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Effect of lactation stage and processing on characteristics of deer milk

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

Interest in non-bovine milk consumption is growing, with scientific evidence for the unique benefits and the growing farming of non-bovine animals such as goat, sheep, and deer. The majority of studies have focused on cow milk and then followed by goat or sheep milk. Deer milk farming and detailed understanding of deer milk characteristics is a niche area of dairy farming that needs detailed exploration to understand whether it has the same processability when subjected to the standard dairy processing developed for cow milk. Along with the processing knowledge of deer milk, it is an interest of the dairy industry to understand how the lactation stage of deer affects the milk composition and its characteristics. This research aimed to understand the variation in milk composition with lactation stage of deer, and to investigate the milk characteristics along with different processing conditions to know its potential dairy products.

Fresh deer milk samples were collected over the lactation period and the milk composition analysed, including fat, protein, and lactose content. The fresh milk was subjected to processing conditions most used in dairy industries such as 75°C/15 seconds with or without homogenization, and 95°C/5 minutes with homogenization. The seasonal and processing-induced changes in milk characteristics were analysed using conventional methods. The colloidal stability of deer milk was investigated in this study by using the different heating (time and temperature combinations) and homogenization conditions. The characteristics of reconstituted deer milk were also studied with the same processing conditions as used for fresh milk and compared with fresh deer milk characteristics.

Deer milk was characterized by a higher content of macronutrients (fat and protein) and minerals compared to cow milk, and it was found that the protein distribution of deer milk was different from cow milk. The study showed that lactational stage of deer milk significantly impacted the fat, protein, minerals distribution in milk. Deer milk protein contained a significantly higher amount of β-casein and a lower amount of α_{s1}-casein than cow milk. Deer milk formed stronger gels when inoculated with rennet or acidified than cow milk, but, like cow milk, heating improved the acid gel strength of deer milk.

The behaviour of both fresh and reconstituted deer milk was almost similar after heat treatment as explained by milk physicochemical properties, but the gelation properties of rennet and acid gel made from reconstituted and fresh deer milk showed difference in terms of gel firmness and gelation time.

The casein micelle size in deer milk was larger than micelles of cow milk. Heat treatment (95°C/5 min) increased the size of casein micelles in deer milk, and the effect was more pronounced as compared to micelles of cow milk, suggesting different mechanisms of casein micelle modification. Study of the colloidal stability of deer milk determined that the extent of whey protein denaturation and their association with casein micelles in deer milk increased with heating intensity like cow milk, but the kinetics of whey protein denaturation and their association with casein micelles differ in deer milk.

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Symbol and abbreviations

BC	Buffer capacity
FA	Fatty acid
GDL	Glucono-δ-lactone
HPLC	High-performance liquid chromatography
IMCU	International milk clotting unit
Tan δ	Tangent delta
MFG	Milk fat globule
MFGM	Milk fat globule membrane
SDS	Sodium dodecyl sulphate
SEM	Standard error of mean
SOP	Standard operation procedure
UC	Ultracentrifuge
v/v	volume/volume
w/v	weight/volume
w/w	weight/weight
α-LA	α-Lactalbumin
β-LG	β-Lactoglobulin
WP	Whey protein

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1. Introduction

Milk and dairy products have evolved to meet the nutritional and physiological needs of consumers. Because of its accessibility and higher production volumes, cattle milk is the most-consumed milk worldwide and has been investigated in detail. Non-cattle milk has been consumed in areas in which the natural climate or geographical conditions is unsuitable for dairy cattle. For example, sheep milk in Europe, buffalo milk in Asia, goat milk in Africa, reindeer milk in northern Scandinavia, etc. The dairy industry is growing and becoming diversified with different types of milk and milk products from non-cattle dairy species including goat, sheep, buffalo, deer, etc. (Park, Haenlein, & Wendorff, 2006).

The total milk production of non-cattle milk increased from ~9% in 1961 to 19% in 2018 (FAOSTAT—Crops, 2020). Interest in non-cattle milk has increased in the past few years due to several reports (Park et al., 2006; Raynal-Ljutovac, Park, Gaucheron, & Bouhallab, 2007; Wang, A. E.-D. A. Bekhit, J. D. Morton, & S. Mason, 2017) about the nutritional benefits of and certain characteristics where non-cattle milk is more similar to human milk than cattle milk. Thus, non-cattle milk is identified as a potential source of milk for the development of infants, children, and elderly nutrition, with specialized products optimized for national profiles. However, only a few non-cattle milks (mainly goat and buffalo) have been studied in detail, whereas milk from some other species, such as deer milk, is not well understood (De Favereau et al., 2014).

In the past few years, the dairy industries of New Zealand have developed many nutritious dairy products (Infant food) from goat and sheep milk, which has boosted their interest and production volumes. By considering the demand for non-cattle milk as well as existing large and advanced deer farming, New Zealand has pioneered the development of a red deer dairy farming system in the last few years. Based on the historical use of deer milk, it is not only a potential source for human consumption but it may also have different medical benefits (Wang et al., 2017).

In addition, products made of non-cattle milk have been investigated as a suitable carrier of probiotics, due to their ability to maintain the activity of beneficial microorganisms for longer during storage. These types of milk provide them a suitable pH, buffering capacity, and high nutrient levels, which makes them suitable for manufacturing dairy products such as fermented milk, yoghurt, ice cream, and cheese (Ranadheera, Naumovski, & Ajlouni, 2018). Deer milk

was also identified as a rich source of nutrition with a different buffering capacity than cow milk, but the characteristics of deer milk under various processing conditions, as well as its lactational study not well understood properly.

This thesis investigates the impact of the lactation stage on the composition of deer milk and the effects of processing conditions (homogenization and heating treatment) on the characteristics of deer milk. Chapter 2 discusses the relevant literature covering the basic details of milk characteristics, the nutritional aspects, and the physicochemical properties of deer milk. Chapter 3 outlines the major protocols used in this work to systematically characterize the impact of processing on physicochemical, gelation properties, and colloidal stability of fresh and reconstituted deer milk in Chapters 4, 5, and 6 respectively. Detailed information regarding the composition and properties of fresh deer milk was discussed in Chapter 4. Heat-induced changes in fresh deer milk were studied, including variation in casein micelle structures and their aggregation, whey protein denaturation, salt balances, etc. The gelation characteristics of fresh deer milk (for acid and rennet gel) were also discussed in this chapter and the impact of processing conditions (heating and homogenization) on these gels was also observed. The processing-induced changes were studied further in Chapter 5, in which the colloidal instability of processed deer milk was investigated at different heat and homogenization conditions. In Chapter 6, deer milk powder properties were investigated to understand the reconstitution properties of the powder, and reconstituted deer milk was studied to understand the physicochemical and gelation properties. Finally, Chapter 7 summarises the outcomes of the study to compare the physicochemical characteristics and technological behaviour of deer and cow milk, and at the end concluding remarks are given on the possible industrial applications of deer milk. Figure 1.0 below provides a schematic outline of individual chapters.

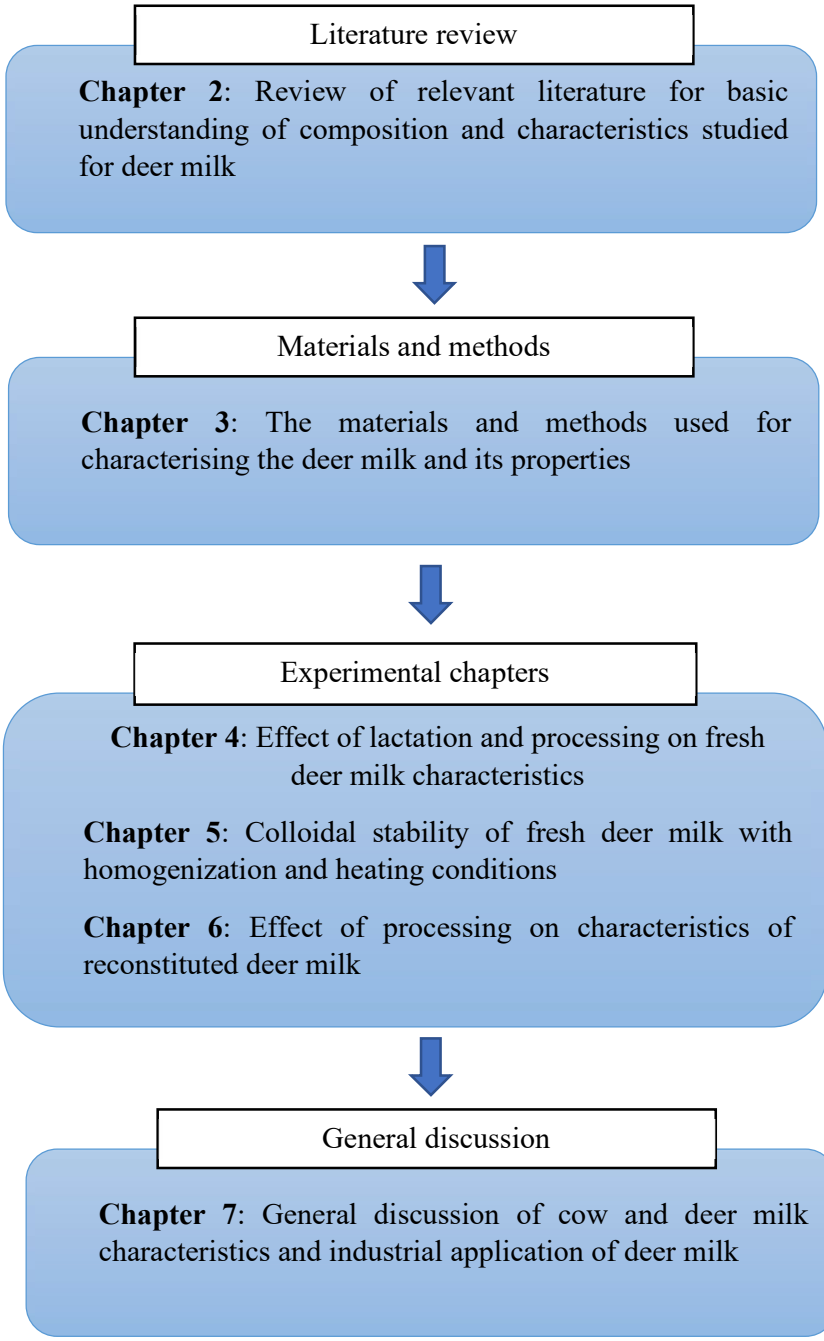

Figure 1:Schematic outline of the thesis

2. Literature review

2.1 Deer milk and farming history

"Deer are ruminants belonging to the family Cervidae. This family class separated from the Bovidae, which includes cattle, buffalo, sheep, and antelope, about 30 million years ago." Some species of deer, such as reindeer, have been used for milking in some parts of the world such as Siberia, Mongolia, and the Russian region (Gaya, Sánchez, Nuñez, & Fernández-García, 2005). Wang et al. (2017) mentioned that "New Zealand has one of the largest populations of farmed deer in the world, although they did not originate from here. In the 19th century, deer were introduced into New Zealand for hunting and meat (venison) but due to suitable weather, the population of deer increased rapidly. The first license to farm deer in New Zealand was issued in 1970 and marked the beginning of deer farming. Most of New Zealand's deer herd are red deer (~85%) followed by fallow deer with 10%, the remaining 5% contain some hybrid breed of deer (Opatha Vithana, 2012). In the past few years, different studies have shown interest in understanding the unique properties of deer milk by considering the development of deer farming and milking process of farmed deer hinds, as well as the high nutritional values of deer milk (Opatha Vithana, 2012; Wang et al., 2017).

