}	98.4 ± 0.2^{a}	95.5 ± 0.5^{a}
	Average	88.5 ± 2.7	96.7 ± 1.4	93.4 ± 2.0

Mean values (Mean ± SD) with different superscripts within the column differ significantly ($P < 0.05$).

Whey protein distribution in heated goat milk (95°C/5 min) is shown in Figure 4.5. Approximately 85% of total WP was found associated with micelles, while the remaining was found in serum aggregates and native forms. Compared to β-LG, α-LA was denatured to a lower

degree under the same heating condition (95°C/5 min) (Table 4.8). Above 95% of β-LG was denatured, most of which was bound to the micelles. However, the denaturation of α-LA was slightly lower (85%) as compared to β-LG, and only 70% of α-LA was found associated with the casein micelles as compared to > 95% for β-LG.

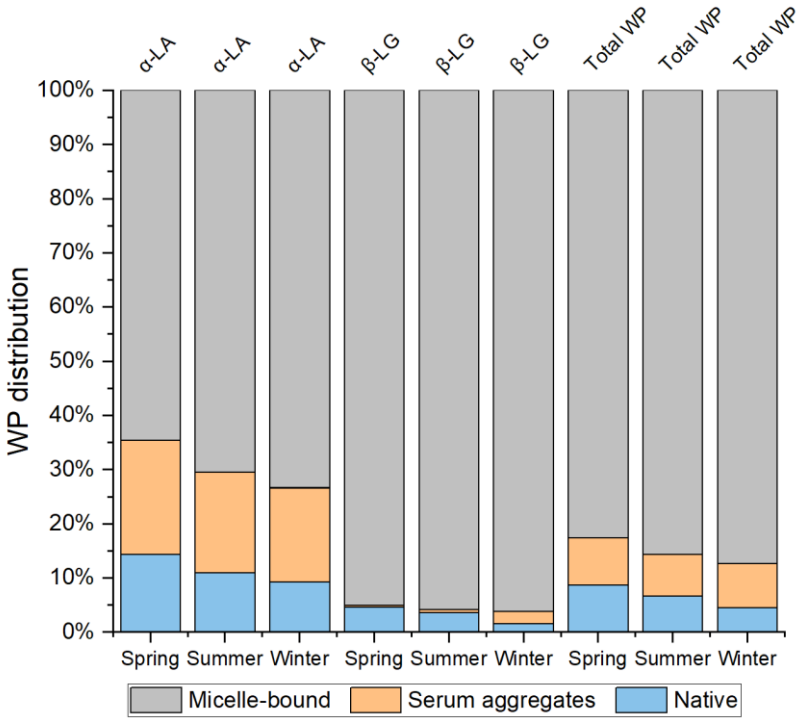


Figure 4.5 Whey protein distribution in goat milk heated at 95°C for 5 min

The lower level of α- LA denaturation was in agreement with Pesic et al. (2012) who reported that α-LA was more heat stable than β-LG in goat milk. These differences between the denaturation levels of α-LA and β-LG have also been observed in cow milk (Lucey et al., 1997; Pesic et al., 2012). In general, α-LA has a strong cation binding site that attracts ionic calcium, and such binding increases the protein stability against heat and denaturing agents (Permyakov & Berliner, 2000).

The proportion of micelle-bound denatured WP in heated milk (95°C/5 min) was higher in winter than in spring, while the opposite trend was found for the proportion of native WP ($P < 0.05$). The proportion of β-LG in milk proteins correlated with the degree of WP denaturation ($P = 0.012$, $r = 0.786$), and to a lesser extent, the level of WP-casein micelle association ($P = 0.065$, $r = 0.637$). A higher proportion of β-LG in winter milk (Table 4.2) likely played a part in promoting the overall whey protein denaturation and their complexation with the micelles. Because β-LG also catalyses the irreversible denaturation of α-LA by forming a disulphide bond (Li et al., 2019).

4.2.3 Effect of seasonality and processing conditions on gelation properties of goat milk

4.2.3.1 Rennet gelation

The effect of seasonality and processing on rennet gelation properties of goat milk is presented in Figure 4.6. Rennet gelation time of goat milk was unaffected by seasonality or processing conditions. Processing showed an impact on the storage modulus of goat milk gel, but the effect was not consistent across the seasons.

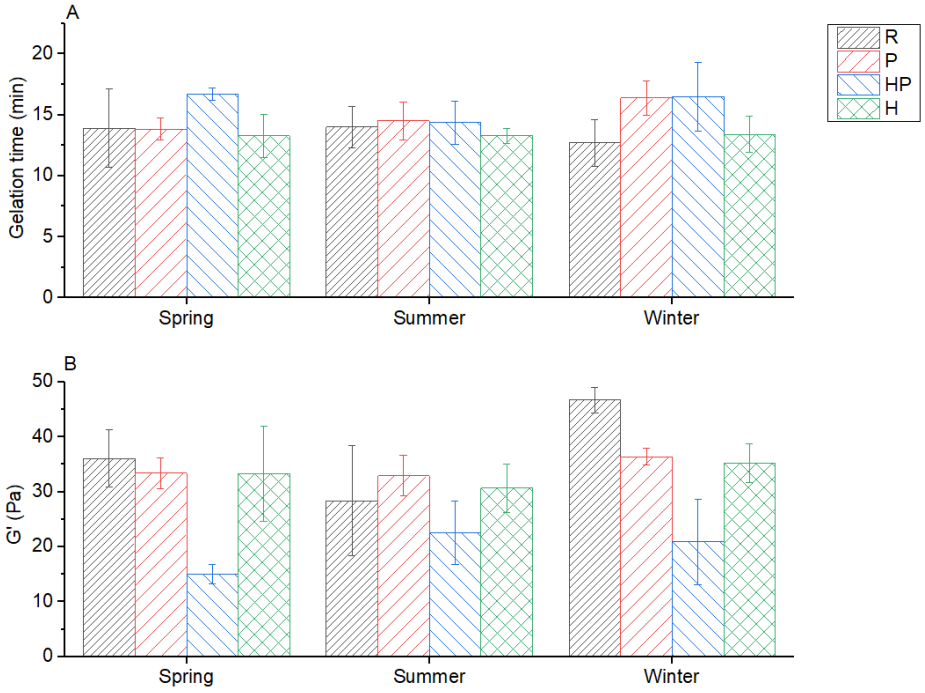


Figure 4.6 Processing effect on rennet gelation time (A) and final storage modulus (B) of goat milk. R - Raw; P - Pasteurised (75°C/15s) without homogenisation; HP - Homogenised and pasteurised (75°C/15s); H - Heated (95°C/5 min) with homogenisation. Error bars indicate the standard deviation of measurements from three different individual experiments.

The average results of rennet gelation properties (gelation time, G', G" and Tan δ) for raw and processed goat milks were summarised in Table 4.8. Rennet gelation time for raw goat milk was 13.5 min, which remained consistent regardless of the seasons or processing conditions. Neither homogenisation nor heat treatments affected the gelation time significantly. The same renneting behaviour of goat milk has been observed in several studies. The coagulation time of goat milk was not influenced by various heat condition, which notably different from that of cow milk (Miloradovic et al., 2020; Montilla et al., 1995; Raynal & Remeuf, 1998).

Table 4.8 Effect of seasonality and processing conditions on rennet gelation properties of goat milk

Properties	Season	R	P	HP	H	* Season × Process interaction
Rennet gelation time (min)	Spring	13.9	13.8	16.7	13.3	NS
	Summer	14	14.5	14.4	13.3	
	Winter	12.7	16.4	16.5	13.4	
	SEM	0.7	0.5	0.7	0.4	
Final storage modulus (G')	Spring	36.1^{aAB}	33.4^{a}	15.0^{b}	33.3^{a}	NS
	Summer	28.4^{B}	32.9	22.5	30.7	
	Winter	46.7^{aA}	36.4^{b}	20.9^{c}	35.2^{b}	
	SEM	3.3	1.0	2.0	1.8	
Final LT (Tan δ)	Spring	0.474^{aA}	0.422^{b}	0.334^{c}	0.337^{c}	P < 0.05
	Summer	0.447^{aAB}	0.433^{b}	0.346^{c}	0.340^{c}	
	Winter	0.435^{aB}	0.420^{b}	0.326^{c}	0.330^{c}	
	SEM	0.007	0.003	0.005	0.002	

R - Raw; P - Pasteurised (75°C/15s) without homogenisation; HP - Homogenised and pasteurised (75°C/15s); H - Heated (95°C/5 min) with homogenisation; NS - Not significant; SEM – Standard error of mean.

^{abc} Mean values with different lowercase superscripts within the same row differ significantly ($P < 0.05$).

^{AB} Mean values with different uppercase superscripts within the same column differ significantly ($P < 0.05$).

* Analysed by two-way ANOVA test.

Storage modulus (G') of raw goat milk gel showed seasonal variation (spring > winter), but gels made of processed goat milks did not show seasonal differences in their G' values. The G' value dropped markedly in the gels made of homogenised milk, but the decrease was not significant during summer ($P > 0.05$). On the other hand, high temperature (95°C/5 min) increased the G' (H > HP) during spring and winter, but not during summer. The overall impact of heating on the rennet gelation properties of goat milk was far less pronounced than those in cow milk. This will be further discussed in Chapter 6.

The seasonal variation in gel strength (G') could be explained by the concentration of milk components. The G' value was positively correlated with total protein, casein, total calcium concentration and colloidal calcium content ($P < 0.05$). The higher concentration of casein and total protein contributes to a coarser gel network, probably by increasing the availability of proteins for gel formation, although the protein content is not a sole factor on the rennet-gel firmness (Guinee et al., 1997; Storry et al., 1983). The amount of CCP and caseins in the micelles have a great influence on rennet-gel development. Increasing CCP (up to 30%) by addition of calcium salt increased the G' of rennet gels made of skim milk (Udabage et al., 2001).

In homogenised milk, the fat globule surface is partially covered by casein micelles to stabilise the newly formed fat globules with disrupted MFGM. This adsorption of proteins promotes the fat globules as an active filler in the gel strand, which has been reported to improve the gel strength in cow milk (Storry et al., 1983; Titapiccolo et al., 2010). However, it was interesting that homogenisation caused a marked decrease in the final G' of goat milk gels (Table 4.8). This indicates that the rennet gelation properties of goat milk response to processing may be different from those of cow milk.

The final LT of raw goat milk gel was lower in winter compared to that in spring, but there was no seasonal variation in the final LT of processed goat milk gels. Both pasteurisation and homogenisation decreased the final LT across all three seasons (Table 4.8).

4.2.3.2 Acid gelation

Figure 4.7 shows the overall processing impact on acid gelation properties of goat milk in different seasons. Overall, the effect of processing was not strong on gelation properties of goat milk, and the gel strength was relatively weak ($G' < 25$ Pa) irrespective of seasonality and processing. The pH profile during the gelation process was unaffected by processing, reaching around 4.00 after 8 hours of acidification as designed from the preliminary trials.

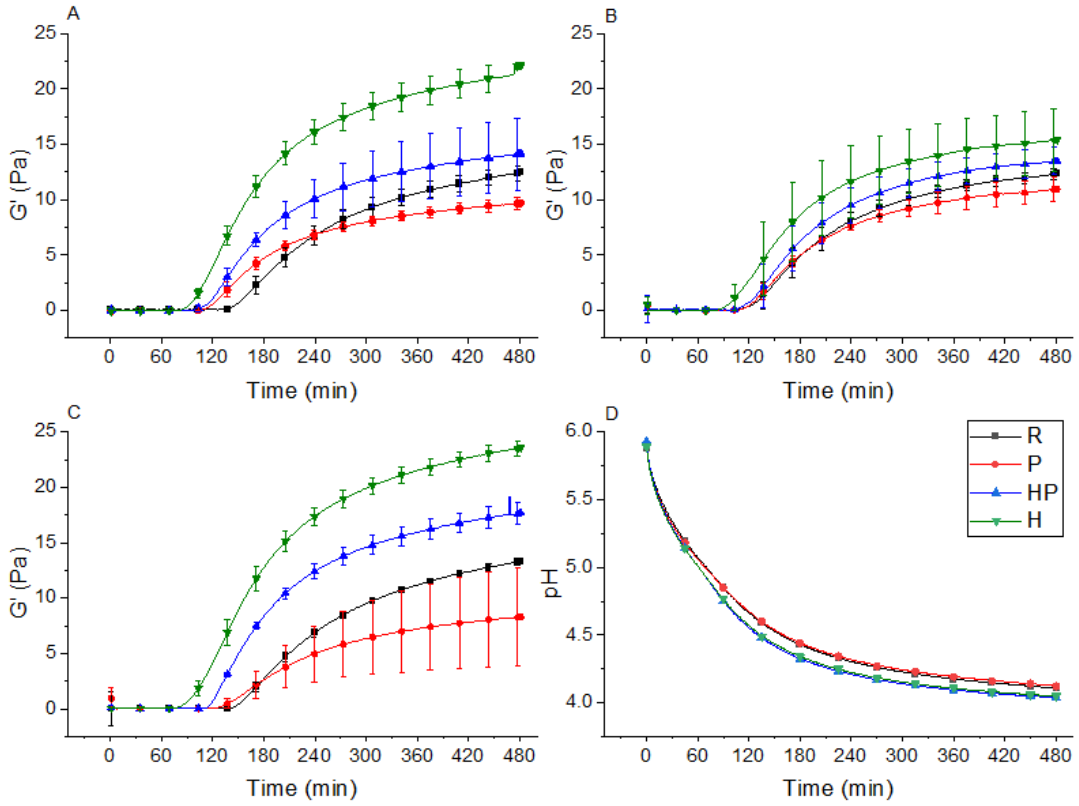


Figure 4.7 Processing effect on acid gelation properties of spring (A), summer (B), and winter (C) goat milk and pH profile during acidification (D). R - Raw; P - Pasteurised (75°C/15s) without homogenisation; HP - Homogenised and pasteurised (75°C/15s); H - Heated (95°C/5 min) with homogenisation. Error bars indicate the standard deviation of measurements from three different individual experiments.

Table 4.9 summarises the acid gelation properties of raw and processed goat milk in different seasons. The average acid gelation time for raw goat milk was 162 min in spring and winter and decreased to 140 min in summer. However, the seasonal effect was insignificant ($P > 0.05$) on the gelation time for any processed milk. Pasteurisation and homogenisation did not cause a consistent change in gelation time, but heat treatment (95°C/5 min) coupled with homogenisation shortened the gelation time significantly. The gelation pH was recorded around isoelectric point (4.50-4.60) for raw and pasteurised milks (unhomogenised and homogenised), however, increased to the range 4.70-4.80 for the milk heated at 95°C for 5 min.

Table 4.9 Effect of seasonality and processing conditions on acid gelation properties of goat milk

Properties	Season	R	P	HP	H	* Season × Process interaction
Acid gelation time (min)	Spring	160.5^{aA}	130.9^{b}	123.8^{bc}	103.4^{c}	NS
	Summer	139.6^{aB}	136.7^{a}	125.6^{ab}	100.9^{b}	
	Winter	163.8^{aA}	150.3^{a}	127.7^{b}	100.9^{c}	
	SEM	4.8	4.5	2.8	1.8	
Gelation pH	Spring	4.55^{b}	4.58^{b}	4.61^{b}	4.80^{a}	$P < 0.05$
	Summer	4.55^{b}	4.55^{b}	4.58^{b}	4.71^{a}	
	Winter	4.55^{c}	4.55^{c}	4.67^{b}	4.85^{a}	
	SEM	0.01	0.01	0.02	0.02	
Final storage modulus (G')	Spring	12.4^{b}	9.6^{b}	14.1^{b}	21.3^{aA}	$P < 0.01$
	Summer	12.3^{bc}	10.9^{c}	13.7^{b}	17.4^{aB}	
	Winter	13.2^{c}	10.9^{d}	17.6^{b}	23.5^{aA}	
	SEM	0.2	0.3	0.8	0.9	
Final LT (Tan δ)	Spring	0.297^{a}	0.280^{b}	0.243^{c}	0.229^{d}	NS
	Summer	0.293^{a}	0.285^{a}	0.247^{b}	0.226^{c}	
	Winter	0.293^{a}	0.282^{b}	0.228^{c}	0.221^{d}	
	SEM	0.002	0.001	0.004	0.002	

R - Raw; P - Pasteurised (75°C/15s) without homogenisation; HP - Homogenised and pasteurised (75°C/15s); H - Heated (95°C/5 min) with homogenisation; NS - Not significant; SEM – Standard error of mean.

^{abcd} Mean values with different lowercase superscripts within the same row differ significantly ($P < 0.05$).

^{AB} Mean values with different uppercase superscripts within the same column differ significantly ($P < 0.05$).

* Analysed by two-way ANOVA test.

The interaction of seasonality and processing on the final G' was significant ($P < 0.01$). The seasonality showed an impact on the final G' of the gel made of heated milk (95°C/5 min) but showed no effect on those made of other types of milk. Pasteurisation without homogenisation decreased the final G' in winter but did not affect the G' in spring and summer. Homogenisation increased the final G' in summer and winter, but the effect was insignificant on the G' in spring.

The most consistent change was the increase of final G' in the gels made of heated milk (95°C/5 min).

The final LT of acid-gel was not influenced by seasonality but was significantly affected by processing conditions. The magnitude of decrease in LT followed the sequence H > HP > P. Pasteurisation decreased the final LT by up to 6%, but this was not significant in summer ($P > 0.05$). Similarly, homogenisation resulted in up to a 24% decrease in the final LT, while heating (95°C/5 min) caused a further 9% decrease in the final LT of goat milk acid-gels (Table 4.9).

Overall, heating goat milk at 95°C for 5 min resulted in shorter gelation time, higher gelation pH and stronger gel formation. These findings were consistent with the heating effect (above 80°C) on acid gelation properties of cow milk (Anema et al., 2004a; Lucey et al., 1999; Lucey et al., 1997). The improved acid gelation properties of heated milk are attributed to the heat-induced denaturation of β-LG and their possible interactions with κ-casein (micellar or serum phase), or among denatured WPs (del Angel & Dalgleish, 2006; Lucey et al., 1998).

Anema et al. (2004a) underlined the significance of soluble denatured WP on the final G' of the acid gel made of heated milk. The denatured WPs in the serum phase increase the aggregating particles, which potentially would be involved in the gel matrix. Lucey and Singh (1997) also suggested the mechanisms behind the increased G' for the gels made of heated milk to be the increased number and strength of bonds between the casein micelles and more active participation of WPs in the gel network.

Increased gelation pH has been observed for heated cow milk and may be explained by the higher isoelectric point of β-LG (~ 5.3) or the reduced hydration barrier against aggregation of casein micelles due to the complex formation (Anema et al., 2004a; Heertje et al., 1985; Lucey & Singh, 1997). However, the gelation pH of heated goat milk was much lower than that of cow milk (above pH 5.00).

The acid gelation properties of heated (95°C/5 min) goat milk seemed to be influenced mainly by the slight variations in milk solids content. The total protein, total casein and fat contents were positively correlated with the G' of heated milk ($P < 0.05$). This was in contrast with New Zealand cow milk, whose acid gelation properties varied significantly over the seasons even when standardised for protein and fat contents (Li et al., 2020). The difference between the species in their gelation behaviour will be discussed in Chapter 6.

4.3 Conclusion

Goat milk was similar to cow milk in the concentration of major and mineral components, but the two milks differ in the proportion of individual proteins. Goat milk contains more β-casein and less α_{s1}-casein than cow milk, which may partially explain the different characteristics and response of goat milk to different processing conditions.

Overall, the impact of seasonality (time of the year) was not strong on compositional and physicochemical characteristics of goat milk, possibly due to the nature of non-seasonal milk production system in which the lactation effect was minimised. Despite the similarities in proximate composition, gelation properties of goat milk were notably different from those of cow milk. The level of improvement in acid gelation properties of goat milk achieved by heating was relatively small.

CHAPTER 5: EFFECT OF SEASONALITY AND PROCESSING ON SHEEP MILK COMPOSITION AND CHARACTERISTICS

5.1 Introduction

Sheep milk has been well characterized for its nutritional aspects (Balthazar et al., 2017; Park et al., 2007; Wendorff & Haenlein, 2017), and processability for cheese and yoghurt (Nguyen et al., 2018; Park, 2007). However, information about the seasonal effect on physicochemical and rheological properties of sheep milk is limited. The objective of this chapter is to investigate the effect of seasonality and processing on physicochemical and gelation properties of sheep milk.

The farming system for dairy sheep in New Zealand was briefly described earlier (Chapter 3). Unlike goat milk being supplied throughout the entire year, sheep milk is produced from spring-lambing flocks for a relatively short period. For this reason, we expect to observe more evident seasonal variation in sheep milk characteristics because of the impact of the progressing stages of lactation.

5.2 Results and discussion

5.2.1 Seasonal variation in sheep milk composition

Table 5.1 shows the composition of major components in sheep milk over the seasons. The average fat and protein contents of sheep milk during the milking seasons were 6.0% and 5.7%, respectively. Fat and protein levels in early- and mid-season sheep milk were consistent, but both increased in the late-season milk by approximately 10%. This increase in fat and protein contents translated into differences in total solids of milk, but these differences were comparatively smaller (< 5%).

Seasonal trends in fat and protein contents of sheep milk resembled those observed for cow milk. In a seasonal-calving system, fat and protein levels in cow milk remained relatively consistent across early-mid seasons, then increased markedly toward the late season (Auldist et al., 1998; Li et al., 2019). The increased amounts are most likely caused by the concentrating effect of lower milk yield during late lactation (Auldist et al., 1998).

Table 5.1 Major components of raw sheep milk

Components	Milking seasons	Concentration (%)
Fat	Early	5.75 ± 0.29^{b}
	Mid	5.68 ± 0.03^{b}
	Late	6.53 ± 0.15^{a}
Protein	Early	5.59 ± 0.08^{b}
	Mid	5.51 ± 0.05^{b}
	Late	6.08 ± 0.16^{a}
Lactose	Early	4.84 ± 0.02^{a}
	Mid	4.83 ± 0.03^{a}
	Late	4.60 ± 0.02^{b}
Total solids	Early	17.17 ± 0.22^{b}
	Mid	16.99 ± 0.03^{b}
	Late	18.21 ± 0.29^{a}

Mean values (Mean ± SD) with different superscript within the column differ significantly ($P < 0.05$).

The changing fat content in milk can be partially attributed to the fibre content in the pasture (Phelan et al., 1982). However, Auldist et al. (2000) concluded that diet is not the primary cause for seasonal variation in milk composition. In sheep milk produced under semi-intensive farming conditions, fat and protein contents similarly increased with advancing lactation (Aganga et al., 2002; Kuchtík et al., 2008).

In comparison, the lactose content in sheep milk decreased in late season compared to that in early- and mid-season (Table 5.1). This trend is similar to that reported for cow milk in a seasonal calving system (Li et al., 2019; Phelan et al., 1982). Lactose plays a vital role in maintaining osmotic equilibrium between alveolar cells of the mammary gland and bloodstream (Fox et al., 2015h; Wendorff & Haenlein, 2017). The concentration of lactose decreases during late lactation to compensate for the increase in sodium and chloride to regulate osmotic pressure in the system (Phelan et al., 1982).

In sheep milk, lactose concentration was reported to be the highest during early lactation, then decreased steadily toward the end of lactation (Mayer & Fiechter, 2012; Pavić et al., 2002). By contrast, lactose content in sheep milk from certain breeds in the Mediterranean region (Karagouniki, Serron and Red Karaman) was not affected by SOL (Polychroniadou &

Vafopoulou, 1985; Yilmaz et al., 2011). It is possible that other factors such as animal breed and regional climate (extreme weather) result in different seasonal effects.

The fractions of casein and whey protein for different seasons are presented in Table 5.2. Total casein accounted for 86.2% of total protein in sheep milk consisting of α_{s1}-, α_{s2}-, β- and κ-caseins at roughly 4:1.5:3.5:1 ratio.

Table 5.2 Fraction of major proteins in sheep milk

Major proteins	Milking season	Percentage of total protein (%)
Total casein	Early	86.6 ± 0.3
	Mid	86.1 ± 0.4
	Late	85.8 ± 0.4
κ-casein	Early	10.1 ± 0.5^{b}
	Mid	12.1 ± 0.6^{a}
	Late	11.3 ± 0.2^{a}
α_{s2}-casein	Early	12.3 ±1.3
	Mid	11.9 ±1.3
	Late	11.4 ± 0.4
α_{s1}-casein	Early	35.0 ± 1.5
	Mid	33.4 ±0.5
	Late	33.9 ± 0.7
β-casein	Early	29.1 ± 0.4
	Mid	28.6 ± 0.6
	Late	29.1 ± 0.6
Total WP	Early	13.4 ± 0.3
	Mid	13.9 ± 0.4
	Late	14.2 ± 0.4
α-LA	Early	4.5 ± 0.4
	Mid	5.1 ± 0.7
	Late	4.5 ± 0.1
β-LG	Early	8.8 ± 0.1^{b}
	Mid	8.8 ± 0.5^{b}
	Late	9.7 ± 0.3^{a}

Mean values (Mean ± SD) with different superscript within the column differ significantly ($P < 0.05$)

The proportion of α_{s1}-, α_{s2}-, and β-casein in sheep milk did not vary significantly over the seasons. However, the portion of κ-casein was the lowest during the early season then increased significantly ($P < 0.05$) in the later seasons.

In sheep milk, α_{s1}- and β-caseins were found to be the dominant caseins, which agreed with the review by Bramanti et al. (2003). They reported that the fraction of α_{s1}- and β-caseins in sheep milk accounted for 35% and 38% of total casein, respectively. In contrast, Ruprichova et al. (2015) reported that the proportion of β-casein alone represented about 70% of total casein in sheep milk. Despite the similar separation method, Ha et al. (2014) determined a lower level of α_{s1}-casein and a much higher amount of β-casein in sheep milk than the current study. The protein quantification in sheep milk, therefore, seems to be divergent in the literature. It is likely that these differences arise due to differences in sheep breeds or genetic variants that may produce milk with different protein composition.

The percentage of sheep milk WP was 13.8 % of total protein, and α-LA and β-LG were quantified at the ratio of 1:2. In cow milk, the α-LA level is positively correlated to the lactose content as it plays an important role in lactose synthesis (Heck et al., 2009; Li et al., 2019). However, the correlation between the levels of two components in sheep milk was not found significant ($P < 0.1$, $r = 0.610$), as the proportion of α-LA, remained consistent throughout the seasons.

The proportion of β-LG in total protein increased significantly (10%) in the late-season sheep milk (Table 5.2), which was found similar to the reports for some ruminant's milk. The level of β-LG showed an increase in cow milk during late lactation (Regester & Smithers, 1991; Sanderson, 1970). Likewise, the concentration of β-LG increased ($P < 0.01$) in goat and sheep milk toward the end of lactation. The changing level of β-LG might be related to its biological function of binding and transporting hydrophobic compounds to the offspring (Hejtmánková et al., 2012).

The mineral components of sheep milk and their seasonal variations are shown in Table 5.3. Macro-minerals in milk - calcium, phosphorus, magnesium, potassium, sodium and chloride are found in significant amounts and other minerals such as copper and zinc at trace level. Average calcium and phosphorus contents were determined to be 200 mg and 160 mg per 100 g milk, respectively. The calcium concentration showed no seasonal variation, and similarly phosphorus content remained relatively consistent except for a slight decrease in mid-season ($P < 0.05$).