2.2 Milk composition

Milk is a nutritional source for all age groups of human beings. Milk is a well-balanced nutritious diet that contains water, fat, proteins, carbohydrates, and minerals. The nutritional values can vary along with species, breed, season, etc. Table 2.1 shows the milk composition of different ruminants, and it is demonstrating that deer milk contains a higher percentage of total solids, including fat and protein content, than goat, cow, and sheep milk.

Table 2.1: Milk composition of different bovine and non-bovine milk

Composition (%)	Deer milk	Cow milk	Sheep milk	Goat milk
Fat	8.0 - 12	3.5 - 4.5	5.4 - 6.2	3.3 - 3.8
Protein	7.5 - 8.5	3.0 - 3.5	5.5 - 6.2	3.0 - 3.2
Lactose	4.5 - 5.5	4.0 - 4.8	4.5 - 4.8	4.2 - 4.5
Total solid	20 - 26	10 - 13	14 - 16	10-12

Table data was taken from Wang et al. (2017)

2.3 Milk Fat

Milk is an oil-in-water emulsion in which fat exists as a dispersed phase and water acts as a dispersed medium. The milk fat globules or droplets are distributed in the milk serum phase as described in Figure 2.1(A). On average around 15 billion milk droplets are distributed per ml of milk. These vary from 0.1 to 20 µm in diameter, the average size of milk fat globule is 3 to 4 µm. All fats are members of the ester family which are components of different alcohol and acids. Milk fat is the combination of different triglycerides (fatty acid esters), diglycerides, monoglycerides, sterols, fatty acids, carotenoids (responsible for the yellowish colour of milk fat), vitamins (A, D, E, and K), etc. as outlined in Figure 2.1(B). Milk fat can easily change its shape and consistency when exposed to different processing conditions such as heat treatment and mechanical treatment (homogenization, pumping, or flowing in pipes during milk processing (Bylund, 2003).

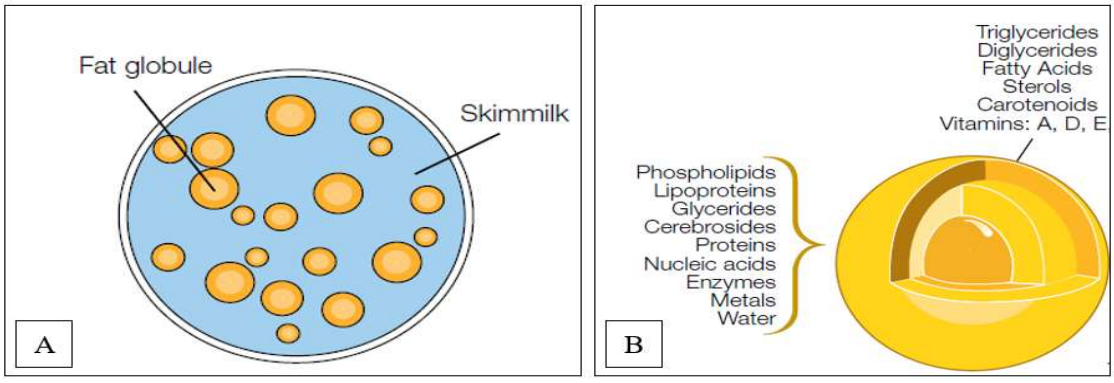

Figure 2.1.: Distribution of fat globules in milk serum phase(A) and structure of fat globules with the distribution of different triglyceride (B). *Reproduced with permission of Bylund (2003)

Milk fat globules are big, but light in weight (density of cow milk fat at 15°C is 0.93 g/cm^3) as compared to the other components of milk. Fat globules are responsible for the creaming in milk due to the separation of fat particles from milk serum which tend to rise towards the top surface of the milk. The creaming process is based on Stokes' law which tells that the rise of dispersed particles (fat globules) towards the surface of an emulsion (milk) is due to the difference in density of serum phase and dispersed particles, as shown in Figure 2.2. Creaming of milk can be delayed or avoided by reducing the size of fat globules with the help of homogenization, however, the creaming rate can be accelerated by aggregation of fat particles without efficient homogenization (after 1st stage of homogenization) due to the influence of

milk proteins (agglutinin). These fat particles' aggregation rises at a faster rate as compared to individual fat particles, but they can be easily disintegrated by a small mechanical (2nd stage of homogenization) or heating process (Bylund, 2003; Walstra, 1990).

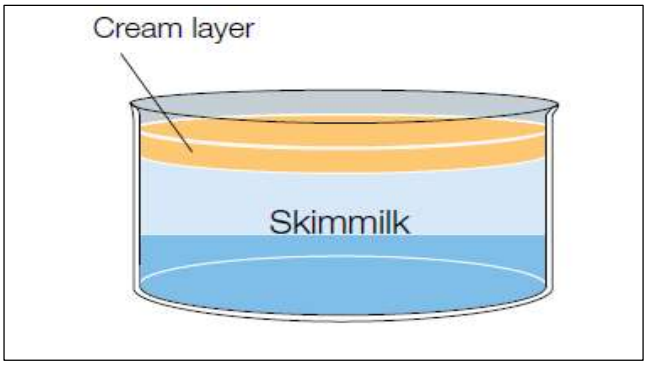

Figure 2.2: Representation of creaming of milk with separation of skim milk and cream layer *Reproduced with permission of Bylund (2003)

2.3.1 Milk Fat Globule Membrane

Milk fat globules are surrounded by a very thin membrane called the milk fat globule membrane (MFGM, 10-50 nm thick). This membrane consists of different compositions including lipoproteins, proteins (around 20 to 60% of the total mass of MFGM, major proteins are *Butyrophilin and glycoproteins)*, nucleic acids, enzymes, phospholipids, and bound water as shown in Figure 2.3. An estimated weight of MFGM is about 3 to 6% of the total weight of milk fat globules. The mass of MFGM is highly dependent on the size of the fat globules and it has been observed that the percentage of membrane weight of MFGM increased with the size of fat globules. Processing conditions such as heat treatment, homogenization, or mixing can affect the structure of the membrane and lead to the mixing of the broken membrane into milk serum. These conditions further favour the adsorption of casein micelles and denatured whey protein over milk fat globules and impact the physicochemical properties of milk and milk products. Figure 2.3 shows the structure of milk fat globules and milk fat globule membrane which is surrounded by whey protein and casein micelles and aggregation of denatured whey protein and casein micelles over milk fat globules after processing damages of MFGM (Bylund, 2003; Walstra, 1990).

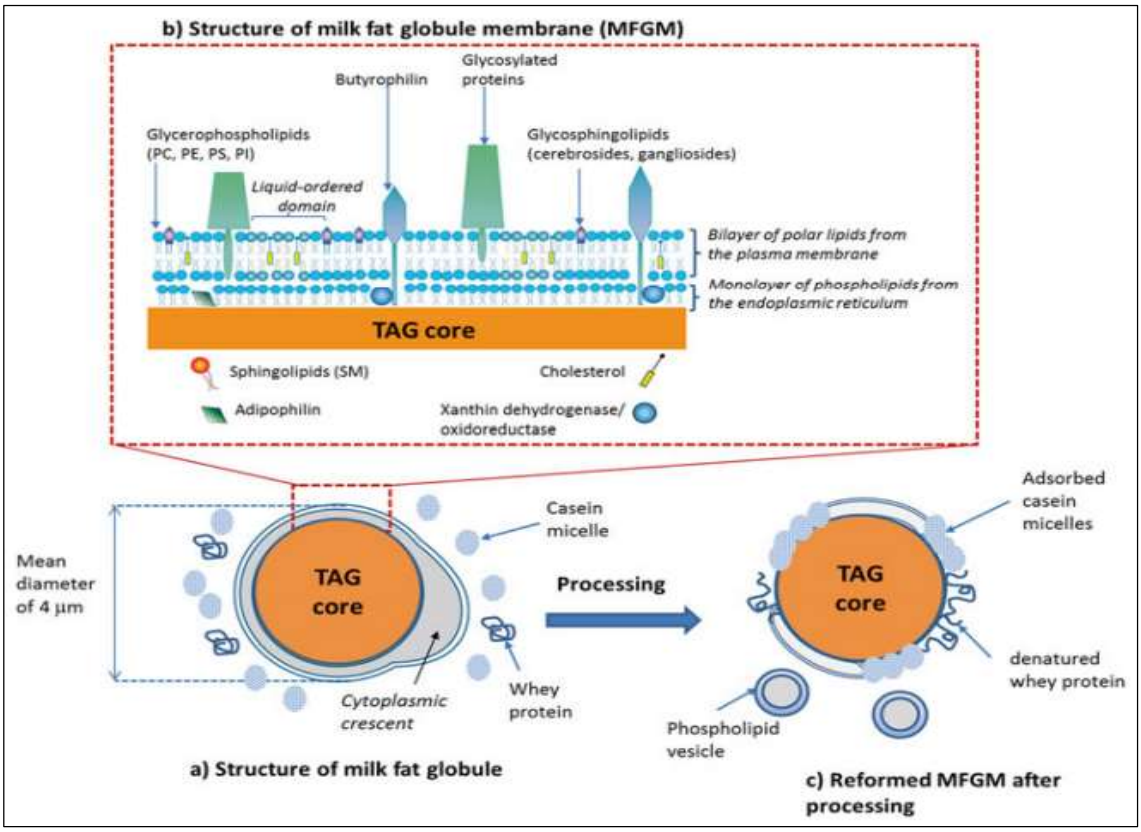

Figure 2.3: Schematic representation of milk fat globule structure and distribution of casein and WP in milk serum(a), the structure of milk fat globule membrane(b), and processing impact on MFGM and aggregation of denatured WP and casein micelles over fat globules *Reproduced with permission of Bylund (2003)

Fat content in deer milk - Milk fat content and composition can vary with the species, diet, stage of the lactation period, and physiological status of deer. However, overall, the consistency of deer milk looks like cream because of the higher amount of fat content (10 to 14 %). The fat content of deer milk is higher than other bovine and non-bovine milk including cow (3.3 to 4.5 %), sheep, goat, etc. The variation of fat content in deer milk has been observed in several studies (Bovolenta, Corazzin, Messina, Focardi, & Piasentier, 2013; Malacarne et al., 2015), which have reported that the fat content of deer milk can vary from 13 – 14.5 % throughout the lactation period of red deer. Malacarne et al. (2015) also studied the distribution of fatty acid of deer milk in detail and reported that the deer milk majorly consisted of the *palmitic(C16:0), stearic acid(C18:0), myristic acid(C14:0), and oleic acid(C18:1)* fatty acids.

Table 2.2: Fat percentage in different deer milk subspecies

Species	Fat % of total milk content	(Origin or lactation stage)
Fellow deer	14.1	Farmed deer, mid-lactation
Red deer	10.8	Farmed deer
Red deer	6.6 - 17.4	Farmed deer, throughout the lactation
Reindeer	13.8 - 16.6	Semi-domestic deer, throughout the lactation
White-tailed deer	7.75	Captive
Black-tailed deer	10.4	Wild deer
Roe deer	11.0	Wild deer
Moose deer	10.5	Wild deer, mid-lactation

*The data of table referred from (Bovolenta et al., 2013; De la Vara et al., 2018; Wang et al., 2017)

2.4 Milk proteins

Proteins are an important part of our diet. The proteins present in our diet are broken down in our digestive system into peptides and amino acids which are transferred to the body cells and used as a constructive material. Many chemical reactions in our body are controlled by different enzymes, which are mostly proteins in nature. Proteins are the combination of many smaller units of amino acids and carboxyl groups (100-200) which are arranged in a specific order as presented in Figure 2.4 (Bylund, 2003).

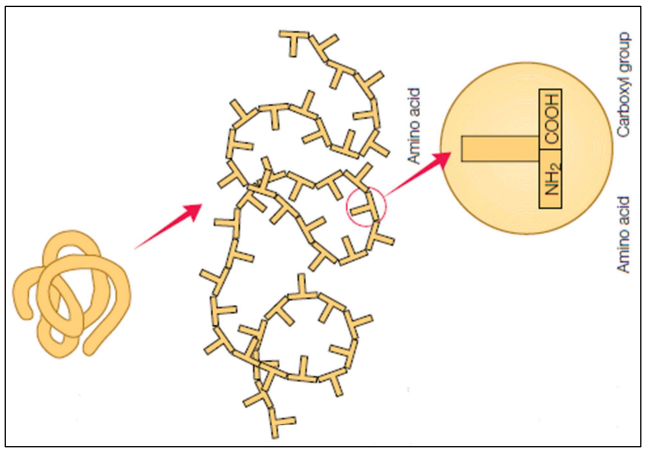

Figure 2.4: Model of protein with a combination of different amino acids and carboxyl groups.*Reproduced with permission from Bylund (2003).

The electrical status of milk proteins

The amino acids present in milk proteins contain an electric charge which defines the pH of the milk. The charge distribution of milk proteins and pH is changed by the addition of acid or base. The electric status of milk proteins and their distribution along with different pH is described in Figure 2.5.

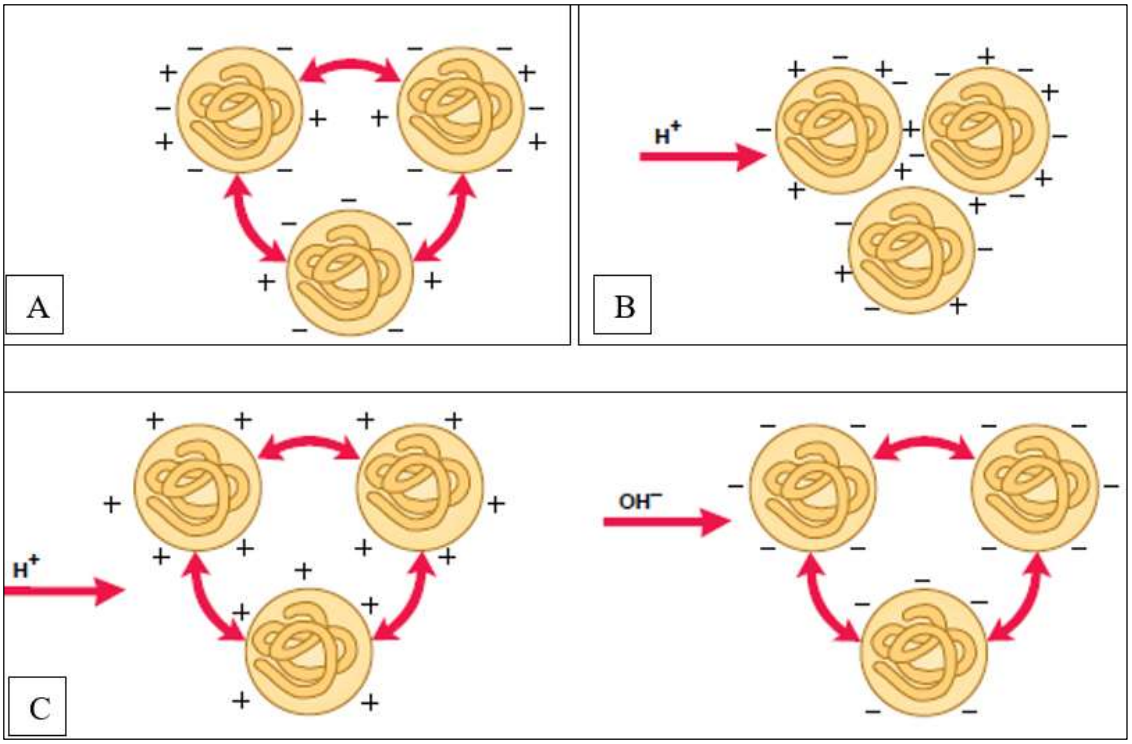

Figure 2.5: Distribution of electrical charges over protein molecules at pH= 6.0 with the net negative charge (A), Isoelectric point for proteins molecules is at pH = 4.7 with an equal positive and negative charge (B) and proteins molecules having net positive charge at pH = 14 respectively (C -left to right). *Reproduced with permission from Bylund (2003).

Figure 2.5 shows that at normal conditions i.e., pH 6.6 to 6.8, the protein molecules contain a net negative charge. Because they are identically charged, protein molecules repel each other and remain separated. The pH of milk is reduced with the addition of acid (H^+) which is adsorbed by protein molecules. At a specific pH = 4.7, the numbers of $NH3^+$ and COO^- groups on the side chain proteins become equal and the net charge on proteins is zero. At this specific pH, the repelling force in between proteins molecules become zero, due to which neighbouring protein molecules start to merge and create a large protein cluster, known as aggregation. This

leads to the precipitation of protein from the solution, which is also called the coagulation of milk. This specific pH at which maximum precipitation of proteins happens is known as the isoelectric point of the protein. With the addition of excess hydrogen ions or acid the proteins carry a more positive charge and begin to repel each other, on the other hand with the addition of a strong alkaline solution (OH^-) all protein molecules carry a negative charge and remain in solution due to repulsion force (Bylund, 2003).

Classes of milk proteins - Milk proteins can be defined in different ways based on their biological functions and physio-chemical properties. Major milk proteins are identified as casein, whey proteins, and milk fat globule membrane proteins. Milk contains various minor proteins also which have been excluded in this review for the sake of clarity (Miocinovic et al., 2016). The casein proteins found as micelles form and are distributed around the fat globules with whey proteins (serum proteins) in the solution of milk. MFGM proteins are the constructive material of the membrane which covers the milk fat globules and can be released by any mechanical action such as homogenization or churning of cream into butter (O'mahony & Fox, 2013).

Casein - The major or dominant class of milk proteins is casein, which is found in micelles form. Casein can easily form polymers with identical or other types of molecules. The casein micelles contain ionizable groups, hydrophobic as well as hydrophilic sites which provide a unique identity to these molecules. These polymers are made from many different molecules which form the colloidal solution in milk and are responsible for the whitish-blue tinge of skim milk. This complex structure is known as casein micelle, it varies in size up to 0.4 microns (Tuinier & De Kruif, 2002). Cow milk casein is categorized into three heterogeneous subgroups, α-casein, β-casein, and k-casein, with 2 to 8 genetic variants. The genetic variation among different proteins is mainly because of a few amino acids. The amino acid of these all three sub-groups of casein have one common hydroxy group which are esterified to phosphoric acids (O'mahony & Fox, 2013)**.** The phosphate group is responsible for binding calcium, magnesium, and some other salts and forming bonds between or within the molecules. Casein micelles, as shown in Figure 2.6, consist of sub micelles with diameters that vary from 10 to 15 nm (nm= 10^{-9} m), and α, β, and k-casein are distributed heterogeneously among different micelles. The calcium salts of α and β caseins are insoluble in water, while the salts of k-casein

are soluble in water. The distribution of most of the *k*-casein is around the surface of micelles due to which the solubility of *k*-casein overcomes the insolubility nature of α and β-caseins and provides the overall solubility of whole micelles in water (Dalgleish & Corredig, 2012). The medium-sized micelles contain around 400-550 sub micelles which bound together. The size of casein micelles depends on the concentration of casein content and the availability of calcium ion (Ca^{2+}) contents in milk. The reduction of the concentration of calcium ions from micelles leads to the disintegration of micelles into sub micelles (Miocinovic et al., 2016; O'mahony & Fox, 2013).

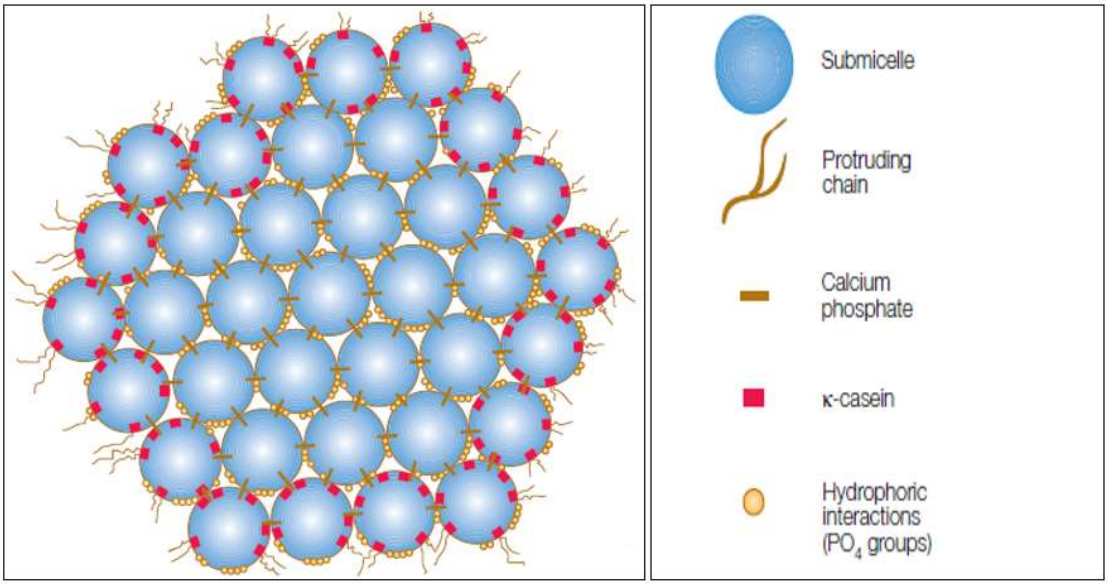

Figure 2.6: The schematic representation of formation and stabilization of casein micelles. *Reproduced with permission from Bylund (2003)

The role of *k*-casein, β-casein and α-casein instability of casein micelles - PO_4 groups and calcium phosphate are distributed in between the sub micelles and their hydrophobic interaction provides the integrity to the casein micelles. The *k*-casein constitutes the hairy type of structure surrounding the sub-micelles of casein due to the presence of hydrophilic C-terminal parts of the carbohydrate group. The negative charge over casein micelles is provided by the *k*-casein which stabilizes the micelles (Walstra, 1990). During cheese processing, the chymosin attack the hydrophilic C-terminal end of *k*-casein present on the surface of micelles, and this breakdown leads to the instability of casein micelles which initiate the aggregation of sub-micelles and finally leads to casein curds. The casein micelles contain surplus negative charges at normal conditions which provide stability due to repelling force and the hydrophilic

site of k-casein holds the water molecules to provide stability in milk. But in the absence of hydrophilic sites water starts to move outside from the structure allowing attracting force to act. New bonds will begin to form with salts such as Calcium hydrophobic bonds and these new bonds help to break down the structure finally, the micelle's structure collapses into a dense curd (Bylund, 2003). The temperature of milk also plays an important role in the stability of casein micelles in milk. The *β*–casein is the most hydrophobic casein present in milk and hydrophobic interactions are weakened when the temperature is lowered. Swaisgood (1982) mentioned that at low-temperature *β*-casein chains commence to dissociate and enhance the leaving rate of Calcium hydroxy phosphate from the micelle's structures to the solution. These changes provide less stability of milk during cheese processing as they are resulting in longer renneting time and softer curd. The *β*-casein is easily hydrolysed by different proteases present in milk after releasing from micelles structures. The hydrolysis of *β*-casein and *α*-casein leads to lower yield during cheese production as loss of protease-peptone fraction in whey. The breakdown of *β*-casein may also be responsible for the formation of different bitter peptides in cheese and leads to off-flavour development during the storage of cheese (Guinee, O'Kennedy, & Kelly, 2006).