Table 5.3 Mineral components of raw sheep milk

Minerals	Season	Total amount
Calcium (g/100g)	Early	0.195 ± 0.006
	Mid	0.199 ± 0.002
	Late	0.207 ± 0.006
Phosphorus (g/100g)	Early	0.162 ± 0.003^{a}
	Mid	0.156 ± 0.000^{b}
	Late	0.162 ± 0.002^{a}
Magnesium (g/100g)	Early	0.016 ± 0.000^{b}
	Mid	0.017 ± 0.000^{b}
	Late	0.020 ± 0.001^{a}
Potassium (g/100g)	Early	0.136 ± 0.003^{a}
	Mid	0.137 ± 0.001^{a}
	Late	0.123 ± 0.005^{b}
Sodium (g/100g)	Early	0.036 ± 0.002^{b}
	Mid	0.038 ± 0.001^{b}
	Late	0.051 ± 0.002^{a}
Chloride (g/100g)	Early	0.076 ± 0.004^{c}
	Mid	0.089 ± 0.001^{b}
	Late	0.103 ± 0.002^{a}
Copper (mg/kg)	Early	0.263 ± 0.031^{a}
	Mid	0.135 ± 0.010^{b}
	Late	0.114 ± 0.014^{b}
Iodine (mg/kg)	Early	0.243 ± 0.038
	Mid	0.191 ± 0.033
	Late	0.239 ± 0.046
Selenium (mg/kg)	Early	0.033 ± 0.001^{b}
	Mid	0.041 ± 0.004^{a}
	Late	0.033 ± 0.004^{b}
Zinc(mg/kg)	Early	6.133 ± 0.231^{a}
	Mid	5.767 ± 0.153^{ab}
	Late	5.567 ± 0.231^{b}

The mean values (Mean ± SD) with different superscript within the column differ significantly ($P < 0.05$).

The other major mineral contents changed significantly in late season. Magnesium level increased by 25% in late season compared to the level in early season. Chloride content increased continuously (up to 35%) toward the end of lactation. There was a 35% decrease in the K/Na ratio in late-season sheep milk as the amount of potassium decreased, and sodium increased. The concentrations of Na and Cl in late-season sheep milk increased along with the decrease in lactose content, which agreed with Phelan et al., (1982). The differences in the mineral contents observed in late-season sheep milk may be associated with feeding practice, however this was not scope of the present work and further experiments are necessary to ascertain this.

The mean concentrations of major minerals in sheep milk were found in close proximate to the findings of a previous study in Europe, except for lower chloride content (Mayer & Fiechter, 2012). The concentrations of calcium and magnesium in sheep milk were much higher than those in cow milk (Moreno-Rojas et al., 1994) and both positively correlated with total protein content ($P < 0.01$). Poulsen et al. (2015) made a conclusion about the same correlation between these minerals and protein content in cow milk. This might be due to a considerable amount of those minerals being associated with casein micelles in the colloidal phase.

Soluble minerals such as Na and K in sheep milk were quantified as lower than the average amounts determined in cow milk (Keogh et al., 1982; Moreno-Rojas et al., 1994). The ratio of Na and K increased in the late season as the concentration of the former increased and the latter decreased. The increase in Na/K ratio is due to the enhanced permeability of mammary epithelium and indicates lower milk secretion (Auldist et al., 1998; Stelwagen et al., 1999). Early-season sheep milk was higher in copper and zinc. The copper content was remarkably higher in early season, then dropped by nearly half in the later seasons. The amount of zinc decreased gradually (up to 10%) toward the end of lactation.

The ratios of some sheep milk constituents are calculated as shown in Table 5.4. The average ratio of Ca/P in sheep milk was 1.25 and varied slightly throughout the seasons. The average amounts of calcium and phosphorus per 1g protein were 35.1 mg and 28.1 mg, respectively and decreased in late season with a higher concentration of protein.

Table 5.4 Seasonal variation in the ratio of some sheep milk constituents

Ratio	Season	Value
Ca/P	Early	1.20 ± 0.02^{b}
	Mid	1.27 ± 0.01^{a}
	Late	1.28 ± 0.02^{a}
Ca/Protein (mg/g)	Early	35 ± 0.8^{ab}
	Mid	36 ± 0.2^{a}
	Late	34 ± 0.7^{b}
P/Protein (mg/g)	Early	29 ± 0.6^{a}
	Mid	28 ± 0.2^{a}
	Late	27 ± 0.5^{b}

The mean values (Mean ± SD) with different superscript within the column differ significantly ($P < 0.05$).

The mineral distribution between soluble and colloidal phase is shown in Figure 5.1. Approximately one-fifth of total calcium in sheep milk was found in the serum part, and this serum calcium concentration remained consistent across all seasons. Soluble inorganic phosphorus was 38.7 % of the total P_i and gradually decreased (by up to 10%) until late season. About 60% of magnesium was determined in the serum phase and showed no seasonal variation.

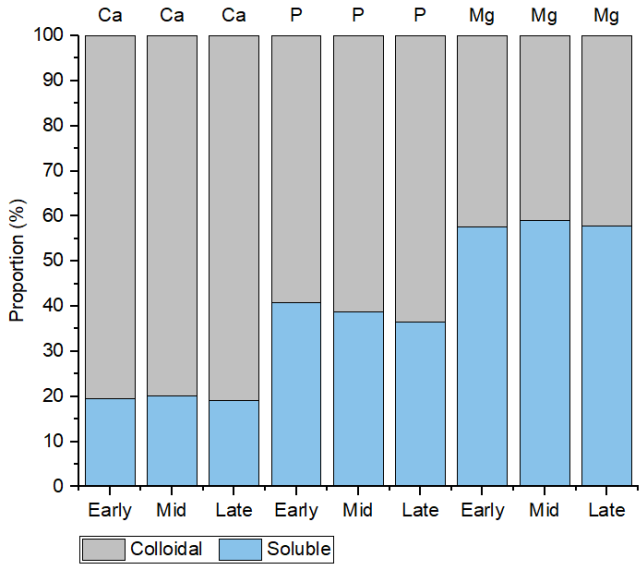


Figure 5.1 Distribution of minerals in sheep milk across different seasons

The major mineral distribution between colloidal and soluble phase in sheep milk was similar to the results in the earlier study of O'connor and Fox (1977). The authors determined soluble calcium and inorganic phosphorus in mid-season sheep milk as 18% and 39% of their total amounts, respectively. In cow milk, the percentage of soluble Ca (~ 30%), and P_i (~ 50%) was found (Holt et al., 1984; Keogh et al., 1982) higher than the averages mentioned above for sheep milk.

5.2.2 Effect of seasonality and processing on sheep milk characteristics

5.2.2.1 Characterisation of sheep milk fat

The DSC thermograms of triglycerides of the early, mid- and late-season sheep milk are shown in Figure 5.2. The melting curves for early- and mid-season sheep milk fat were almost identical in shape and showed the major peaks around 10°C. In comparison, the late-season milk triglycerides displayed different melting behaviour in the range of -10 to 25°C. Also, the melting points of late-season sheep milk fat were higher than those observed in earlier seasons.

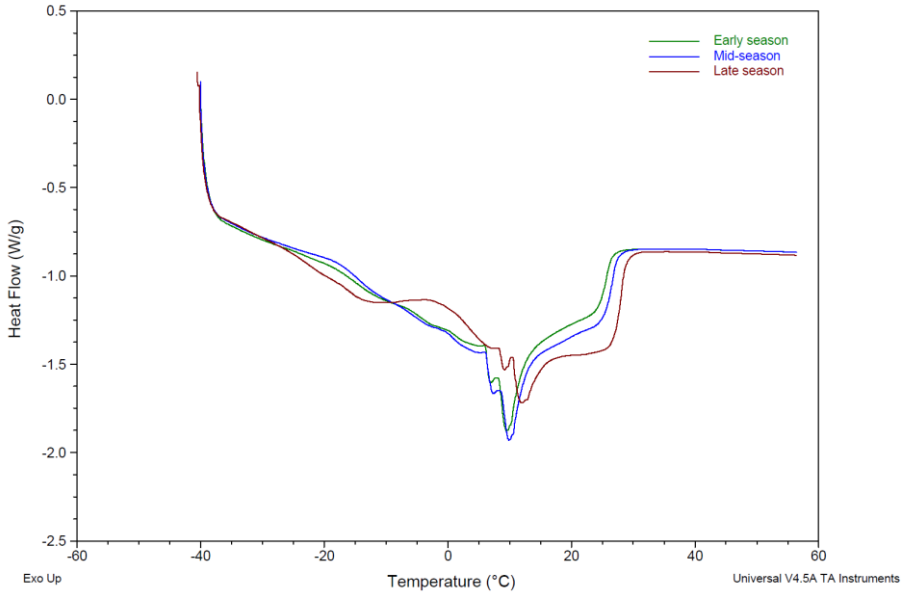


Figure 5.2 DSC melting curves for sheep milk fat from different seasons

Table 5.5 shows the seasonal variation in three melting fractions of sheep milk fat. The average proportions of fat in LMF and MMF were 59.4% and 26.3%, respectively, with no seasonal variation. But, the proportion of fat in HMF was significantly higher ($P < 0.05$) in the late season than in early and mid-season.

Table 5.5 Seasonal variation in melting behaviour of sheep milk fat

Melting range	Season	Fraction (%)
LMF (< 5°C)	Early	59.8 ± 1.1
	Mid	58.5 ± 2.8
	Late	59.7 ± 0.9
	Average	59.4 ± 1.7
MMF (5 - 20°C)	Early	27.0 ± 0.7
	Mid	27.5 ± 2.3
	Late	24.3 ± 1.1
	Average	26.3 ± 2.0
HMF (> 20°C)	Early	13.2 ± 0.5^{b}
	Mid	13.9 ± 0.5^{b}
	Late	16.0 ± 0.5^{a}
	Average	14.4 ± 1.3

The mean values (Mean ± SD) with different superscript within the column differ significantly ($P < 0.05$).

The seasonal difference in the melting curves can be attributed to the changing fatty acid composition over the seasons. The increased HMF during late season indicates a higher concentration of long-chain SFAs primarily contributed by C_{16} (Lopez, 2020). The variation in HMF in sheep milk triglycerides coincided with the typical seasonal trend observed for New Zealand cow milk triglycerides. Typically, long-chain SFA and solid fat contents are the greatest during summer and the lowest during spring (Auldist et al., 1998; Norris et al., 1973). In the current study, sheep milk was sampled across spring (Early and Mid) and summer (Late), hence making it possible to observe a clear difference between the two seasons (time of the year). As concluded by Auldist et al. (1998), summer milk fat was harder irrespective of SOL, suggesting a possible role of other factors, such as diet, in altering the triglyceride composition. In New Zealand, summer pasture is more mature than spring and autumn pasture, containing more SFAs. This might have led to the highest HMF in late-season sheep milk fat (Norris et al., 1973).

Figure 5.3 shows the SDS-PAGE gel image for MFGM proteins in raw sheep milk obtained from three milking seasons. The major proteins were identified as MUC 1 (228 kDa), Xanthine oxidase (XO) (125 kDa), Butyrophilin (66 kDa) and PAS 6/7 (48 kDa). There was no significant difference in the proportions of the major MFGM proteins over the seasons.

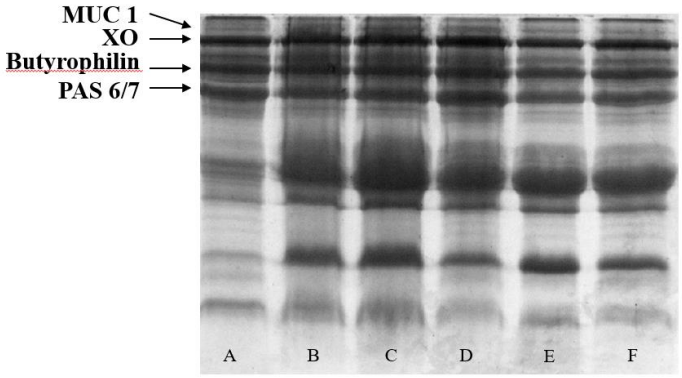


Figure 5.3 Identification of major MFGM proteins in sheep milk fat obtained in early (A), mid (B-C) and late (D-E) season

5.2.2.2 Physicochemical properties

The impact of seasonality and processing conditions on physicochemical properties of raw and processed sheep milk are summarised in Table 5.6. All properties except for ionic calcium concentration were significantly ($P < 0.05$) influenced by seasonality and processing.

The average pH for raw sheep milk was 6.60 throughout the milking seasons and showed little variation during early and mid-season but decreased significantly in the late season. Processing conditions did not affect the pH of raw milk *(*$P > 0.05$). The average buffering capacity for raw sheep milk was calculated as 0.040 (dB/dpH) and none of the processing types affected the BC. But late-season raw and processed milks had higher buffering capacity ($P < 0.05$) than the milks from earlier seasons. Buffering capacity mainly depends on the compositional properties of milk, particularly protein and mineral constituents (Salaün et al., 2005). In the current study, the correlation between protein concentration and BC of sheep milk was not significant ($P > 0.05$), although both variables followed similar seasonal patterns.

Table 5.6 Effect of seasonality and processing conditions on physicochemical characteristics of sheep milk

Physicochemical properties	Season	R	P	HP	H	*Season × Process interaction
pH	Early	6.64^{A}	6.67^{A}	6.67^{A}	6.65	NS
	Mid	6.62^{A}	6.65^{A}	6.63^{AB}	6.60	
	Late	6.54^{B}	6.57^{B}	6.58^{B}	6.56	
	SEM	0.02	0.02	0.02	0.02	
Buffering capacity (dB/dpH)	Early	0.039^{aB}	0.038^{abB}	0.035^{bC}	0.037^{abB}	NS
	Mid	0.040^{B}	0.038^{B}	0.038^{B}	0.038^{B}	
	Late	0.043^{A}	0.041^{A}	0.041^{A}	0.042^{A}	
	SEM	0.001	0.001	0.001	0.001	
Ionic calcium (mM)	Early	2.53	2.56	2.50	2.33	NS
	Mid	2.66	2.64	2.54	2.42	
	Late	2.92	2.68	2.67	2.73	
	SEM	0.09	0.05	0.05	0.08	
Ethanol stability	Early	52.5^{A}	ND	ND	ND	ND
	Mid	53.3^{A}	ND	ND	ND	
	Late	48.3^{B}	ND	ND	ND	
	SEM	0.9	-	-	-	
Casein micelle size (nm)	Early	183.8^{bA}	188.1^{bA}	n/a	205.9^{aB}	P < 0.01
	Mid	173.3^{bC}	178.0^{bB}	n/a	210.1^{aB}	
	Late	179.1^{bB}	182.2^{bAB}	n/a	264.3^{aA}	
	SEM	1.63	1.90	-	9.92	
Fat globule size (D [4,3])	Early	4.60^{aA}	4.65^{a}	0.58^{b}	0.58^{b}	NS
	Mid	4.51^{aAB}	4.40^{a}	0.56^{b}	0.66^{b}	
	Late	4.40^{aB}	4.40^{a}	0.52^{b}	0.54^{b}	
	SEM	0.04	0.06	0.01	0.03	
Viscosity (cP)	Early	4.44^{abB}	4.34^{bB}	4.67^{abB}	4.85^{aB}	P < 0.05
	Mid	4.61^{bAB}	4.34^{cB}	4.55^{bB}	4.76^{aB}	
	Late	4.85^{abA}	4.61^{bA}	4.86^{abA}	6.34^{aA}	
	SEM	0.08	0.05	0.05	0.03	

R - Raw; P - Pasteurised (75°C/15s) without homogenisation; HP - Homogenised and pasteurised (75°C/15s); H - Heated (95°C/5 min) with homogenisation; ND - Not determined; NS - Not significant; SEM – Standard error of mean.

^{abc} Mean values with different lowercase superscripts within the same row differ significantly ($P < 0.05$).

^{ABC} Mean values with different uppercase superscripts within the same column differ significantly ($P < 0.05$).

*Analysed by two-way ANOVA test.

The average concentration of ionic calcium in raw sheep milk was 2.70 mM, and it was not significantly affected by seasonality or processing conditions. Ethanol stability of sheep skim milk was consistent across early to mid-season and decreased in late season (Late > Early, Mid). As shown in Table 5.7, the sheep milk with lower pH had lower ethanol stability. The ionic calcium concentration was positively correlated with total protein and calcium, and negatively correlated with ethanol stability.

Table 5.7 The correlations between the physicochemical properties of sheep milk

	Protein	**Total Ca**	**Total P_i**	**Ethanol stability**	**Ca^{2+}**	**pH**
Total Ca	0.812**					
Total P_i	0.587	0.364				
Ethanol stability	-0.863**	-0.723*	-0.368			
Ca^{2+}	0.727*	0.811**	0.292	-0.750*		
pH	-0.814**	-0.576	-0.150	0.865**	-0.607	
BC	0.644	0.512	0.191	-0.728*	0.536	-0.804**

* $P < 0.05$.

** $P < 0.01$.

The sigmoidal relationship between milk pH (6.0-7.2) and ethanol stability has been established earlier by Horne and Parker (1981). Lin et al. (2006) also observed that an increasing amount of total calcium elevated ionic calcium concentration in milk further, leading to lower pH and poorer ethanol stability. It has been suggested that increasing Ca^{2+} concentration results in a higher amount of CCP and increases the micelle stability, which would require a higher concentration of ethanol for inducing coagulation of milk (Horne & Parker, 1981).

Ethanol stability of sheep milk at natural pH (Table 5.6) was much lower than that of cow milk (Horne & Parker, 1981; Li et al., 2019; Tsioulpas et al., 2007). Lower ethanol stability of late-season sheep milk and its correlation with protein content was in agreement with a recent report for seasonal cow milk (Li et al., 2019). The authors report that a higher amount of protein, particularly casein, in late season milk may be responsible for promoting ethanol-induced aggregation of casein micelles.

The average diameter of casein micelle was measured as 178.7 nm for raw sheep milk. The interaction effect of seasonality and processing was significant ($P < 0.01$) on casein micelle size. Heating milk at 95°C increased the micelle size but the extent of increase was different in each

season. In both raw and pasteurised milk, the casein micelles size was largest in the early season and smallest in the mid-season, whereas heated milk had the biggest micelle size in late season (Late > Early, Mid). There was a strong correlation ($P < 0.001$, $r = -0.950$) found between the fraction of κ-casein and the micelle size. The increased proportion of κ -casein during the early season can be attributed to the decreased size of casein micelles as κ-caseins are located on the micelle surface. The same correlation between micelle size and proportion of κ-casein was also reported in the earlier studies on cow milk (Dalgleish et al., 1981; Davies & Law, 1983).

The average diameter of fat globule in raw sheep milk was measured 4.5 μm with slight seasonal variation. The diameter of MFG was bigger in early season compared with that in late season (Table 5.6). Homogenisation reduced the fat globule size 7-8 times as normally expected. Pasteurisation did not change the natural size of MFG ($P > 0.05$). The reduced fat globule size in pasteurised (HP) and heated (H) milks with homogenisation were not significantly different ($P > 0.05$). Bigger fat globules during early season is in agreement with the seasonal trend observed for cow MFG size and can be explained by the limited synthesis of MFGM materials during early lactation (Fleming et al., 2017; Li et al., 2019; Wiking et al., 2004).

The viscosity of raw sheep milk was measured as 4.64 cP as an average of all-season results. The season × processing interaction effect was significant on viscosity ($P < 0.05$). Overall, late-season raw and processed milks had a higher viscosity than early and mid-season milks. Homogenisation increased the viscosity of mid-season milk, but it did not change the viscosity of early- and late-season milks (Table 5.6). The viscosity of heated milk (95°C for 5 min) increased the viscosity compared to that of unhomogenised and pasteurised (P) milk in all seasons ($P < 0.05$). The extent of this increase was greater (37%) in late season than in early and mid-season (up to 11%).

The seasonal change in viscosity of fresh whole milk can be related to its fat and TS concentrations (Park, 2007; Spreer, 1998). In this study, there was no significant correlation found between the viscosity of raw sheep milk and the dry matter contents ($P > 0.05$). But the viscosity of heated sheep milk was strongly correlated with the micelle size ($P < 0.01$, $r = 0.848$). It has been suggested that the association of denatured WP with casein micelles increases the particle size in heated milk. This heat-induced change in the micelle structure increases the volume fraction, which would consequently result in higher viscosity (Anema & Li, 2003; Jeurnink & De Kruif, 1993).

5.2.2.3 Heat-induced changes

Figure 5.4 shows the denaturation level of whey proteins in sheep milk after two different heat treatments. Heating milk at 95°C for 5 mins resulted in a greater extent of whey protein denaturation than pasteurisation (75°C/15s). The average denaturation level of total WP was 17% in pasteurised milk, whereas almost complete denaturation (98%) of total WP was achieved in heated milk (95°C for 5 min). The denaturation levels of α-LA and β-LG were 96% and 99%, respectively.

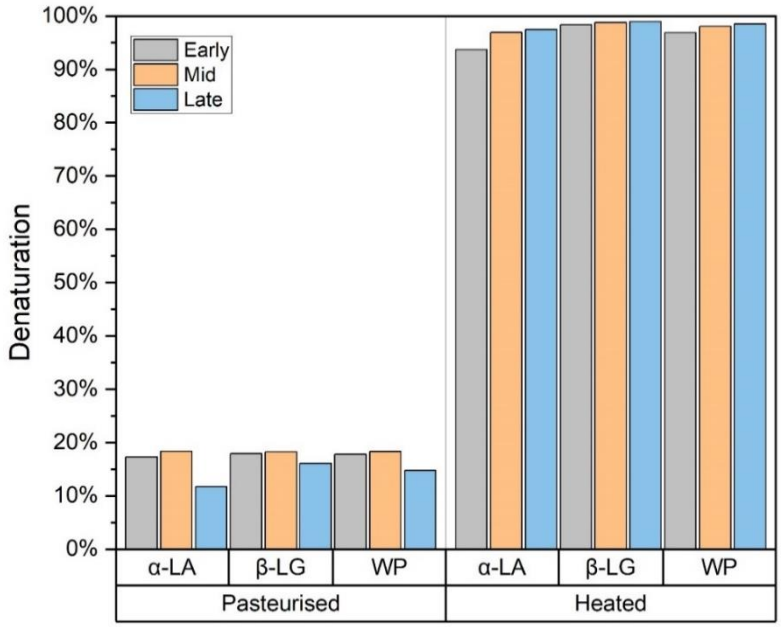


Figure 5.4 Denaturation level of whey proteins after heat treatment determined by HPLC

Seasonal variation in denaturation levels of sheep milk WP is presented in Table 5.8. The denaturation level of WPs in pasteurised milk showed no significant difference across the seasons. On the other hand, the denaturation level of α-LA in heated milk (95°C/5 min) was slightly lower in the early season than the consistent levels across mid-and late seasons. As a result, the total WP denaturation in early-season heated milk was lower than those in mid-and late-season heated milks.

Table 5.8 Effect of heat treatments on denaturation level (%) of whey proteins in sheep

Process	Season	α-LA	β-LG	WP
75°C/15s	Early	17.3 ± 5.2	17.9 ± 0.4	17.8 ± 1.9
	Mid	18.4 ± 2.6	18.3 ± 2.5	18.3 ± 2.2
	Late	11.7 ± 4.7	16.1 ± 7.3	14.7 ± 6.3
	Average	15.8 ± 4.9	17.4 ± 4.0	17.0 ± 3.9
95°C/5 min	Early	93.7 ± 0.8[b]	98.4 ± 0.4	96.9 ± 0.1[b]
	Mid	96.9 ± 0.3[a]	98.8 ± 0.3	98.1 ± 0.3[a]
	Late	97.5 ± 0.1[a]	99.0 ± 0.2	98.5 ± 0.1[a]
	Average	96.0 ± 1.8	99.0 ± 0.4	98.0 ± 0.8

[ab] Mean values (Mean ± SD) with different superscript within the same column differ significantly ($P < 0.05$).

Figure 5.5 demonstrates the WP distribution in sheep milk heated at 95°C for 5 min. More than 90% of total WP was found associated with micelles, and the level of serum aggregates and native whey proteins together accounted for up to 5% of total WP.

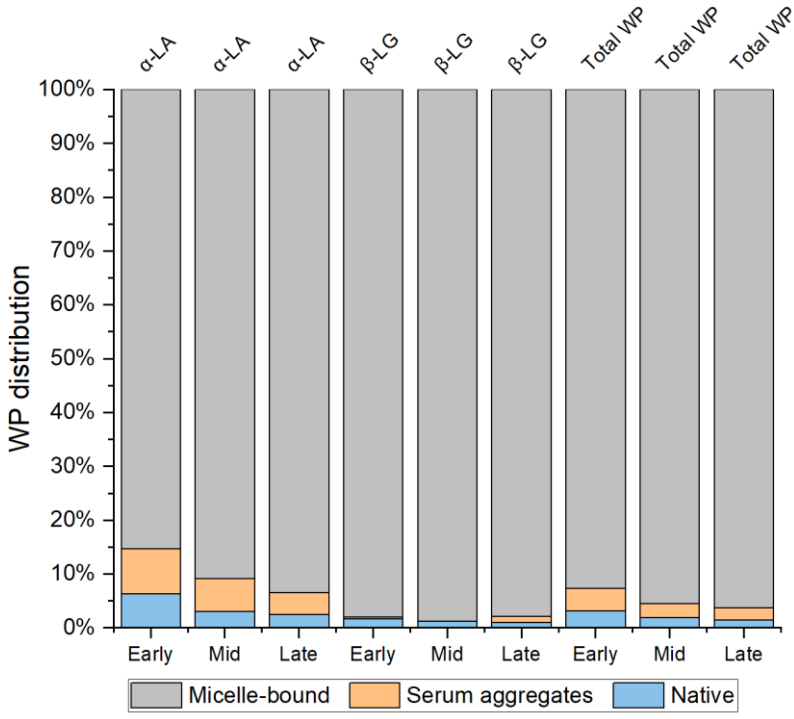


Figure 5.5 Whey protein distribution in sheep milk heated at 95°C for 5 min

The extent of WP denaturation and their association with casein micelles in heated sheep milk (95°C for 5 min) was significantly lower in early season than in mid-and late seasons ($P < 0.01$). Li et al., (2019) found a similar seasonal pattern for the proportion of micelle-bound WP in cow milk heated at 90°C for 6 min and its correlation with the proportion of β-LG in the total WP.

The proportion of micelle-bound WP was positively correlated with the denaturation level of total WP ($P = 0.001$, $r = 0.895$) and ionic calcium concentration ($P = 0.050$, $r = 0.666$), while the denaturation level of WP was positively correlated with the fraction of WP in total proteins ($P < 0.041$, $r = 0.687$). Smits and Van Brouwershaven (1980) found a reduced association of β-LG with casein micelles with lowering ionic calcium concentration in the milk system heated at 90°C. The ionic calcium probably influences the surface properties of micelles by binding with ionic groups of the proteins and affects the interaction between β-LG and casein micelles (Smits & Van Brouwershaven, 1980).

5.2.3 Effect of seasonality and processing on gelation properties of sheep milk

5.2.3.1 Rennet gelation

The overall processing effect on rennet gelation properties of sheep milk in three milking seasons is shown below (Figure 5.6). Rennet gelation time of raw and processed sheep milk was relatively consistent, while G' value decreased with processing. Table 5.9 shows the average values for rennet gelation properties of sheep milk as affected by seasonality and processing conditions.

The average rennet gelation time of raw sheep milk was 14.4 min and was unaffected by seasonality. Processing did not show a consistent effect on gelation time over the seasons. During early and mid-season, the processing effect was insignificant on the gelation time. In the late season, however, pasteurisation (without homogenisation) prolonged the gelation time significantly ($P < 0.05$).