Whey Proteins - Milk serum proteins present in milk are commonly referred to as whey protein. Generally, casein is recovered from skim milk with precipitation method by using acidic conditions, and the remaining group of proteins in milk are considered as milk serum proteins, also known as whey proteins. The denaturation/aggregation of whey proteins by heat or the combination of heat and pH adjustment is most commonly used in dairy industries to recover whey protein from milk. Tuinier and De Kruif (2002) reported that denaturation of whey protein initiated with the heat treatment as mentioned above and creates a complex structure with casein micelles. These complex structures decrease the availability of casein micelles to be attacked by rennet during cheese production and hinder rennet from binding with calcium. The process occurs differently when milk is heated at a high temperature, as the curd will not release whey protein due to the formation of small bridges within and in between casein micelles. Whey protein is subdivided into four different groups, *α*-lactalbumin (contains the highest nutritional values among all other whey proteins), *β*-lactoglobulin, Immunoglobulin, and minor proteins (Guinee et al., 2006). The α-lactalbumin from most of the bovine and non-bovine milk plays an important role to provide the synthesis of lactose in the udder of animals. The *β*-lactoglobulin is the dominant whey protein in cow milk which starts to denature when the milk is heated above 60 °C, mainly due to sulphur based amino acid which is highly reactive

to heat. At high treatment, the sulphurous compounds (hydrogen sulphide groups) begin to release from β-lactoglobulin molecules and resulted in the "cooked flavour" in high heat-treated milk. Immunoglobulin and other minor whey proteins- are extremely heterogeneous and usually isolated along with whey during cheese processing to use in pharmaceutical or food industries (Walstra, 1990).

Milk fat globules membrane proteins – membrane proteins are the constructive material of milk fat globule membrane which protect the fat globules and stabilize the emulsion. These proteins contain lipid residues, known as lipoproteins which provide the hydrophobic nature of protein towards the fat surfaces. The protein membrane breaks down under processing conditions such as heat treatment or homogenization due to which the constructive material (phospholipids and lipoproteins) of membrane protein is exposed to different enzymes present in milk and causes rancid flavour due to enzymatic reactions (Bylund, 2003).

Protein distribution in deer milk – It has been reported in several studies, as shown in Table 2.3, that deer milk contains the highest protein content among all other mammalian species including cow, buffalo, sheep, goat, etc. Opatha Vithana (2012) studied the milk characterization of deer milk and compared it with bovine and non-bovine milk. In this study, he reported that deer milk contains almost double the amount of protein content (~8.2%) of cowmilk (~4.1%) and goat milk (~ 4.0%). Opatha Vithana (2012) mentioned that in deer milk β-casein is dominant (~33 g/L), like sheep milk (β-casein 36 g/L) as compared to β-casein present in cow milk (~ 15 g/L) and goat milk (~26 g/L). The distribution of κ-casein (~5/g/L) and α_{s1}-casein-like content (~6.9 g/L) in deer milk was determined as significantly lower ($P < 0.05$) as compared to a cow, goat, and sheep milk. These differences in milk protein and their distribution in deer milk generate interest for a deeper study of the protein characterization and effect of heat treatment on deer milk protein. The protein distribution of milk is majorly impacted during the processing of milk (heat treatment) which further affects the physicochemical properties of milk and milk products (Wang et al., 2017).

Table 2.3: Protein content and distribution in different bovine and non-bovine milk

Components(g/L)	Deer Milk	Cow Milk	Sheep Milk	Goat Milk
Proteins (Min-Max)	8.8[a]	30–39	45–70	30–52
Total casein	87[a]	24.6 – 28.0	41.8 – 46.0	23.3 – 46.3
α_{s1}-Casein	6.9	8 – 10.7	15.4 – 22.1 [a]	0 [b] – 13.0 [a]
α_{s2}-Casein	0.8	2.8 – 3.4	ND	2.3 – 11.6
β-Casein	32.9	8.6 – 9.3	15.6–17.6	0 [b] -29.6
κ-Casein	5.0	2.3 – 3.3	3.2–4.3 [a]	2.8 – 13.4
γ-Casein	ND	0.8	ND	ND
Total whey protein	0.6[a]	5.5 – 7.0	10.2 – 11.0	3.7 – 7.0
β-Lactoglobulin	0.98	3.2 – 3.3	6.5 – 8.5 [a]	1.5 – 5.0
α-Lactalbumin	2	1.2 – 1.3	1–1.9 [a]	0.7 – 2.3
Immunoglobulins	ND	0.5 – 1.0	0.7	ND
Serum albumin	ND	0.3 – 0.4	0.4 – 0.6 [a]	ND
Lactoferrin	2.6	0.02–0.5	0.8	0.02–0.2

[a] Reported as a percentage and it was not possible to convert g/L, [b] Reported as an absence of αs-2 and β-Casein in the specific type of milk, ND: Reported as not detected.

*The table data referred from (Claeys et al., 2014; Ha, Bekhit, McConnell, Mason, & Carne, 2014; Opatha Vithana, 2012; Wang et al., 2017).

2.5 Lactose

Milk contains a wide range of carbohydrates such as lactose, galactose, glucose, and other oligosaccharides. Lactose, also known as milk sugar, is the most dominant carbohydrate of milk. The lactose content of milk has been discussed in detail for different types of milk because of lactose allergy in lactose intolerance humans. Martinez-Ferez et al. (2006) determined that some oligosaccharides which are usually associated with lipids or proteins have the potential to favour the growth of intestinal flora and affect the gastrointestinal and inflammatory process in the human digestion system.

2.6 Minerals

Milk is a rich source of minerals. These minerals play an important role to provide the nutritional and functional characteristics of milk. Milk minerals are divided into two parts, major minerals such as sodium (Na), Potassium (K), calcium (Ca), phosphorous (P), chloride (Cl), and magnesium (Mg), and minor minerals, including iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn). The mineral ions Na^+, K^+, Cl^- maintain the osmotic pressure in milk along with lactose. Calcium, phosphorous, and magnesium play an important role to provide the stability to casein micelles and are very sensitive to processing conditions such as acid and heat treatments. The concentration of milk minerals can depend on many factors, such as species, lactational stage, breed, etc. Malacarne et al. (2015) reported that the average ash content of red deer milk can vary from 1.1% to 1.8% of total milk composition. Opatha Vithana (2012) determined the mineral contents of red deer milk and mentioned that red deer milk contains higher amounts of calcium, potassium, chromium, and zinc as compared to a cow, sheep, and goat milk (Table 2.4). All these minerals are important for the biochemical process and functionality of humans. The results of this study indicate that deer milk is a rich source of nutritional minerals that can be used in food for people requiring high amounts of minerals such as the elderly, patients with poor appetite, and infants (Malacarne et al., 2015).

Table 2.4: Mineral distribution in different bovine and non-bovine milk

Composition	Deer Milk	Cow Milk	Goat Milk	Sheep Milk
Sodium (mg/100g)	27.2-157.6	45-73	30-41	44-58
Potassium (mg/100g)	81-139	140-185	160-180	136-140
Calcium (mg/100g)	233-418	113-120	130-158	195-200
Phosphorous (mg/100g)	158-315	87.04	115-122	124-158
Magnesium (mg/100g)	13-28	9	12	18-21

*The table data referred from (Khan et al., 2019; Malacarne et al., 2015; Wang et al., 2017)

2.7 Lactation variation

The physicochemical properties of milk such as buffering capacity, ethanol stability, and viscosity have been correlated with the compositional properties of milk. Milk composition can be affected by lactation stages as well as different species. Changes in milk composition can impact the physicochemical properties of milk. Several studies reported that usually the fat and protein content of cow milk increases as the lactation period progresses, as compared to lactose remains stable through the lactation period (S. Li, Ye, & Singh, 2019). Malacarne et al. (2015) also reported the same variation of lactation stage with deer milk and mentioned that the fat and protein content of deer milk increased significantly with the lactation period ($P < 0.05$), whereas lactose did not change significantly.

2.8 Physicochemical properties of milk

2.8.1 pH

The acidity of milk or any dilute solution is defined as the pH of the solution, which indicates the concentration of hydrogen ions present inside the solution. The pH of fresh cow milk ranges from 6.5 to 6.7 at 25 °C. The pH value of milk depends on different factors such as temperature and the acidity of milk (presence of lactic acid). Mathematically pH of any solution is expressed as the negative logarithm to the base 10 of the hydrogen ion concentration expressed in molarity, i.e., $pH = -\log [H^+]$. The pH values of deer milk are mentioned as in a similar range to those found in other commercial dairy species such as cow, buffalo, and sheep (Berruga et al., 2021).

2.8.2 Buffering capacity

The ability of any solution to resist the change in pH or acidity with the addition of acid or base. Buffering capacity of milk mainly depends on the presence of acid buffers and base buffers. Milk contains different types of acidic and alkaline groups that affect the buffering action of milk over a wide pH range. The major buffer components in milk are protein content (casein), citrate, bicarbonates, colloidal calcium phosphate, and dissolved CO_2 (Salaün, Mietton, & Gaucheron, 2005). The buffering capacity of cow milk increases with the concentration of protein content, free inorganic, and organic phosphates and it has been observed that the milk shows the maximum buffering capacity at pH 5.0 on acidification due

to the release of free inorganic and organic phosphates due to solubilization of colloidal calcium-phosphate (CCP). The impact of buffer expressed as a buffering index(dB/dpH) and the measurement of buffering capacity observed with the addition of acid and base. The differential ratio of dB/dpH was firstly introduced by Van Slyke (1922) to define the buffer effect.

$$\frac{dB}{dpH} = \frac{(The\ volume\ of\ acid\ or\ base\ added) \times (normality\ of\ acid\ or\ base)}{(Volume\ of\ sample) \times (pH\ change\ produced)} \qquad \text{Eq. 2.1}$$

The buffering capacity of deer milk is higher than cow, goat, or sheep milk due to slow acidification or low pH drop during fermentation. This could be due to the presence of a higher amount of protein and mineral contents (P, Ca, and Mg) in deer milk which affects the pH drop of milk during fermentation (Opatha Vithana, 2012).