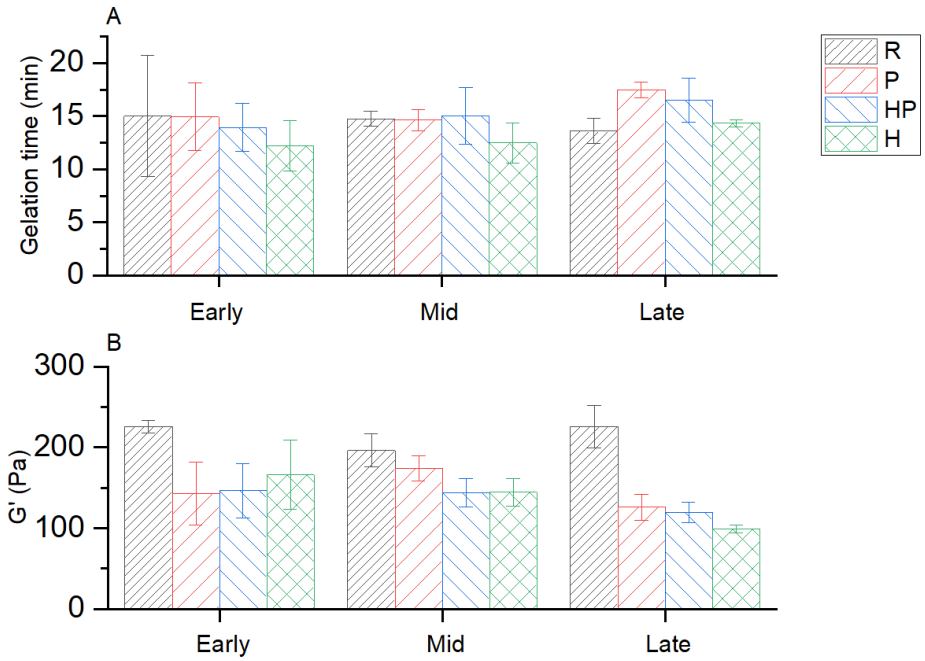


Figure 5.6 Processing effect on rennet gelation time (A) and final storage modulus (B) of sheep milk; R - Raw; P - Pasteurised (75°C/15s) without homogenisation; HP - Homogenised and pasteurised (75°C/15s); H - Heated (95°C/5 min) with homogenisation. Error bars indicate the standard deviation of measurements from three different individual experiments.

Processing did not cause a consistent change in the final G' of sheep milk rennet-gel. The final G' was not affected by processing in the early season, but it decreased sharply after pasteurisation in the late season. Seasonality affected the final G' of heated milk gels only. The final G' of heated milk decreased in late season compared to that in early season (Table 5.9). Hence, the overall result doesn't lead to a solid conclusion about seasonal and processing effects on the rennet gelation properties of sheep milk.

In both raw and pasteurised (unhomogenised) sheep milk, final LT increased in the late season (Late > Early), indicating reduced elasticity of rennet milk gels. Pasteurisation decreased the final LT by up to 5 % in early and late season ($P < 0.05$), but not in mid-season. Homogenisation caused a decrease in the final LT ($P < 0.05$), and this reduction (16-17%) was consistent across all seasons.

The rennet gelation time of sheep milk was negatively correlated with the final G' ($P < 0.01$, $r = -0.586$) based on all results for raw and processed sheep milks ($n = 36$). The shorter the gelation time, the firmer the rennet-gel formed. The stronger rennet-gel firmness was also associated with smaller casein micelles. There was a negative correlation found between the final G' and the

micelle size ($P < 0.05$, $r = -0.738$) when the results for heated milk (95°C/5 min) were used for the correlation analysis ($n = 9$).

Table 5.9 Effect of seasonality and processing conditions on rennet gelation properties of sheep milk

Properties	**Season**	**R**	**P**	**HP**	**H**	*** Season × Process interaction**
Rennet gelation time (min)	Early	15.0	14.9	13.9	12.2	NS
	Mid	14.7	14.6	15.0	12.4	
	Late	13.6^{b}	17.4^{a}	16.5^{ab}	14.3^{ab}	
	SEM	1.0	0.7	0.8	0.6	
Final storage modulus (G')	Early	178.9	142.8	146.4	166.0^{A}	NS
	Mid	196.2^{a}	173.9^{ab}	143.8^{b}	144.4^{bAB}	
	Late	226.0^{a}	126.0^{b}	119.6^{b}	99.3^{bB}	
	SEM	16.2	10.3	7.9	12.5	
Final LT (Tan δ)	Early	0.384^{aB}	0.370^{bB}	0.319^{c}	0.331^{c}	$P < 0.05$
	Mid	0.384^{aB}	0.376^{aAB}	0.323^{b}	0.326^{b}	
	Late	0.402^{aA}	0.384^{bA}	0.327^{c}	0.329^{c}	
	SEM	0.003	0.002	0.001	0.002	

R - Raw; P - Pasteurised (75°C/15s) without homogenisation; HP - Homogenised and pasteurised (75°C/15s); H - Heated (95°C/5 min) with homogenisation; NS - Not significant; SEM – Standard error of mean.

^{abc} Mean values with different lowercase superscripts within the same row differ significantly ($P < 0.05$).

^{AB} Mean values with different uppercase superscripts within the same column differ significantly ($P < 0.05$).

* Analysed by Two-way ANOVA test.

Several studies also found that naturally smaller casein micelles in milk resulted in faster coagulation rate and stronger gel firmness. The smaller casein micelles are thought to interact with each other more readily as they have a larger surface area, so that stronger gel forms upon renneting (Glantz et al., 2010; Guinee et al., 1997; Logan et al., 2015; Pellegrini et al., 1997). Although it should be noted that the micelle size measurements in heated milk were used for the current correlation analysis, not the measurements in raw milk samples.

The reduced final LT indicates improved elasticity of rennet-gel made of homogenised milk. In the gels made of non-homogenised milk, the fat globules are passively entrapped in a gel matrix without any interaction with the caseins (Storry et al., 1983). On the other hand, homogenised fat globules covered with proteins (mainly casein) can act as active fillers in the gel network. The enhanced properties of homogenised milk gel are attributed to the increased population of fat globules and better incorporation of them inside the gel matrix (Guinee et al., 1997; Lodaite et al., 2009; Storry et al., 1983).

5.2.3.2 Acid gelation

The processing impact on acid gelation properties of seasonal sheep milk is shown in Figure 5.7. The most prominent finding was the heating effect (95°C/5 min) on acid gelation time and gel strength. The sheep milk heated at 95°C for 5 min had the shortest gelation time and the highest final G'. During the gelation process, the pH profile was unaffected by processing, reaching around 4.2 after 8 hours of acidification as designed from the preliminary trials.

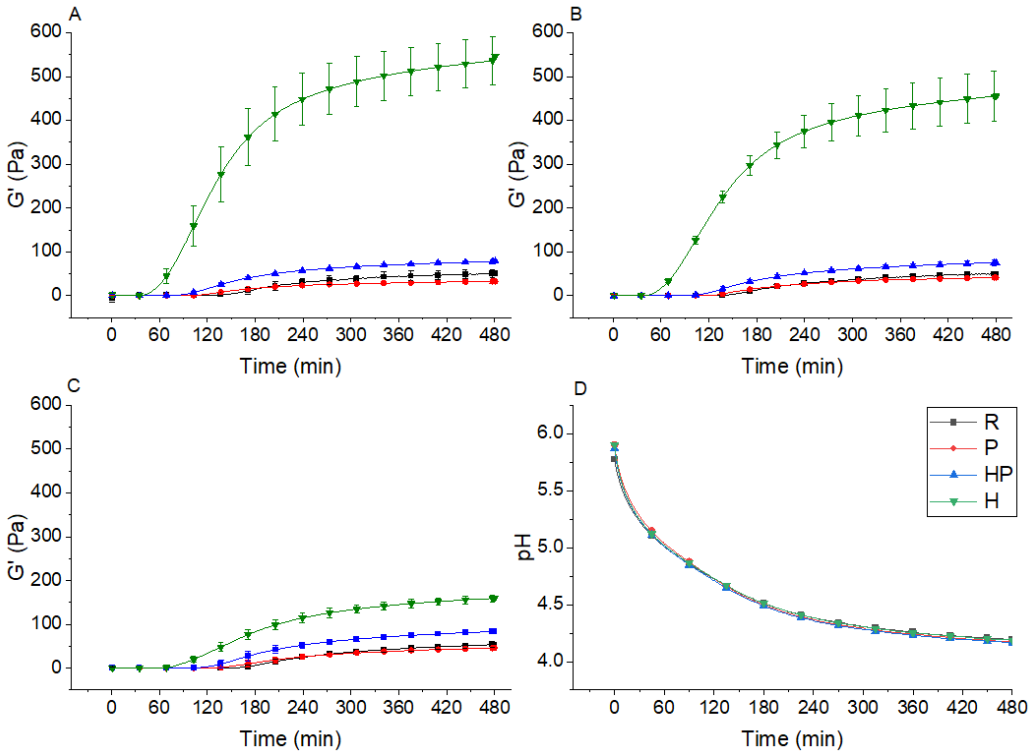


Figure 5.7 Processing effect on acid gelation properties of early-season (A), mid-season (B), late-season (C) sheep milk and pH profile during acidification (D). R - Raw; P - Pasteurised (75°C/15s) without homogenisation; HP - Homogenised and pasteurised (75°C/15s); H - Heated (95°C/5 min) with homogenisation. Error bars indicate the standard deviation of measurements from three different individual experiments.

Table 5.10 summarises the acid gelation properties of raw and processed sheep milks for three milking seasons. The average acid gelation time of raw sheep milk was 154.1 min and remained consistent across the seasons. Processing of milks decreased the gelation time. The gelation time for processed sheep milks was much longer in late season compared to those in early season. Regarding the processing effect, both homogenisation and heating (95°C for 5 min) reduced gelation time significantly ($P < 0.05$).

Table 5.10 Effect of seasonality and processing conditions on acid gelation properties of sheep milk

Acid gelation properties	**Season**	**R**	**P**	**HP**	**H**	*** Season × Process interaction**
Acid gelation time (min)	Early	144.0^{a}	115.0^{abB}	86.4^{bB}	46.1^{cB}	NS
	Mid	149.0^{a}	133.1^{aAB}	105.9^{bAB}	47.9^{cB}	
	Late	169.4^{a}	151.2^{abA}	126.3^{bA}	75.0^{cA}	
	SEM	6.3	6.6	6.9	4.8	
Gelation pH	Early	4.68^{c}	4.72^{cA}	4.88^{bA}	5.13^{aA}	$P < 0.05$
	Mid	4.61^{c}	4.67^{cAB}	4.78^{bB}	5.10^{aA}	
	Late	4.59^{c}	4.61^{cB}	4.71^{bB}	4.93^{aB}	
	SEM	0.02	0.02	0.03	0.03	
Final storage modulus (G')	Early	50.0^{b}	32.2^{bB}	77.8^{b}	535.4^{aA}	$P < 0.01$
	Mid	49.9^{b}	41.0^{bAB}	74.9^{b}	454.4^{aA}	
	Late	53.4^{c}	45.7^{cA}	83.2^{b}	158.9^{aB}	
	SEM	2.2	2.3	1.7	58.7	
Final LT (Tan δ)	Early	0.309^{a}	0.295^{a}	0.241^{b}	0.245^{b}	NS
	Mid	0.306^{a}	0.308^{a}	0.245^{b}	0.245^{b}	
	Late	0.315^{a}	0.299^{a}	0.248^{b}	0.239^{b}	
	SEM	0.004	0.003	0.002	0.001	

R - Raw; P - Pasteurised (75°C/15s) without homogenisation; HP - Homogenised and pasteurised (75°C/15s); H - Heated (95°C/5 min) with homogenisation; NS - Not significant; SEM – Standard error of mean.

^{abc} Mean values with different lowercase superscripts within the same row differ significantly ($P < 0.05$).

^{AB} Mean values with different uppercase superscripts within the same column differ significantly ($P < 0.05$).

*Analysed by Two-way ANOVA test.

The gelation pH of processed sheep milk decreased in the late season compared to the value during early- and mid-seasons, but the gelation pH of raw milk was unaffected by seasonality (Table 5.10). The raw and pasteurised sheep milk gelled around a similar isoelectric point (4.6-4.7). However, homogenisation increased the gelation pH from the average value of 4.67 to 4.79, and heating at 95°C for 5 min further increased the gelation pH to 5.10.

There was a significant season × processing interaction effect ($P < 0.01$) on the final G' of sheep milk acid-gels. Heating milk at 95°C for 5 min increased the G' dramatically in early- and mid-season but caused a much lower extent of increase in G' of late-season milk gel. The pasteurisation effect on the final G' was not significant ($P > 0.05$), whereas homogenisation increased the G' in the late season but not in early- and mid-season. Seasonality had no impact on the final LT of the acid gels made from raw and processed sheep milks. Similar to what was observed for rennet-gels, homogenisation decreased the final LT of sheep milk acid gels by 20-25% ($P < 0.05$). In contrast, heat treatments showed no impact on the final LT of sheep milk acid gels.

Overall heating impact on the acid gelation properties of sheep milk was found to be similar to that reported for cow milk (Anema et al., 2004a; Lucey et al., 1999; Lucey et al., 1997). As discussed earlier (Chapter 4), the improved acid gelation properties, particularly shorter gelation time and elevated G' value, are largely attributed to the heat-induced interactions between denatured β-LG and κ-casein and aggregation of β-LG. Interestingly, the late-season sheep showed the worst gelation behaviour in response to heating despite having the highest protein content.

Similarly, Underwood and Augustin (1997) and Li et al. (2020) reported the most inferior acid gelation properties for late-season/lactation cow milk. The degree of glycosylation of κ-casein might be responsible for the delay in acid-gel formation by increasing the negative charge on the micelle surface and compromising the hydrophobic interaction of the proteins (Li et al., 2020).

5.3 Conclusion

Sheep milk has been investigated over three milking seasons aligned with the stages of lactation: early, mid and late. The sheep milk composition and physicochemical properties were broadly constant in early- and mid-season but changed significantly in the late season. Such variation in sheep milk properties might be due to the inconsistency of seasonal milk quality.

The sheep milk produced during early-and mid-season can be suitable for yoghurt production with superior gel firmness. However, the performance of late-season sheep milk was inferior in acid-induced gelation regardless of higher solid contents. These observations may suggest that

sheep milk acid gelation behaviour is largely influenced by the physicochemical properties rather than the compositional properties. Further investigation is needed to understand the factors influencing acid gelation properties of sheep milk.

CHAPTER 6: INTERSPECIES COMPARISONS AND GENERAL DISCUSSIONS

Chapter 4 and Chapter 5 mainly demonstrated the impacts of seasonal variations and processing conditions on milk properties of each species. This chapter will focus on interspecies comparisons, overviewing the main characteristics of goat and sheep milk investigated in this study and comparing them with those of cow milk. The discussion will mainly focus on the interspecies differences in process-induced changes to the milk and their functional properties. Besides displaying the compositional differences between species, this chapter also aims to illustrate whether the same processing treatment had the same impact on certain properties of milk from different species. Also, the discussion will focus on how seasonality impacts the processing-induced changes in cow, goat and sheep milk properties differently.

6.1 Comparison between the milk compositions and their seasonal patterns

Table 6.1 shows the variation in goat and sheep milk composition. Overall, goat milk components showed a lesser degree of variation over the seasons due to year-round kidding. In goat milk, the most significant change was observed in the fat content (CV 10.2%), indicating strong seasonal (time of the year) influence on the milk fat level. Sheep milk was characterised with higher solid contents (fat and protein) than goat and cow milk and became more concentrated during the late season. This change was similar to the seasonal or lactational pattern of New Zealand cow milk composition predominantly influenced by SOL (Auldist et al., 1998; Li et al., 2019).

The proportions of individual proteins in the three types of milk were different, as shown in Table 6.2. Goat milk had a higher level of total WP and α-LA but a comparatively lower level of α_{s1}-casein than sheep and cow milk. The most abundant protein in goat milk was β-casein, whereas the most dominant protein in sheep and cow milk was α_{s1}-casein. The ratio of β-LG to α-LA was the lowest in goat milk (1.36 versus 1.94 in sheep and 3.25 in cow milk).

Table 6.1 The variation of goat and sheep milk composition

Composition	Goat			Sheep		
	Mean ± SD	Min - Max	CV%	Mean ± SD	Min - Max	CV%
Protein (%)	3.19 ± 0.08	3.09 – 3.30	2.4	5.72 ± 0.28	5.45 – 6.26	5.0
Fat (%)	3.79 ± 0.39	3.30 – 4.27	10.2	5.99 ± 0.44	5.43 – 6.70	7.4
Lactose (%)	4.40 ± 0.05	4.28 – 4.45	1.2	4.76 ± 0.12	4.58 – 4.85	2.5
Ca (g/100g)	0.114 ± 0.005	0.108 – 0.121	4.8	0.200 ± 0.007	0.189 – 0.210	3.3
Mg (g/100g)	0.014 ± 0.001	0.013 – 0.015	4.0	0.018 ± 0.002	0.016 – 0.021	10.9
K (g/100g)	0.203 ± 0.009	0.195 – 0.220	4.2	0.132 ± 0.007	0.119 - 0.138	5.5
Na (g/100g)	0.037 ± 0.001	0.035 – 0.038	3.3	0.042 ± 0.007	0.035 – 0.052	16.3
P (g/100g)	0.100 ± 0.003	0.095 – 0.104	2.8	0.160 ± 0.003	0.156 – 0.165	2.1
Cl (g/100g)	0.164 ± 0.008	0.153 – 0.175	4.8	0.089 ± 0.012	0.071 – 0.105	13.4

Table 6.2 Protein composition of goat, sheep, and cow milk

	Goat	Sheep	Cow [a]
Casein/WP	4.13	6.23	4.99[*]
Casein content (% of total casein)			
α_{s1}-casein	13.5	39.6	43.6
α_{s2}-casein	11.0	13.8	5.3
β-casein	54.8	33.6	31.0
κ-casein	20.6	13.0	20.1
Whey protein content (% of total WP)			
α-LA	42.8	34.0	23.5
β-LG	58.2	66.0	76.5
β-LG/ α-LA	1.36	1.94	3.25

[a] Calculated using data from Ha et al. (2014).

[*] Calculated using data from Auldist et al. (1998).

Figure 6.1 shows the major proteins in goat and sheep milk. The band intensity suggests that the most abundant protein in goat milk was β-casein, which agreed with the quantification of individual proteins using HPLC (Table 4.2). The α_{s2}-casein in sheep milk showed faster mobility

than its counterpart in goat milk and co-migrated with the α_{s1}-casein in the acrylamide gel. This difference in protein mobility might be attributed to their molecular mass.

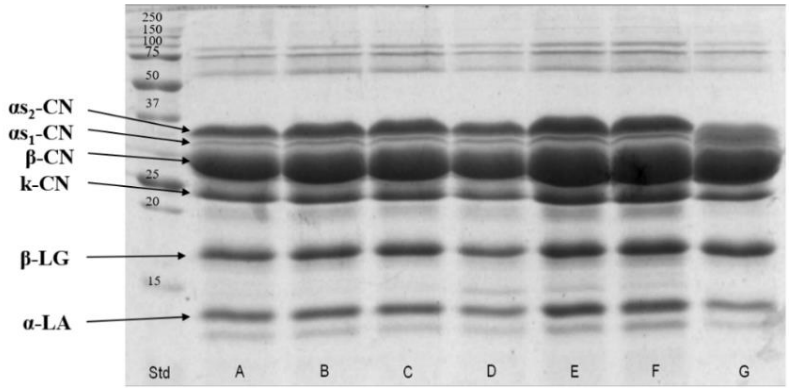


Figure 6.1 Fractions of major proteins in early- (A-C) and mid- (D-F) season goat milk as compared with those in early-season sheep milk (G); Std – Protein standard marker

6.2 Process-induced changes in goat and sheep milk

There are a number of changes occurring in milk upon heating, such as denaturation of WP and modification of salt equilibria. Here the main discussion will be on the denaturation of WP and the subsequent changes in the milk protein system, due to its relevance to the functional properties of milk. When heating goat and sheep milk at 95°C for 5 min, both showed a significant level of WP denaturation (> 90%). But, α-LA in goat milk was more heat stable than the counterpart in sheep milk. The denaturation levels of α-LA in heated goat and sheep milk (95°C/5 min) were 88.5% and 96.1%, respectively. Consequently, the total WP denaturation (α-La + β-LG) in heated goat milk was also lower than that in heated sheep milk ($P < 0.001$).

The interspecies difference in the denaturation kinetics could be partially attributed to the β-LG/α-LA ratio in milk (Table 6.2), considering that β-LG is more heat-sensitive than α-LA (Raynal & Remeuf, 1998). Also, the lower β-LG/α-LA ratio in goat milk means less availability of β-LG to promote the denaturation of α-LA, because β-LG plays a role in catalysing the denaturation of latter protein (Li et al., 2019).

Figure 6.2 demonstrates the distribution of WP in goat and sheep milk heated at 95°C for 5 min. The proportion of micelle-bound WP in heated goat milk was lower than that in heated sheep milk.

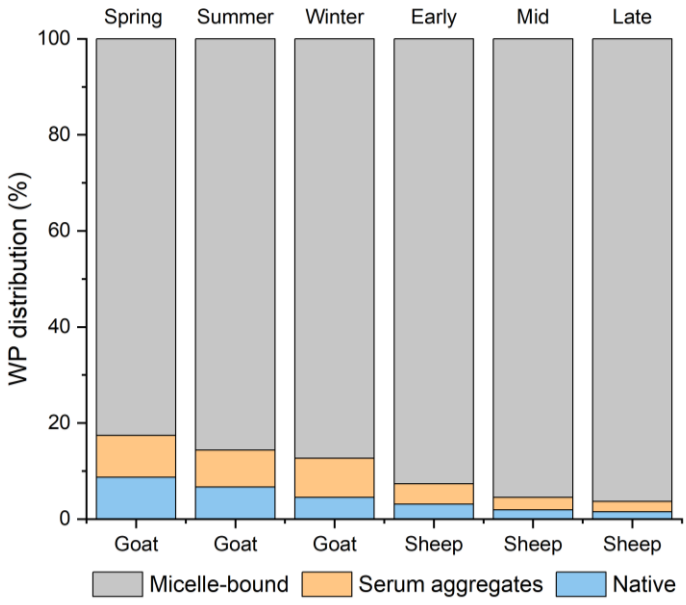


Figure 6.2 Distribution of whey proteins in goat and sheep milk heated at 95°C for 5 min

Table 6.3 compares the distribution of denatured WPs in heated goat and sheep milk. The proportion of micelle-bound WP within the denatured whey protein in heated goat milk was still lower than that in sheep milk ($P < 0.001$). The casein micelle-whey protein association seemed to be more favoured in sheep milk than in goat milk, independent of the denaturation level of total WP.

Table 6.3 The distribution of denatured WP (Mean ± SD) between the micellar and the serum phase of goat and sheep milk after being heated at 95°C for 5 min

	Goat			**Sheep**	
	Micelle-bound WP (%)	Serum WP (%)		Micelle-bound WP (%)	Serum WP (%)
Spring	90.4 ± 0.4	9.6 ± 0.4	Early	95.6 ± 0.5	4.4 ± 0.5
Summer	91.8 ± 1.0	8.2 ± 1.0	Mid	97.3 ± 0.1	2.7 ± 0.1
Winter	91.5 ± 1.6	8.5 ± 1.6	Late	97.8 ± 1.4	2.2 ± 1.4

Li et al. (2019) determined the WP distribution in heated cow milk (90°C/6 min). The proportion of serum aggregates in heated cow milk (~ 15%) was higher compared to those in goat and sheep milk (8.2% and 3.0%. respectively). Similarly, Pesic et al. (2012) found that about 30% of denatured WP formed soluble complexes in heat-treated cow milk (90°C/10 min), whereas no

soluble WP complex was determined in goat milk after the same heat treatment. As discussed in earlier chapters, the degree of WP denaturation and their association with casein micelles correlated with WP fraction, particularly β-LG, and the ionic calcium concentration. Also, the level of serum-phase κ-casein dissociated from the micelle upon heating may affect the WP-casein interaction by competing with the surface κ-caseins (Li et al., 2019).

Heat treatment (95°C/5 min) increased the micelle size in three types of milk. It has been demonstrated that the association of denatured WP with casein micelles is primarily responsible for the increase in micelle size upon heating, although some aggregation of casein micelles could contribute to the heat-induced change in micelle size (Anema & Li, 2003).

As shown in Table 6.4, the extent of increase in micelle size after heat treatment was in the following order: sheep > goat > cow. Heating sheep milk at 95°C for 5 min increased the average micelle diameter by up to 85 nm. In contrast, heating cow milk under similar conditions (90°C/ 6min) resulted in only a 10 nm increase in the micelle size (Li et al., 2019). Raynal and Remeuf (1998) observed a similar difference between heated cow, goat and sheep milk. When the milk was heated at 90°C, cow milk retained its natural micelle size regardless of holding time (up to 10 min), while sheep milk had a maximum 75% increase in the micelle size.

Regarding the seasonal impact on the heat-induced increase in casein micelles size, the maximum increase was observed in winter goat milk and late-season sheep milk. Li et al., (2019) found that heating cow milk at 90°C for 6 min also increased the micelle size, but the extent of increase was relatively small regardless of milking seasons. Interestingly, however, UHT treatment (140°C for 5 s) resulted in much more significant increases in the mean casein micelle size of cow milk, the extent of which increased with progressing lactation stages, similar to that in sheep milk observed in the present study.

Table 6.4 Heat-induced change in micelle size for goat, sheep, and cow milk

Milk	Natural micelle size (nm)	The micelle size after heating (nm)	The maximum increase (%)
Goat	212	245	+50 nm / 24%
Sheep	179	227	+85 nm / 48%
Cow *	160	169	+10 nm / < 10%

* Milk heated at 90°C for 6 min (Li et al., 2019).

The mechanism of heat-induced modification of micelle size in goat and sheep milk might differ due to the casein micelle characteristics. The micelles in goat and sheep milk are more mineralised

and less hydrated than the micelles in cow milk (Park et al., 2007). Low heat stability is a well-known characteristic of goat and sheep milk, and the fouling of heat exchangers is quite an issue during the thermal processing of these types of milk (Raynal-Ljutovac et al., 2007). According to Raynal and Remeuf (1998), the lower colloidal stability in goat and sheep milk could favour the aggregation of micelles and increase the population of larger micelles. Therefore, the larger increase in goat and sheep micelle size upon heating might be caused by the aggregation of micelles rather than the association of WP with casein micelles.

6.3 Gelation behaviours of sheep and goat milk

6.3.1 Rennet gelation

Table 6.5 shows the rennet-induced gelation behaviour of cow, goat and sheep milk from mid-season or summer. The cow milk data was obtained from the preliminary trials for comparison. Homogenisation coupled with pasteurisation improved the firmness of cow milk rennet-gel (expressed as G'), but not those of goat and sheep milk rennet-gels. High-temperature heat treatment (95°C/5 min) caused a sharp drop in the final G' of both cow and sheep milk rennet gels (R > H). However, this heat-induced decrease was much larger for cow milk (about four times) than sheep milk (36%).

The detrimental effect of high-temperature heat treatment on the rennet gelation properties of cow milk has been widely reported. The impaired rennet gel properties (extended gelation time and lower G') are mainly attributed to the formation of WP/casein complexes, which leads to the inhibition of κ-casein hydrolysis during the first stage of rennet coagulation (Singh & Waungana, 2001; Vasbinder et al., 2003; Waungana et al., 1996). The heating effect on the rennet gelation properties of cow, goat and sheep milk agreed with the observation of Raynal and Remeuf (1998). The authors hypothesised that the micelle aggregation requires a lesser degree of κ-casein hydrolysis in goat and sheep milk than in cow milk.