2.8.3 Ionic calcium

Calcium is distributed in the aqueous and colloidal phases of milk. Around 33% of calcium is present in the soluble form including ionic calcium (Ca^{2+}) which accounts for approximately 10% of total calcium at the normal pH of the milk. The presence of Ca^{+2} has been related to the colloidal stability, coagulation properties, heat stability of milk with different heating treatments, and processing conditions (Zamberlin, Antunac, Havranek, & Samaržija, 2012). The heat stability, pH, and ionic calcium concentration of milk are correlated with each other, and it is reported that the ionic concentration increased with the reduction in milk pH as well as heat stability under similar heating conditions (Lewis, 2011). The heating of milk at different temperature conditions leads to the reduction of solubility of calcium phosphate, and this might cause a change in Ca^{2+} concentration (Omoarukhe & Lewis, 2010). Ranjith (1995) reported that boiling of milk decreased Ca^{2+} from 2.78 to 2.09 mM and Ultra-high temperature (UHT) treatment shows a larger reduction than pasteurization. The ionic calcium majorly affects the stability of casein micelles and their ability to aggregate especially during the rennet coagulation. It has been stated that a higher concentration of Ca^{2+} in milk promotes rennet coagulation and reduces the gelation time as free calcium ions may neutralize the negatively charged casein residues and helps in the faster aggregation of micelles with each other. M. J. Lin, Lewis, and Grandison (2006) mentioned that the concentration of Ca^{2+} in milk can vary with the species and lactation period and it has been reported that the level of ionic calcium

concentration in cow milk can vary between 1.43 and 2.50 mM throughout the lactation period. Silanikove, Shapiro, and Shamay (2003) reported that the concentration of Ca^{2+} in goat and sheep milk can vary in the range of 1.7 – 3.7 and 2.1 – 4.3 respectively.

2.8.4 Ethanol stability

Ethanol stability is defined as the lowest concentration of added ethanol (aqueous) that results in the coagulation in milk, this varies from one species to another. Ethanol stability is correlated with the heat stability of milk and is considered as a freshness indicator of milk in various studies. (De la Vara et al., 2018; Horne, 2016). De la Vara et al. (2018) determined the ethanol stability of different species of milk, including cow, goat, deer, and sheep. It has been observed that Ethanol stability of cow milk (80-85 % of ethanol v/v) was highest, followed by sheep and deer milk (60-65 % of ethanol v/v). Goat milk had the lowest ethanol stability (50-55 % of ethanol v/v). The higher concentration of ionic calcium and Na/K ratio majorly correlated with the lower ethanol stability of non-bovine milk as compared to cow milk (Horne, 2016).

2.8.5 Viscosity

The physical behaviour of a fluid to resist its flow due to internal frictions and resistance between the kinetic motion of liquid during flow and a surface is considered as the viscosity of the liquid. The viscosity of milk is mainly dependent on its components, such as protein (mainly casein), lactose, and milk fat globules. The viscosity of milk can be influenced by various factors such as pH, temperature, storage, and processing conditions (homogenization or heating (Sutariya, Huppertz, & Patel, 2017). The viscosity of milk increases with the increasing pH of milk, probably due to swelling of micelles, and it also increases with a decrease in pH of milk because of protein aggregations. The viscosity of milk also depends on the ionic equilibria of milk components and minerals. (Fox, Uniacke-Lowe, McSweeney, & O'Mahony, 2015). Y. Li, Joyner, Carter, and Drake (2018) reported that the presence of a larger number of fat globules in high-fat milk and large size casein micelles resulted in the increase of viscosity with homogenization and heating conditions because of the resistance of fat particles to the flow of milk. The average viscosity of deer milk has been reported to be higher (~3.12 cP) than other species of milk such as sheep milk (~ 2.48 cP), cow milk (~2.13 cP), and goat milk (~2.12 cP). This is most likely due to the presence of higher fat and protein contents (Berruga et al., 2021).

2.9 Processing impact on physicochemical properties of milk

2.9.1 Homogenization

Homogenization is a process that breaks the fat globules into smaller ones, this reduces the creaming and improves the stability of milk and milk products. Homogenization is required to produce recombined or reconstituted milk by blending milk fat with milk powder (Bylund, 2003). Figure 2.7 shows the schematic representation of the homogenization process with two-stage homogenizers. Milk is forced through the narrow gap (~0.1 mm) at high velocity which produces the turbulence and cavitation forces which help reduce the size of fat globules to approximately 1µm in diameter. The surface area of fat droplets increases by 5 to 6 fold as compared to native fat globules (Bylund, 2003).

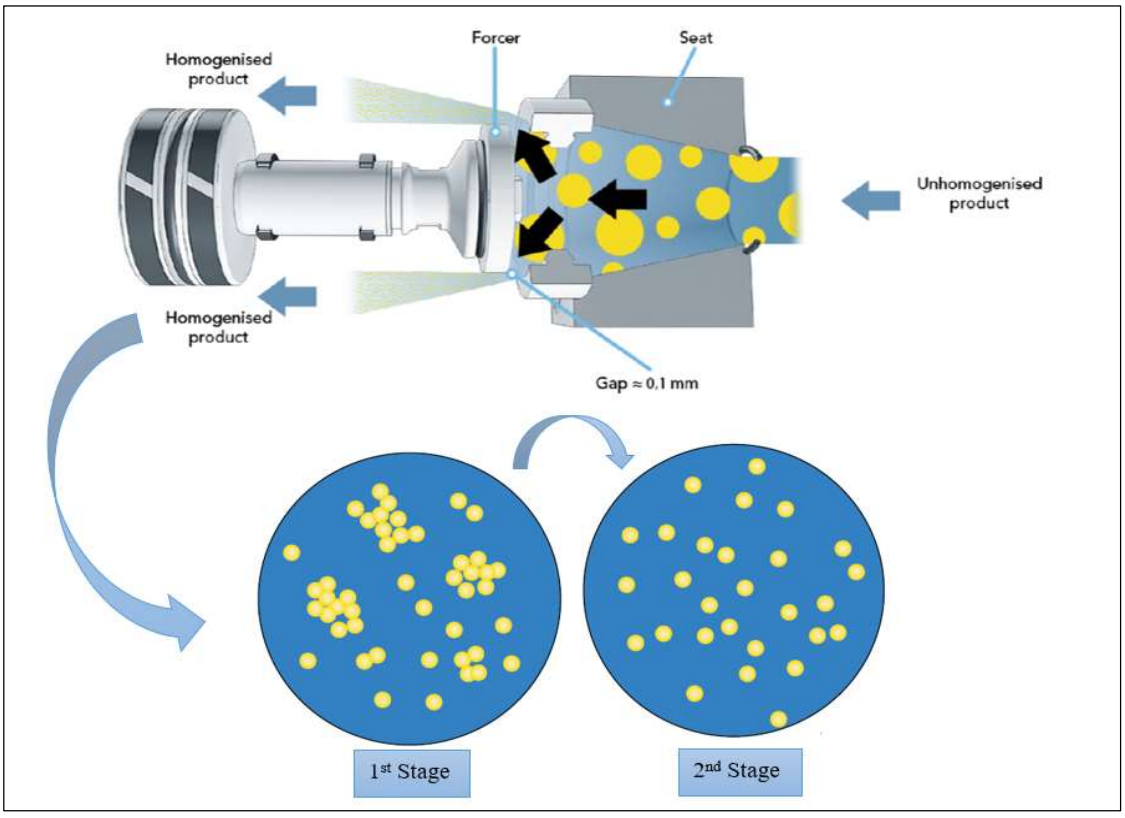

Figure 2.7: The schematic representation of homogenization of milk with 2 stage homogenizers. *Reproduced with permission of Bylund (2003)

The fat globule membrane also breaks during the homogenization process, the original membrane materials are not enough to stabilize the newly formed small fat globules and they

start to aggregate with each other with adsorption of proteins from milk plasma. So, the process needs 2nd stage homogenization to separate those aggregations for further stability of milk droplets. This protein surface coverage over small fat globules leads to the increased volume fraction of fat and increases the viscosity of milk (Walstra, 1990). Two stage-homogenization is used for most dairy processing by maintaining the total 20-25 MP pressure at 50 to 60 °C to break the fat globules and 4-5 MP pressure in the second stage to disrupt the aggregation of small fat globules which may occur after the first stage (Ciron, Gee, Kelly, & Auty, 2010).

Effects of homogenization on physical properties of milk and milk products (Bylund, 2003).

- Smaller fat globules lead to a no-creaming effect in milk.
- Better mouthfeel and uniform distribution of fat globules.
- Homogenization can reduce heat stability.
- Homogenization can increase the viscosity of high-fat content milk.

2.9.2 Thermal treatment

Heat treatment is a process used for different milk products to inactivate pathogenic microorganisms to provide microbiological safety in milk. Heat treatment is the combination of heating temperature and time. The most common treatments in the dairy industry are pasteurization and high heat treatment, which can be used in batch or continuous systems. The pasteurization of milk can be done in two different ways, low-temperature and long-time (LTLT) when milk is heated at 63 °C for 30 minutes or, high-temperature short time (HTST) pasteurization where the milk is heated at 72-75 °C for 15-20 sec. The high heat treatment includes heating of milk 95 °C for 5 minutes (Yoghurt processing) and ultra-heat treatment (UHT) where milk is heated at 135-140 °C for 2-5 sec or without holding (Bylund, 2003). Heat treatment extends the shelf-life of milk and milk products but also results in physicochemical changes in milk, these are strongly dependent on temperature. The discussion here is largely focused on treatments above 65 °C. The heat-induced changes in milk include reduction in the concentration of soluble calcium, denaturation, and aggregation of whey protein, increase in viscosity, size of fat globules, and casein micelles. The heating of milk at 65 °C or above causes irreversible structural changes in casein micelles and whey protein such as aggregation and denaturation of WP (Singh, 2004).

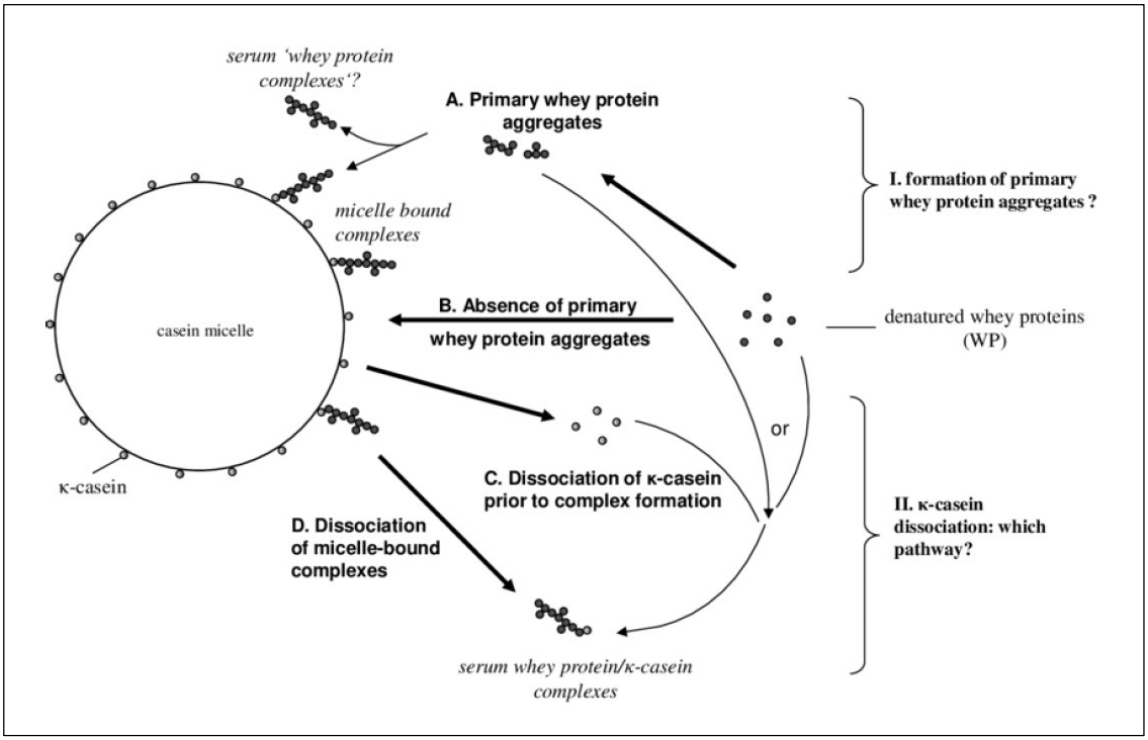

Figure 2.8: The schematic representation of formation heat-induced whey protein and k-casein complexes in heated skim milk. *Reproduced with permission from Donato and Guyomarc'h (2009).

The distribution of whey protein in milk can be affected by heating intensity as it begins to associate with κ-casein to form whey protein- κ-casein complexes, while other parts of whey protein can aggregate together with di-sulfate bonds, which further impact the physicochemical properties of milk (Dave & Singh, 2019). Figure 2.8 gives a schematic representation of heat-induced changes in whey protein and casein micelles. Heat treatment can also affect the salt balance equilibria and their interaction with casein micelles in milk. Heat treatment reduces the solubility of calcium phosphate of milk so that it starts to associate with casein micelles in the colloidal phase. This mechanism explains the reduction of ionic calcium concentration also found with high heat treatment (above 65 °C). (Singh, 2004). The heat-induced changes in the structure of whey protein due to denaturation cause the association of casein micelles with each other and denatured whey protein, which increases the volume fraction in milk. These changes further explain the increase in the size of casein micelles and the viscosity of milk. However, viscosity is also correlated with other factors such as protein and fat content, homogenization pressure and pH of milk, etc. Ye, Singh, Taylor, and Anema (2004) reported that a slight change in pH of milk from 6.7 to 6.5 resulted in a higher level of whey protein denaturation

and association of casein micelles which increased the size of casein micelles, volume fraction, and viscosity of milk.

2.9.3 Gelation properties of milk

Gelation of milk initiates with the precipitation of casein micelles and due to the complex structure of casein micelles, the stability of micelles depends on many factors such as pH, mineral distribution, etc. During the processing of different dairy products, milk is processed with two different kinds of gel formation, acid gelation and rennet gelation (J. Lucey, Munro, & Singh, 1999).

Acid gelation- The processing of yogurt involves the acidification of milk whereas milk undergoes acidification due to the fermentation of lactose into lactic acid by lactic acid bacterial cultures. The pH of milk drops when acid-producing bacteria are allowed to grow in milk at specific temperature conditions. Alternatively, Glucono-delta-lactone (GDL) is a commonly used chemical acidifier for acid gelation in milk. GDL reduces the pH of milk after hydrolyzation like yogurt fermentation and results in gel formation in milk (J. Lucey et al., 1999). Firstly, the colloidal calcium phosphate (CCP) which is present with casein micelles begin to dissolve and form ionized calcium, which further penetrates the structure of micelles and creates a strong calcium bond. Due to acidification, the pH of milk starts to drop and approaches the isoelectric point of casein micelles (pH 4.6). At pH 4.6 the casein micelles lose their integrity resulting in the aggregation of micelles forming a three-dimensional network. Acid gelation is a complicated process that mainly depends on different factors such as aggregation of casein micelles, milk salt balance, particularly the distribution of CCP, the protein distribution of the milk, and the pH range (Heertje, Visser, & Smits, 1985; J. Lucey et al., 1999). A different study has been done regarding the acid gelation of cow and goat milk. It is reported that cow milk provides better gelation properties compared to goat milk by comparing the gelation properties including gelation time, Tan δ, or final modulus (G'). The distribution of protein in goat milk especially the lower level of α_{s1}-casein is considered the prime factor for the poor texture of the gel, as compared to cow milk. (Miocinovic et al., 2016; Nguyen, Afsar, & Day, 2018; Roy, Ye, Moughan, & Singh, 2020a).

Nguyen et al. (2018) reported that milk with a higher amount of protein and solid contents produced a dense and firmer textured gel during the acidification process. Roy et al. (2020a)

also mentioned that sheep, deer, and buffalo milk provided a stronger gel formation than cattle and goat milk due to the presence of a higher amount of protein and solid content. However rheological properties of milk and milk gel depend on other factors also such as casein micelles size, whey protein distribution, mineral content, processing conditions, and the interaction of the components. Most of the acid gelation studies focused on cow, goat, and sheep milk, but knowledge of the acid gelation property of deer milk is still limited and the processing impact on acid gelation of deer milk has rarely been reported.

Rennet gelation - Rennet gelation is an important step during the processing of cheese. Most of the prototype enzymes (rennet) tend to attack and split the bond between amino acids 105 (phenylalanine) and 106 (methionine). The amino acid chain from 106 to 169 is hydrophilic and soluble and this part of κ-casein is released with the whey in the cheese-making process. On the other hand, the amino acid chain from 1 to 105 is insoluble and remains in the curd along with β and αs-caseins in the presence of calcium bridging and calcium ions known as para κ-casein (Dave & Singh, 2019). The breaking of the bond of 105 – 106 κ-casein molecule is known as the primary phase of rennet action, whereas the phase of syneresis and coagulation is considered as the second phase of rennet action (Bylund, 2003). The content of total solids, protein, and minerals is considered the prime factors impacting the rennet gelation properties of milk and it has been reported that milk with a higher amount of total solids, protein, and minerals resulted in a shorter coagulation time and more consistent curd structure during the cheese-making process (Hilali, El-Mayda, & Rischkowsky, 2011). The presence of α_{s1}-casein was reported as the important factor to provide the firmness of gel during the rennet gelation process and milk with a higher amount of α_{s1}-casein (cow milk) produced better gelation properties with firm curd, whereas goat milk with lower content of α_{s1}-casein produced a weaker curd (Dimassi et al., 2005). Ha et al. (2014) proposed that deer milk has good potential to produce high yield and firm curd/cheese after rennet gelation due to the presence of a higher amount of protein and solid content in deer milk. However, the detailed study regarding the rennet gelation and processing impact on rennet gelation of deer milk has rarely been reported.

Processing impact on gelation properties of milk - The processing of milk has important factors that affect the properties of milk during gelation. Heat treatment is a major and necessary step in the dairy industry to provide food safe from bacterial growth, or functional improvement of milk and milk products. Heat treatment directly impacts the physicochemical

properties of milk, which further affects the gelation properties during acid and rennet gelation. Heat treatment majorly affects the structure of whey protein and casein micelles which results in the variation of their interaction as discussed in Section 2.4.2. This further impacts the protein-protein interaction of milk during the gelation process. Donato and Guyomarc'h (2009) reported that heat treatment of milk at 85-95 °C for 10-15 minutes favors the yogurt making process by increasing the interaction of proteins in the gel network, also the denatured whey protein shows high water binding capacity and participates in the gel network which reduces the syneresis in yoghurt. Heat treatment induces the denaturation of whey protein and produces the gel network of different WP aggregates and micelles when they are acidified in the presence of casein micelles, which resulted in a lower gelation time and a firmer gel (S. Li et al., 2019; Lucey, Teo, Munro, & Singh, 1997).

Pre-heat treatment of milk is also required for food safety in cheese processing, but the high-intensity heat treatment reduced the rennet gelation properties of cow milk by increasing the longer gelation time and weak gel network. As discussed earlier, heat treatment increases the denaturation level of whey protein as well as their interaction with casein micelles (especially with κ-casein), which blocks the interaction of rennet enzymes that are supposed to attack the amino acid bond (105-106) of κ-casein. This interaction delays the primary stage of rennet coagulation and results in porous structure or weak rennet gel networks (Dave & Singh, 2019). However, different types of milk can behave differently with heating intensity, for example, the heating of cow milk at 85 °C for 30 minutes resulted in a longer rennet gelation time but the same heating effect did not cause any significant change on rennet gelation time of goat milk. Pesic, Barac, Stanojevic, and Vrvić (2016) proposed that there is a difference in whey protein and casein interaction under high heat treatment in cow and goat milk. In this study, it is mentioned that the intense heating effect in goat milk decreased the interaction of *k*-casein and denatured whey protein, which further explains the reason behind the unaffected rennet gelation time of goat milk even after high heat treatment, as described in Figure 2.9. Many studies have been done regarding the processing impact on gelation properties of cow and goat milk, but there is not much-published work investing the processing or heating impact on gelation properties of deer milk.

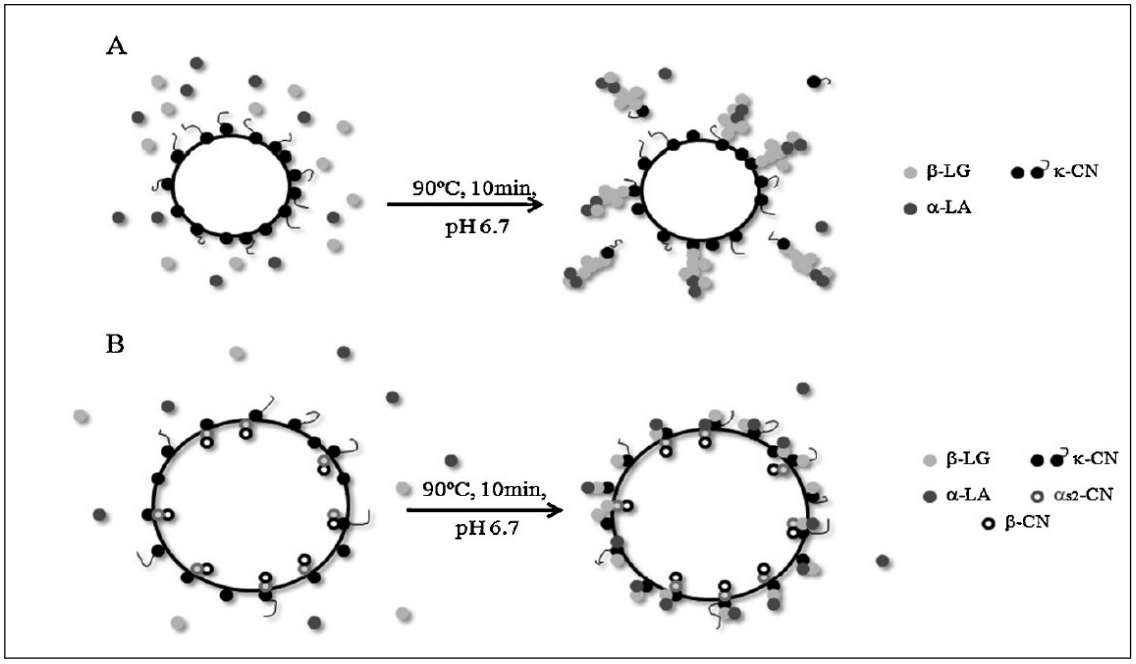

Figure 2.9: The schematic representation of the interaction of denatured whey protein and casein micelles at high intense heat treatment (90 °C for 10 minutes at pH 6.7) in cow milk (A) and goat milk (B). *Reproduced with permission from Pesic et al. (2016).