On the other hand, goat milk was not significantly influenced by processing conditions. The seasonal variation in the final G' of raw goat milk gel (Table 4.8) was mainly associated with milk components such as protein and calcium. This may suggest that the adjustment of milk components can partially control the renneting properties of goat milk.

Table 6.5 Comparison between rennet gelation properties of cow, goat and sheep milk

	Cow				**Goat**				**Sheep**			
Process	R	P	HP	H	R	P	HP	H	R	P	HP	H
Gelation time (min)	17.3	-	11.6	20.2	14.0	14.5	14.4	13.3	14.7	14.6	15.0	12.4
Final G' (Pa)	58.5	-	95.0	14.0	28.4	32.9	22.5	30.7	196.2[a]	173.9 [ab]	143.8[b]	144.4[b]

R - Raw; P - Pasteurised (75°C/15s) without homogenisation; HP - Homogenised and pasteurised (75°C/15s); H - Heated (95°C/5 min) with homogenisation.

[ab] Mean values with different lowercase superscripts within the same row differ significantly ($P < 0.05$).

6.3.2 Acid gelation

The interspecies difference in acid-induced gelation behaviour of milk is shown in Table 6.6. Among the three types of milk, goat milk formed the weakest gel (G' < 18 Pa) irrespective of processing conditions. This observation was in agreement with the previous studies that reported more porous microstructure for goat milk acid-gel than those made from other ruminant's milk (Nguyen et al., 2018; Roy et al., 2020b; Wang et al., 2019). All three types of heated milk (H), had decreased acid gelation time and increased final G' compared to pasteurised milk (HP). However, the extent of increase in the final G' was dramatic for cow and sheep milk (4 to 6-fold), in comparison to that for goat milk (27%).

These findings suggest that goat milk does not possess the same functionality as cow or sheep milk when acidified. Such distinct gelation behaviour of goat milk might be attributed to casein composition, particularly the level of α_{s1}-casein, and their characteristics.

The acid gelation properties of goat milk did not show a considerable seasonal variation. In contrast, the late-season sheep milk formed comparatively weaker acid-gel despite having higher protein content than the early- and mid-season milk. In the same way, the strength of acid-gel made from heated cow milk decreased in the late season (Li et al., 2020). But, the extent of decrease was much greater for sheep milk than that for cow milk. As shown in Table 6.7, the late-season G' value for heated sheep milk decreased almost 3-fold from the mid-season value, whereas the same decrease was less than 50% for heated cow milk.

Table 6.6 Comparison between acid gelation properties of mid-season cow, goat and sheep milk

	Cow				Goat				Sheep			
Process	R	P	HP	H	R	P	HP	H	R	P	HP	H
Gelation time (min)	133.0	-	78.4	50.3	139.6 ^{a}	136.7^{a}	125.6^{ab}	100.9^{b}	149.0^{a}	133.1^{a}	105.9^{b}	47.9^{c}
G'	29.4	-	126.0	519.4	12.3 ^{bc}	10.9^{c}	13.7^{b}	17.4^{a}	49.9^{b}	41.0^{b}	74.9^{b}	454.4^{a}
Gelation pH	4.85	-	5.05	5.24	4.55^{b}	4.55^{b}	4.58^{b}	4.71^{a}	4.61^{c}	4.67^{c}	4.78^{b}	5.10^{a}

R-Raw; P-Pasteurised (75°C/15s) without homogenisation; HP-Homogenised and pasteurised (75°C/15s); H-Heated (95°C/5 min) with homogenisation.

^{abc} Mean values with different lowercase superscripts within the same row differ significantly ($P < 0.05$).

Table 6.7 Comparison between the acid gelation properties of heated cow and sheep milk

Acid gelation properties	Season	* Cow milk	Sheep milk
Gelation time (min)	Early	43.5 ± 0.8^{B}	46.1 ± 2.0^{B}
	Mid	43.6 ± 2.3^{B}	47.9 ± 1.4^{B}
	Late	64.8 ± 4.4^{A}	75.0 ± 6.8^{A}
Gelation pH	Early	5.29 ± 0.04^{A}	5.13 ± 0.01^{A}
	Mid	5.26 ± 0.04^{A}	5.10 ± 0.03^{A}
	Late	5.07 ± 0.04^{B}	4.93 ± 0.02^{B}
Final G' (Pa)	Early	891.2 ± 21.8^{A}	535.4 ± 53.9^{A}
	Mid	764.4 ± 31.2^{A}	454.4 ± 57.2^{A}
	Late	513.4 ± 58.0^{B}	158.9 ± 8.1^{B}

^{AB} Mean values (Mean ± SD) with different superscripts within the same column differ significantly ($P < 0.05$).

* Milk with standardised protein (4.6%) and fat content (4.0%) heated at 90°C for 6 min (Li et al., 2020).

Compared to heated cow and sheep milk, heated goat milk had much lower gelation pH and G' value (Table 6.6). The acid gelation properties of heated goat milk seemed to be similar to those of unheated cow milk, which had a gelation pH below 4.9. It is possible that acid gel strength (G') is always low when the gelation pH is lower than a certain value, e.g. 4.9, regardless of the milk types. Above this value, the G' value would increase with increasing gelation pH (with intense heat treatment). This could explain why heating increased the gelation pH of goat milk from 4.7 to close to 4.9, but the G' value was still very low. The gelation pH of heated cow milk decreased in the late season, but it was still above pH 5.0 (Table 6.7). However, the gelation pH of late-season sheep milk was 4.9, which might be falling in a sensitive range, above which the G' would increase with increasing gelation pH.

This chapter highlighted the main differences between goat, sheep and cow milk in their process-induced changes. To fully explain the mechanism behind the process-induced changes in goat and sheep milk, which were distinct from that in cow milk, the unique characteristics of these types of milk need to be investigated in detail.

6.4 Possible industrial applications and the directions of future research

- **New product development based on the functionality of goat and sheep milk**

The current finding on process-induced changes in goat and sheep milk can be used for assessing their suitability for a certain type of dairy products or developing new products based on their technological functionalities. For example, sheep milk obtained across early and mid-season can be used for yoghurt production owing to its superior acid gelation properties.

- **Standardisation of product made from sheep milk**

The extensive recording of seasonal goat and sheep milk data (composition, thermal properties of fat, physicochemical properties, heat-induced changes, and gelation properties) can help local manufacturers deal with product inconsistency associated with seasonality. Further solutions need to be explored to standardise the textural quality of yoghurt made from sheep milk as the late-season milk exhibits different acid gelation behaviour.

- **Digestion behaviour of goat and sheep milk**

Some non-bovine milks are considered to form softer curds during gastric digestion and are more easily digested than cow milk (Roy et al., 2020a). The consistency of the curd has a defining impact on the digestion pathway of milk, such as protein hydrolysis and the release of fat globules (Mulet-Cabero et al., 2019; Ye et al., 2017). Goat and sheep milks are well characterized regarding their physicochemical and gelation properties; however, little is known about their digestion behaviours. Further study is required to understand the digestion behaviour of goat and sheep milks in relation to their physicochemical characteristics and process applications.

- **Making yoghurt with a mixture of goat and sheep milk**

Goat milk forms a weak acid gel, and by itself is not suitable for yoghurt making. However, it would be interesting to make yoghurt from a mixture of goat and sheep milk and measure the improvement in the acid gelation properties and the sensory acceptability.

- **Identification of goat or sheep milk adulteration with cow milk, based on the differences in protein composition and characteristics**

Inadequate supply of milk can be easily turned into an opportunity for milk adulteration and additional financial gain (Azad & Ahmed, 2016). Goat and sheep milk adulteration with cow milk may cause serious health issues among consumers with allergies to cow milk. Based on the

differences in protein composition and characteristics, qualification and quantification methods can be used for detecting adulterants in goat and sheep milk.

6.5 Limitations of research

- The information on the animal breed and feed was not available for both species, although many studies found a link between these factors and milk composition. Further studies in this area should consider these effects while discussing the results.
- The heat treatments have been selected to mimic commercial heat treatments used for cow milk processing. It would be interesting to look at the kinetics of heat-induced changes in goat and sheep milk using a range of temperature and holding time.
- It is not known what impact stage of lactation has on goat milk composition. The milk used in this study was mixed milk from herds with different kidding to ensure a consistent milk composition throughout the year.
- Other commercially relevant heat treatments such as batch pasteurization (63°C for 30 minutes) or retorting (121°C for 15 minutes) were not considered for comparison in this study.
- It is not known what impact does drying of these milks has on the physicochemical and technological properties. This was not considered in the scope of the study.

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APPENDICES

Appendix 1. Mineral analysis of goat and sheep milk

Hill Laboratories
TRIED, TESTED AND TRUSTED
R J Hill Laboratories Limited
28 Duke Street Frankton 3204
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Hamilton 3240 New Zealand
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T +64 7 858 2000
E mail@hill-labs.co.nz
W www.hill-laboratories.com

Certificate of Analysis

Client:	Massey University	**Lab No:**	2240070	SPv1
Contact:	Siqi Li	**Date Received:**	12-Sep-2019	
	C/- The Registrar	**Date Reported:**	25-Sep-2019	
	Private Bag 11222	**Quote No:**	101146	
	Manawatu Mail Centre	**Order No:**	PN 478428	
	Palmerston North 4442	**Client Reference:**		
		Submitted By:	Siqi Li	

Sample Type: Dairy Products (liquid)

	Sample Name:	Goat Perm E1 11-Sep-2019	Sheep Perm E1 11-Sep-2019			
	Lab Number:	2240070.3	2240070.4			
Calcium	g/100g as rcvd	0.033	0.037	-	-	-
Magnesium	g/100g as rcvd	0.0091	0.0093	-	-	-
Phosphorus	g/100g as rcvd	0.050	0.069	-	-	-

Sample Type: Milk

	Sample Name:	Goat E-1 11-Sep-2019	Sheep E-1 11-Sep-2019			
	Lab Number:	2240070.1	2240070.2			
Calcium	g/100g as rcvd	0.111	0.189	-	-	-
Magnesium	g/100g as rcvd	0.0135	0.0161	-	-	-
Potassium	g/100g as rcvd	0.20	0.132	-	-	-
Sodium	g/100g as rcvd	0.038	0.035	-	-	-
Phosphorus	g/100g as rcvd	0.100	0.161	-	-	-
Iron	mg/kg as rcvd	< 0.5	< 0.5	-	-	-
Copper	mg/kg as rcvd	0.102	0.29	-	-	-
Iodine	mg/kg as rcvd	0.22	0.27	-	-	-
Selenium	mg/kg as rcvd	0.030	0.034	-	-	-
Zinc	mg/kg as rcvd	3.7	6.0	-	-	-
Chloride*	g/100g as rcvd	0.164	0.071	-	-	-

Summary of Methods

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.
Unless otherwise indicated, analyses were performed at Hill Laboratories, 28 Duke Street, Frankton, Hamilton 3204.

Sample Type: Milk

Test	Method Description	Default Detection Limit	Sample No
Biological Materials Digestion	Nitric and hydrochloric acid micro digestion, filtration.	-	1-4
TMAH Digestion	Tetramethylammonium hydroxide micro digestion, filtration. P.A.Fecher, I.Goldman and A.Nagengast. Journal of Analytical Atomic Spectrometry, 1998, 13, 977-982.	-	1-2
Calcium	Biological materials digestion. Analysis by ICP-OES.	0.00010 g/100g as rcvd	1-4
Magnesium	Biological materials digestion. Analysis by ICP-OES.	0.00005 g/100g as rcvd	1-4
Potassium	Biological materials digestion. Analysis by ICP-OES.	0.0004 g/100g as rcvd	1-2
Sodium	Biological materials digestion. Analysis by ICP-OES.	0.0005 g/100g as rcvd	1-2
Phosphorus	Biological materials digestion. Analysis by ICP-OES.	0.0002 g/100g as rcvd	1-4
Iron	Biological materials digestion. Analysis by ICP-OES.	0.5 mg/kg as rcvd	1-2
Copper	Biological materials digestion. Analysis by ICP-MS.	0.005 mg/kg as rcvd	1-2
Iodine	TMAH digestion. Analysis by ICP-MS. J. Anal. At. Spectrom., 1998, 13, 977 - 982.	0.0010 mg/kg as rcvd	1-2
Selenium	TMAH digestion. Analysis by ICP-MS.	0.002 mg/kg as rcvd	1-2
Zinc	Biological materials digestion. Analysis by ICP-MS.	0.10 mg/kg as rcvd	1-2

IANZ ACCREDITED LABORATORY

This Laboratory is accredited by International Accreditation New Zealand (IANZ), which represents New Zealand in the International Laboratory Accreditation Cooperation (ILAC). Through the ILAC Mutual Recognition Arrangement (ILAC-MRA) this accreditation is internationally recognised.
The tests reported herein have been performed in accordance with the terms of accreditation, with the exception of tests marked *, which are not accredited.