2.10 Reconstituted milk and properties

Milk is a perishable food product and some parts of the world have very limited milk production of their own. Fresh milk has a very short shelf life and can be spoiled by the presence of many different bacteria and exposure to sunlight. Therefore, the distribution of fresh milk is quite difficult in some parts of the world, especially when the producer and consumers are located at a longer distance. The production of fresh milk is also limited throughout the year due to the limited lactation period of animals, thus it is difficult to provide fresh milk throughout the year. In such conditions dairy industries convert fresh milk into milk powder to increase the shelf life of the product and process the reconstituted or recombined milk during the lean season or in places where milk production is limited. Reconstitution of milk is an alternative method to produce milk that closely resembles fresh milk. Reconstituted milk is produced by mixing water with skim milk powder (SMP) or whole milk powder (WMP) with different processing conditions such as blending, mixing, homogenization, and heat treatment. Reconstitution of milk powder mainly depends on the characteristics of milk powder (rheological or reconstituted

properties). The reconstitution characteristics of milk powder depend on many factors (Bylund, 2003);

- Milk composition and physicochemical characteristics
- Wettability
- Bulk-density and dispersibility
- The particle size of milk powder
- Processing of milk powder

The properties of milk powder and their impact on the reconstitution process as well as physicochemical process have been discussed in detail and it has been observed that cow milk powder with particle size around 200 μm diameter provided the optimum results. (Augustin, Cheng, & Clarke, 1999). Neff and Morris (1968) studied the impact of the processing of cow milk powder on the gel characteristics of reconstituted milk and found that the denaturation level of whey protein impacted the resistance of yogurt to syneresis and strengthened the gel network. The process and properties of reconstituted cow milk or goat milk have been discussed in several studies (Augustin et al., 1999; Neff & Morris, 1968) but the information regarding deer milk powder and reconstituted deer milk is still limited.

2.11 Summary of literature

The above discussion indicated that deer milk composition differs from cow and other non-bovine milk. The milk composition of milk majorly affects the physicochemical properties of milk, and these properties are likely to be affected by variation in milk components due to lactation and processing conditions.

The current knowledge of lactation and processing conditions variation in milk has mainly focused on cow milk with some attention to goat or sheep milk. Deer milk has been well characterized for its nutritional values or compositional characteristics, but processing-induced changes and technological properties have not been extensively studied. So, understanding the seasonal variation in deer milk composition and processing-induced changes is important for the dairy industry to optimize the processibility of deer milk.

The objectives of this study are:

1. To investigate the effect of lactation and processing on characteristics of deer milk.

2. To investigate the effect of processing on characteristics of reconstituted deer milk.

3. To gain mechanistic insights behind the colloidal stability of deer milk with homogenization and heating treatment.

3. Materials and methods

3.1 Materials

The chemicals and reagents were purchased from Sigma-Aldrich (Auckland, New Zealand) and the water used for dilution was purified by Milli-Q apparatus (Millipore Corp., Bedford, MA, USA). All fresh deer milk and milk powder samples were provided by Pamu Deer Milk (Wellington, New Zealand). The lactational period for deer milk was divided into 3 parts by considering the total time of milking (22 weeks). The early lactation was considered from 0 to 50 days, followed by mid-lactation from 51 to 100 days, and finally, late lactation from 101 to 150 days. The fresh milk samples for "Chapter 4" (Impact of processing and lactation on characteristics of deer milk) were received throughout the full lactation period of deer from deer farms in Gore, New Zealand whereas the milk samples for "Chapter 5" (The investigation of processing stability of deer milk) were received at the end of lactation from the deer farms in Taupo, New Zealand. Full cream deer milk powder was used for the investigation of physicochemical properties of the powder, as well as to understand the processing impact on the characteristics of reconstituted deer milk. Sample preparation and workflow diagram for all three experimental chapters are described in detail in the specific chapter. Table 3.1 summarized the overview of material used in different chapters during this study.

Table 3.1: Summary of materials used in different chapters

Deer milk/powder	Farm	Chapters	Lactation
Fresh deer milk	Gore-deer farm	Chapter 4	Early, mid and late lactation
Fresh deer milk	Taupo-deer farm	Chapter 5	Late lactation
Full cream deer milk powder	N/A	Chapter 6	N/A

3.2 Methods

3.2.1 Sample preparation and processing

The aliquots of fresh whole milk samples were stored at 4 ºC and -20 ºC for proximity and mineral analysis respectively. The remaining whole milk samples were processed within 24 hours at the Food Pilot plant of Massey University (Palmerston North, New Zealand). All three experimental chapters (4^{th}, $5^{th,}$ and 6^{th}) of this study used different processing conditions (heat treatment and homogenization) depending on the objective of the investigation, and the processing details are described in the respective chapter. The processed samples were preserved with sodium azide (0.02% w/w) for further analysis. The processed and non-processed whole milk samples were used for the analysis of physicochemical (fat globule size, viscosity, microscopy) and gelation properties of deer milk. The skimming of milk was done by centrifugation process (Thermo Scientific Multifuge X3R; 3,000g/15min at 10°C) and the skimmed samples were used for analysis (viscosity, casein micelles size, microscopy, and heat-induced changes).

Serum sample preparation – Serum samples of deer milk were prepared from processed and raw skim milk to analyse the heat-induced changes in deer milk protein with the reversed-phase HPLC method. The preparation of serum samples was done with two different methods, acetic acid precipitation and ultracentrifugation method.

Acetic acid precipitation- The acid precipitation method as described by Vasbinder and De Kruif (2003) modified for deer milk for pH adjustment with preliminary studies. The deer skim milk samples (0.4 g) were mixed with 780 µL Q water (around 40°C) in a 2 mL tube. 60 µL of 10% acetic acid was added to the sample and mixed properly with a vortex. The mixture was kept for 10 minutes without any disturbance and mixed with Sodium Acetate (60 µL) and Milli-Q water (700 µL). The whole mixture was again mixed properly with vortex and kept for 60 minutes. The samples were centrifuged at 3000*g* for 5 minutes to separate the serum and the supernatant as described by Vasbinder and De Kruif (2003). The supernatant samples were stored at -80 °C for further RP- HPLC.

Ultracentrifugation (UC) - The serum sample of deer skim milk was obtained by ultracentrifugation process ("Sorval WX Ultra 100, Thermo Scientific"; 63,000*g*/60min at 20

°C). The clear supernatant was collected in a 1.5 mL tube after ultracentrifugation as described by Vasbinder and De Kruif (2003). The supernatant samples were stored at -80 °C for future RP- HPLC analysis.

3.2.2 Compositional analysis

The proximate analysis of deer milk (fat, crude protein, lactose, and solid contents) was determined with "Milkoscan FT1 (Foss Electric, Denmark)". The complete mineral analysis of all milk samples used for this study was measured by Hill Laboratories (Hamilton, New Zealand). The concentrations of calcium, magnesium, potassium, sodium, and phosphorus were determined by inductively coupled plasma optical emission spectrometry. The concentration of copper, iodine, selenium, and zinc was measured by inductively coupled plasma-mass spectrometry, and chloride content was determined by potentiometric titration (AOAC 971.27). The serum samples recovered from the acetic acid precipitation method were stored at -20 °C and used for the determination of protein composition.

The protein composition of deer milk was done by the "RP-HPLC (Reversed-Phase - High - Performance Liquid Chromatography)" method. Reversed-Phase High-Performance Liquid chromatography is a widely used technique to identify heat-induced changes in milk protein and the sample preparation (Table3.2) was performed as described by Bobe, Beitz, Freeman, Lindberg, and Chemistry (1998). The skim milk and serum samples produced from raw, pasteurized, and heated skim milk were mixed with the same amount of solution A (Table 3.2) as described by Bobe, Beitz, Freeman, and Lindberg (1998). The samples were incubated for 60 minutes at room temperature and then centrifuged using "MiniSpin® plus centrifuge" (14000g/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 a syringe filter (0.2µm) and transferred into HPLC vials.

Table 3.2: Recipes for the solutions A and B used for sample preparation (per 100mL)

Chemicals	**solution A**	**solution B**
Bis-Tris	2.1 g	-
GDN-HCl (Guanidine HCl)	57.3g	43.0g
Sodium Citrate	0.157g	-
DTT (Dithiothreitol)	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. A reversed-phase C18 column (Aeris Widepore 3.6μm XB-C18 RP; Phenomenex, Torrance, CA) was used to separate the major proteins in the samples. Solvent A (Acetonitrile, water, and trifluoroacetic acid at a ratio of 100:900:1 (v/v/v)) and Solvent 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, Beitz, Freeman, and Lindberg (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 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 the next sample (9 min). The total run time was 45 min, and the flow rate was 0.6mL/min. The fractions of major proteins in deer milk were calculated from the area under chromatogram peaks for the corresponding proteins and expressed in percentage.

$$\text{Fraction of individual protein (\%)} = \frac{Peak\ area\ of\ individuals\ proetin}{Peak\ area\ of\ total\ proteins} \times 100 \qquad \text{Eq.3.1}$$

The extent of the whey protein casein micelle association was calculated as the difference in whey protein concentration between raw and heated milk serum samples. 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;

$$\text{Serum WP (\%)} = \frac{Peak\ area\ of\ WP\ in\ heat\quad serum}{dx Peak\ area\ of\ WP\ in\ raw\ serum} \times 100 \qquad \text{Eq.3.2}$$

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

Casein and Whey protein determination through HPLC Method – Different type of casein protein identified with the help of HPLC chromatograms and cow's HPLC chromatograms have been considered as a reference to identity the high concentrated proteins in deer milk. Whey protein chromatograms were also observed in this study but due to lack of references or research the identification of whey protein in deer milk considered as out of scope of this study.