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Certificate of Analysis

Client:	Massey University	**Lab No:**	2254838	SPv1
Contact:	Siqi Li	**Date Received:**	08-Oct-2019	
	C/- The Registrar	**Date Reported:**	17-Oct-2019	
	Private Bag 11222	**Quote No:**	101146	
	Manawatu Mail Centre	**Order No:**	PN482428	
	Palmerston North 4442	**Client Reference:**		
		Submitted By:	Siqi Li	

Sample Type: Dairy Products (liquid)

Sample Name:		E2 - G Perm	E2 - S Perm	E3 - Goat Perm	E3 - Sheep Perm	
Lab Number:		2254838.5	2254838.6	2254838.7	2254838.8	
Calcium	g/100g as rcvd	0.034	0.040	0.034	0.037	-
Magnesium	g/100g as rcvd	0.0093	0.0097	0.0099	0.0092	-
Phosphorus	g/100g as rcvd	0.052	0.065	0.050	0.064	-

Sample Type: Milk

Sample Name:		E2 Milk Goat	E2 Milk Sheep	E3 Goat (Hill) Sigi	E3 Sheep (Hill) Sigi	
Lab Number:		2254838.1	2254838.2	2254838.3	2254838.4	
Calcium	g/100g as rcvd	0.109	0.196	0.118	0.20	-
Magnesium	g/100g as rcvd	0.0132	0.0163	0.0143	0.0166	-
Potassium	g/100g as rcvd	0.20	0.138	0.20	0.138	-
Sodium	g/100g as rcvd	0.037	0.036	0.037	0.038	-
Phosphorus	g/100g as rcvd	0.099	0.160	0.103	0.165	-
Iron	mg/kg as rcvd	< 0.5	< 0.5	< 0.5	< 0.5	-
Copper	mg/kg as rcvd	0.104	0.27	0.114	0.23	-
Iodine	mg/kg as rcvd	0.27	0.26	0.27	0.20	-
Selenium	mg/kg as rcvd	0.035	0.034	0.031	0.032	-
Zinc	mg/kg as rcvd	3.4	6.0	3.7	6.4	-
Chloride*	g/100g as rcvd	0.161	0.079	0.153	0.078	-

Analyst's Comments

The samples arrived thawed and measured 4.3 °C. The laboratory kept the samples chilled and analysed them the next day. Given the matrix and the analytes requested we don't believe there was any impact on the results.

Summary of Methods

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.
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Calcium	Biological materials digestion. Analysis by ICP-OES.	0.00010 g/100g as rcvd	1-8
Magnesium	Biological materials digestion. Analysis by ICP-OES.	0.00005 g/100g as rcvd	1-8
Potassium	Biological materials digestion. Analysis by ICP-OES.	0.0004 g/100g as rcvd	1-4
Sodium	Biological materials digestion. Analysis by ICP-OES.	0.0005 g/100g as rcvd	1-4
Phosphorus	Biological materials digestion. Analysis by ICP-OES.	0.0002 g/100g as rcvd	1-8
Iron	Biological materials digestion. Analysis by ICP-OES.	0.5 mg/kg as rcvd	1-4
Copper	Biological materials digestion. Analysis by ICP-MS.	0.005 mg/kg as rcvd	1-4

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Certificate of Analysis

Client:	Massey University	**Lab No:**	2288420	SPv1
Contact:	Siqi Li C/- The Registrar Private Bag 11222 Manawatu Mail Centre Palmerston North 4442	**Date Received:** **Date Reported:** **Quote No:** **Order No:** **Client Reference:** **Submitted By:**	06-Dec-2019 17-Dec-2019 101146 PN493113 Siqi Li	

Sample Type: Dairy Products (liquid)

Sample Name:		M1 S-perm	M2 S-perm	M3 S-perm	M4 G-perm	M6 G-perm
Lab Number:		2288420.6	2288420.7	2288420.8	2288420.9	2288420.10
Calcium	g/100g as rcvd	0.040	0.039	0.041	0.034	0.032
Magnesium	g/100g as rcvd	0.0096	0.0097	0.0099	0.0097	0.0088
Phosphorus	g/100g as rcvd	0.060	0.060	0.061	0.051	0.045

Sample Type: Milk

Sample Name:		M2 Sheep Milk	M3 Sheep Milk	M4 Sheep Milk	M4 Goat Milk	M6 Goat Milk
Lab Number:		2288420.1	2288420.2	2288420.3	2288420.4	2288420.5
Calcium	g/100g as rcvd	0.197	0.199	0.20	0.113	0.108
Magnesium	g/100g as rcvd	0.0164	0.0166	0.0165	0.0134	0.0132
Potassium	g/100g as rcvd	0.138	0.137	0.137	0.21	0.21
Sodium	g/100g as rcvd	0.038	0.039	0.038	0.036	0.035
Phosphorus	g/100g as rcvd	0.156	0.156	0.156	0.099	0.095
Copper	mg/kg as rcvd	0.145	0.125	0.134	0.098	0.082
Iodine	mg/kg as rcvd	0.21	0.153	0.21	0.26	0.29
Selenium	mg/kg as rcvd	0.043	0.037	0.044	0.029	0.027
Zinc	mg/kg as rcvd	5.8	5.6	5.9	3.3	3.2
Chloride*	g/100g as rcvd	0.088	0.089	0.090	0.173	0.175

Summary of Methods

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively simple matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis. A detection limit range indicates the lowest and highest detection limits in the associated suite of analytes. A full listing of compounds and detection limits are available from the laboratory upon request.
Unless otherwise indicated, analyses were performed at Hill Laboratories, 28 Duke Street, Frankton, Hamilton 3204.

Sample Type: Milk

Test	Method Description	Default Detection Limit	Sample No
Biological Materials Digestion	Nitric and hydrochloric acid micro digestion, filtration.	-	1-10
TMAH Digestion	Tetramethylammonium hydroxide micro digestion, filtration. P.A.Fecher, I.Goldman and A.Nagengast. Journal of Analytical Atomic Spectrometry, 1998, 13, 977-982.	-	1-5
Calcium	Biological materials digestion. Analysis by ICP-OES.	0.00010 g/100g as rcvd	1-10
Magnesium	Biological materials digestion. Analysis by ICP-OES.	0.00005 g/100g as rcvd	1-10
Potassium	Biological materials digestion. Analysis by ICP-OES.	0.0004 g/100g as rcvd	1-5
Sodium	Biological materials digestion. Analysis by ICP-OES.	0.0005 g/100g as rcvd	1-5
Phosphorus	Biological materials digestion. Analysis by ICP-OES.	0.0002 g/100g as rcvd	1-10
Copper	Biological materials digestion. Analysis by ICP-MS.	0.005 mg/kg as rcvd	1-5
Iodine	TMAH digestion. Analysis by ICP-MS. J. Anal. At. Spectrom., 1998, 13, 977 - 982.	0.0010 mg/kg as rcvd	1-5
Selenium	TMAH digestion. Analysis by ICP-MS.	0.002 mg/kg as rcvd	1-5
Zinc	Biological materials digestion. Analysis by ICP-MS.	0.10 mg/kg as rcvd	1-5
Chloride*	2% nitric acid extraction, potentiometric titration. AOAC (OMA) 971.27, 18th edition (modified).	0.00010 g/100g as rcvd	1-5

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Certificate of Analysis

Client:	Massey University	**Lab No:**	2402758	SPv1
Contact:	Siqi Li	**Date Received:**	17-Jul-2020	
	C/- The Registrar	**Date Reported:**	29-Jul-2020	
	Private Bag 11222	**Quote No:**	101146	
	Manawatu Mail Centre	**Order No:**	PN517718	
	Palmerston North 4442	**Client Reference:**		
		Submitted By:	Siqi Li	

Sample Type: Dairy Products (liquid)

Sample Name:		L1-G Perm	L2-G Perm	L3-G Perm		
Lab Number:		2402758.4	2402758.5	2402758.6		
Calcium	g/100g as rcvd	0.033	0.035	0.033	-	-
Magnesium	g/100g as rcvd	0.0094	0.0093	0.0094	-	-
Phosphorus	g/100g as rcvd	0.050	0.047	0.047	-	-

Sample Type: Milk (liquid)

Sample Name:		Goat L-1	Goat L-2	Goat L-3		
Lab Number:		2402758.1	2402758.2	2402758.3		
Calcium	g/100g as rcvd	0.118	0.121	0.121	-	-
Magnesium	g/100g as rcvd	0.0141	0.0140	0.0145	-	-
Potassium	g/100g as rcvd	0.197	0.195	0.195	-	-
Sodium	g/100g as rcvd	0.035	0.036	0.038	-	-
Phosphorus	g/100g as rcvd	0.104	0.102	0.103	-	-
Copper	mg/kg as rcvd	0.112	0.124	0.129	-	-
Iodine	mg/kg as rcvd	0.190	0.176	0.25	-	-
Selenium	mg/kg as rcvd	0.032	0.025	0.030	-	-
Zinc	mg/kg as rcvd	3.9	3.7	4.0	-	-
Chloride*	g/100g as rcvd	0.157	0.158	0.163	-	-

Summary of Methods

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively simple matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis. A detection limit range indicates the lowest and highest detection limits in the associated suite of analytes. A full listing of compounds and detection limits are available from the laboratory upon request. Unless otherwise indicated, analyses were performed at Hill Laboratories, 28 Duke Street, Frankton, Hamilton 3204.

Sample Type: Milk (liquid)

Test	Method Description	Default Detection Limit	Sample No
Biological Materials Digestion	Nitric and hydrochloric acid micro digestion, filtration.	-	1-6
TMAH Digestion	Tetramethylammonium hydroxide micro digestion, filtration. P.A.Fecher, I.Goldman and A.Nagengast. Journal of Analytical Atomic Spectrometry, 1998, 13, 977-982.	-	1-3
Calcium	Biological materials digestion. Analysis by ICP-OES.	0.00010 g/100g as rcvd	1-6
Magnesium	Biological materials digestion. Analysis by ICP-OES.	0.00005 g/100g as rcvd	1-6
Potassium	Biological materials digestion. Analysis by ICP-OES.	0.0004 g/100g as rcvd	1-3
Sodium	Biological materials digestion. Analysis by ICP-OES.	0.0005 g/100g as rcvd	1-3
Phosphorus	Biological materials digestion. Analysis by ICP-OES.	0.0002 g/100g as rcvd	1-6
Copper	Biological materials digestion. Analysis by ICP-MS.	0.005 mg/kg as rcvd	1-3
Iodine	TMAH digestion. Analysis by ICP-MS. J. Anal. At. Spectrom., 1998, 13, 977 - 982.	0.0010 mg/kg as rcvd	1-3
Selenium	TMAH digestion. Analysis by ICP-MS.	0.002 mg/kg as rcvd	1-3
Zinc	Biological materials digestion. Analysis by ICP-MS.	0.10 mg/kg as rcvd	1-3
Chloride*	2% nitric acid extraction, potentiometric titration. AOAC (OMA) 971.27, 18th edition (modified).	0.00010 g/100g as rcvd	1-3

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Certificate of Analysis

Client:	Massey University	**Lab No:**	2326953 SPv1
Contact:	Siqi Li	**Date Received:**	21-Feb-2020
	C/- The Registrar	**Date Reported:**	04-Mar-2020
	Private Bag 11222	**Quote No:**	101146
	Manawatu Mail Centre	**Order No:**	PN503091
	Palmerston North 4442	**Client Reference:**	
		Submitted By:	Siqi Li

Sample Type: Dairy Products (liquid)

Sample Name:		L1 S-Perm	L2 S-Perm	L3 S-Perm	M7 Goat Perm	
Lab Number:		2326953.5	2326953.6	2326953.7	2326953.8	
Calcium	g/100g as rcvd	0.039	0.038	0.041	0.036	-
Magnesium	g/100g as rcvd	0.0113	0.0117	0.0121	0.0115	-
Phosphorus	g/100g as rcvd	0.058	0.059	0.061	0.057	-

Sample Type: Milk (liquid)

Sample Name:		L1 Sheep	L2 Sheep	L3 Sheep	Goat M7	
Lab Number:		2326953.1	2326953.2	2326953.3	2326953.4	
Calcium	g/100g as rcvd	0.21	0.20	0.21	0.108	-
Magnesium	g/100g as rcvd	0.0197	0.0199	0.021	0.0146	-
Potassium	g/100g as rcvd	0.128	0.123	0.119	0.22	-
Sodium	g/100g as rcvd	0.049	0.051	0.052	0.038	-
Phosphorus	g/100g as rcvd	0.163	0.160	0.163	0.099	-
Copper	mg/kg as rcvd	0.126	0.116	0.099	0.064	-
Iodine	mg/kg as rcvd	0.186	0.26	0.27	0.33	-
Selenium	mg/kg as rcvd	0.032	0.030	0.037	0.023	-
Zinc	mg/kg as rcvd	5.7	5.3	5.7	3.3	-
Chloride*	g/100g as rcvd	0.101	0.103	0.105	0.173	-

Summary of Methods

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively simple matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis. A detection limit range indicates the lowest and highest detection limits in the associated suite of analytes. A full listing of compounds and detection limits are available from the laboratory upon request. Unless otherwise indicated, analyses were performed at Hill Laboratories, 28 Duke Street, Frankton, Hamilton 3204.

Sample Type: Milk (liquid)

Test	Method Description	Default Detection Limit	Sample No
Biological Materials Digestion	Nitric and hydrochloric acid micro digestion, filtration.	-	1-8
TMAH Digestion	Tetramethylammonium hydroxide micro digestion, filtration. P.A.Fecher, I.Goldman and A.Nagengast. Journal of Analytical Atomic Spectrometry, 1998, 13, 977-982.	-	1-4
Calcium	Biological materials digestion. Analysis by ICP-OES.	0.00010 g/100g as rcvd	1-8
Magnesium	Biological materials digestion. Analysis by ICP-OES.	0.00005 g/100g as rcvd	1-8
Potassium	Biological materials digestion. Analysis by ICP-OES.	0.0004 g/100g as rcvd	1-4
Sodium	Biological materials digestion. Analysis by ICP-OES.	0.0005 g/100g as rcvd	1-4
Phosphorus	Biological materials digestion. Analysis by ICP-OES.	0.0002 g/100g as rcvd	1-8
Copper	Biological materials digestion. Analysis by ICP-MS.	0.005 mg/kg as rcvd	1-4
Iodine	TMAH digestion. Analysis by ICP-MS. J. Anal. At. Spectrom., 1998, 13, 977 - 982.	0.0010 mg/kg as rcvd	1-4
Selenium	TMAH digestion. Analysis by ICP-MS.	0.002 mg/kg as rcvd	1-4
Zinc	Biological materials digestion. Analysis by ICP-MS.	0.10 mg/kg as rcvd	1-4
Chloride*	2% nitric acid extraction, potentiometric titration. AOAC (OMA) 971.27, 18th edition (modified).	0.00010 g/100g as rcvd	1-4

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