3.2.3 Physicochemical properties analysis

Milk Fat globule (MFG) size - The size distribution of milk fat globules in raw and processed deer milk was determined by "Malvern MasterSizer 2000 (Malvern Instruments Ltd., Malvern, UK)" using the laser diffraction method (Static light scattering). This technique works on the principle of scattering of monochromatic laser light by fat globules/casein micelles without using SDS solution and of fat globules with SDS solution. This scattered light is received by detectors at different angles. These received signals are then converted into particle size distribution using theoretical models (Truong, Palmer, Bansal, & Bhandari, 2016). The particle size distribution is described as the distribution of all particles present in milk that can scatter the light (mostly fat and casein micelles). But to capture one peak graph of fat globules it is important to disassociate the casein micelles and other aggregation from the milk samples. So milk samples were diluted (1:2 v/v) in a 2% SDS solution containing 50 mM EDTA (pH 6.7) to dissociate the casein micelles and other aggregation from the milk sample as mentioned in Ye at al. (2002). The diluted samples were added in dispersant (RO water) within the laser obscuration range of 10 – 12 %. The measurement of each sample was triplicated and the average volume mean (D [4,3]) was reported as the diameter of MFG.

Figure 3.1: Demonstration of "Malvern Master-Sizer 2000 (Malvern Instruments Ltd, Malvern, UK)".

Casein micelles size - The average casein micelles size was measured by Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK) at 25 ºC and the method was upgraded as described in Anema (1997) by considering the deer milk composition and to obtain the attenuation level in between 4 to 6 for the measurement. The skim milk samples were diluted 100 times with a calcium imidazole buffering solution (pH 6.7). The diluted samples were filtered with 0.45µm polyvinylidene fluoride syringe filters to remove the interfering large particles. The measurement of each sample was triplicated to reduce measurement errors.

Figure 3.2: Demonstration of "Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK)"

pH and Buffering capacity (BC) - The pH of raw and processed deer milk samples was measured using a HI-2202 edge®blu pH meter (Hanna Instruments, Woonsocket, RI). The BC of raw and processed deer milk was analyzed by acid titration as described by Park et al. (1991) in duplicate. A 25 mL of milk sample was titrated with additions of 1 mL of 0.5 M hydrochloric acid under stirring until the pH value dropped to 4.2. The pH of milk was recorded after each

step of acid addition. The measurement of each sample was triplicated and the BC was calculated by the Van Slyke equation as presented in Equation 3.1.

Van Slyke equation (BC) $$\frac{dB}{dpH} = \frac{\text{mL acid added} \times \text{Normality of acid}}{\text{The volume of milk} \times \text{pH change}}$$ Eq. 3.1

Figure 3.3: Demonstration of "HI-2202 edge®blu pH meter (Hanna Instruments, Woonsocket, RI)"

Ionic calcium concentration (Ca^{2+}) - The Ca^{2+} concentration of raw and processed deer milk was determined by Orion 9720BNWP calcium selective electrode (Thermo Scientific, USA) and Eutech pH 700 pH/mV meter (Thermo Scientific, USA). The combination of five different calcium chloride solutions (1.0, 2.0, 3.0, 4.0, and 5.0 mM) was used to produce the calibration curve. Milk samples and $CaCl_2$ solutions were kept at the same temperature (20 °C) before the measurements. The electrode was calibrated with all five $CaCl_2$ solutions and the measured values were recorded for further analysis. The electrode was dipped in each milk sample after completion of calibration until the mV values were stabilized. The Ca^{2+} concentration was calculated from the recorded mV values using a linear equation and the measurement of each sample was triplicated as described in Li, Ye, and Singh (2020b).

Ethanol stability - Ethanol stability of deer milk samples was determined by mixing an equal volume of milk and ethanol of different concentrations (55%, 57.5%, 60%, and 65%) with continuous stirring and observation. The highest concentration of ethanol that did not induce coagulation was considered as the ethanol stability of deer milk. The measurement of each sample was triplicated.

Viscosity - The viscosity of deer milk samples was determined by using an 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). A shear rate sweep from 10^{-2} to 10^{-3} s^{-1} was performed for 180 seconds. The average data of triplicates were taken as viscosity results from the shear rate range of 10-100 s^{-1} (stable range for measuring the viscosity results).

Microscopy- The structure of casein micelles and fat globules in deer milk samples was imaged using transmission electron microscopy (TEM). Samples for microscopy were prepared by Raoul Solomon and Yanyu He at Manawatu Microscopy and Imaging Centre of Massey University, Palmerston North (New Zealand) by following the method as described by Li, Ye, and Singh (2021). Milk samples were imaged with a Tecnai G2 Spirit BioTWIN (FEI company, Czech Republic) paired with a Veleta TEM camera (Olympus SIS Germany) at 11500/45000*X* magnification with the scale of 500nm and 1 micrometer.

3.2.4 Heat-induced whey protein denaturation and whey protein-casein micelle association

The native protein was determined for processed and raw milk to estimate the heat-induced protein denaturation. The native proteins were separated from casein micelles and denatured whey protein using acetic acid precipitation as described above and the supernatant containing the native whey proteins was collected for the analysis by RP-HPLC as described in section 3.2.2. The milk serum obtained by the ultracentrifugation method was also used to characterize the soluble aggregates of denatured whey proteins. The proportion of whey proteins forming aggregates in the serum phase was calculated as the difference between the total amount of whey protein denaturation and whey protein that is associated with casein micelle. Each sample was prepared and analyzed at least in duplicate. The 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;

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

Denatured WP (%) = 100 – $Native\ WP$ (%) Eq. 3.5

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

Serum aggregates (%) = $Serum\ WP$ (%) – $Native\ WP$ (%) Eq. 3.6

Casein

3.2.5 Gelation properties analysis

Gelation properties of raw and processed deer milk were measured by AR-G2 magnetic bearing rheometer (TA Instruments, Crawley, West Sussex, UK) with Peltier concentric cylinder geometries 1 that include a standard cup (r=15mm) and DIN rotor (r=14mm, h= 44mm). Rheology Advantage Instrument Control AR software (Version) was used for operating the instrument.

Figure 3.4: Demonstration of "AR-G2 magnetic bearing rheometer (TA Instruments, Crawley, West Sussex, UK)"

Rennet gelation - The rennet gelation was determined as described by Glantz, Lindmark Månsson, Stålhammar, and Paulsson (2011). The milk samples were prewarmed to 32 ºC and pH was adjusted to 6.5 by using 0.5 M HCl solution during the rennet gelation analysis for all milk samples. The rennet gelation was induced by chymosin (HANNILASE® XP 1050 NB; Christian Hansen A/S, Horsholm, Denmark). The pre-warmed milk was inoculated with chymosin at the concentration of 38 international milk clotting units (IMCU)/L of milk with

continuous stirring for 2 minutes. The inoculated milk was transferred (20 mL) into a rheometer cup and analysis was done at 32 ºC for 60 minutes running time. The time difference between the inoculation of the milk sample and the beginning of the test was recorded to determine the actual gelation time and other corresponding properties (G' and loss tangent). Rheology Advantage Data Analysis software (v5.7.0) was used for analyzing the data file. Gelation time was defined as the point at which the storage modulus (G') turns greater than 1 Pa and the measurement of each sample was triplicated.

Acid gelation - The acid gelation was determined with an AR-G2 magnetic bearing rheometer (TA Instruments, Crawley, West Sussex, UK). The milk samples were acidified by glucono-δ-lactone (GDL), and their gelation properties were measured by an 8-hour low-amplitude oscillation test at 30 °C as modified by Skelte G. Anema, Lee, Lowe, and Klostermeyer (2004) and Li et al. (2020b). The concentrations of GDL used for deer milk were selected as 3% (wt/wt) to achieve approximate pH of 4.2 after 8 hours of acidification. The deer milk samples (100 mg) were prewarmed from 4 °C to 30 °C and mixed with GDL powder (3 g) under continuous mixing for 2 minutes. Then 20 mL of inoculated milk sample was transferred to Rheometer and the rest of the sample was transferred to a jacketed beaker connected with a water bath set at 30 °C for pH measurement. The pH of acidified milk was recorded by HI-2202 Edge®blu pH meter (Hanna Instruments, Woonsocket, RI) at a 1-minute interval during the oscillation test.

The time difference between the inoculation/acidification of the milk sample and the beginning of the test was recorded to determine the actual gelation time and other corresponding properties (G', G'', gelation pH, final pH and loss tangent). Rheology Advantage Data Analysis software (v5.7.0) was used for analyzing the data file. Gelation time was defined as the point at which the storage modulus (G') turns greater than1 Pa and the measurement of each sample was triplicated.

4. Effect of lactation and processing on fresh deer milk characteristics

4.1 Introduction

The composition and nutritional quality of deer milk have been well studied and reviewed in the literature review (Chapter 2) and outcomes suggest that deer milk composition differs from cow and other non-bovine milk. Chapter 2 also outlined that some studies have been done for the physicochemical properties of deer milk by De la Vara et al. (2018) & Roy et al. (2020a). The composition of deer milk raises the interest to explore the processing impact on the physicochemical and gelation characteristics of deer milk due to higher fat and protein content. In addition, limited information is available regarding the sensitivity of deer milk towards heat treatments or processing parameters. The composition and physicochemical properties of milk are subjected to lactation variations and these variations also impacted the heat-induced changes in physicochemical properties of different types of milk (Li et al., 2019). Therefore, understanding the impact of processing conditions and lactational variation on deer milk characteristics is important for the further development of different dairy products. The objective of this study was to explore the effect of the lactation period of deer on milk composition and to study the impact of processing conditions (heating and homogenization) on the physicochemical and gelation characteristics of deer milk.

4.2 Material and methods

Deer milk samples were supplied by Pamu Deer Milk, from a farm located in Gore, New Zealand throughout the lactation period. The lactation period of deer was divided into three parts based on a total of 150 days, early lactation (0 to 50 days), mid (51 to 100 days), and late lactation (101 to 151). A similar division of the lactation period for deer was mentioned by Luick (1974) based on the number of weeks. The compositional changes in deer milk were studied throughout the lactation period of deer whereas the impact of processing on physicochemical and gelation characteristics of fresh deer milk was investigated only from the mid to late lactation period. Detailed procedures regarding methods of analysis and processing are described in “Chapter 3”.

4.2.1 Sample preparation

The aliquots of fresh milk were stored at -20 ºC for mineral analysis and another part of milk was preserved at 4 °C with sodium azide (0.02% w/w). The milk was processed within 24 hours of milk reception for further analysis. The skimming of processed and non-processed milk was done by centrifugation to prepare the skim milk and serum samples as described in "Chapter 3".

4.2.2 Milk processing

The milk samples were processed within 24 hours at the Food Pilot plant of Massey University (Palmerston North, New Zealand). The milk was homogenized with a two-stage homogenizer (20 MPa in the first stage and 5 MPa in the second stage at 65°C). Milk samples were treated with three different processing conditions (pasteurization (75°C/15 s) without homogenization, pasteurization (75°C/15 s) with homogenization, the higher degree of heating (95°C/5 min) with homogenization. Milk was heated with an indirect UHT system and a preheated water bath was used to complete the holding process for heated milk under similar heating conditions. All three processed milk samples were followed by immediate cooling (20°C) using ice water and stored at 4 °C with the addition of sodium azide (0.02% w/w) for further analysis within 72 hours.

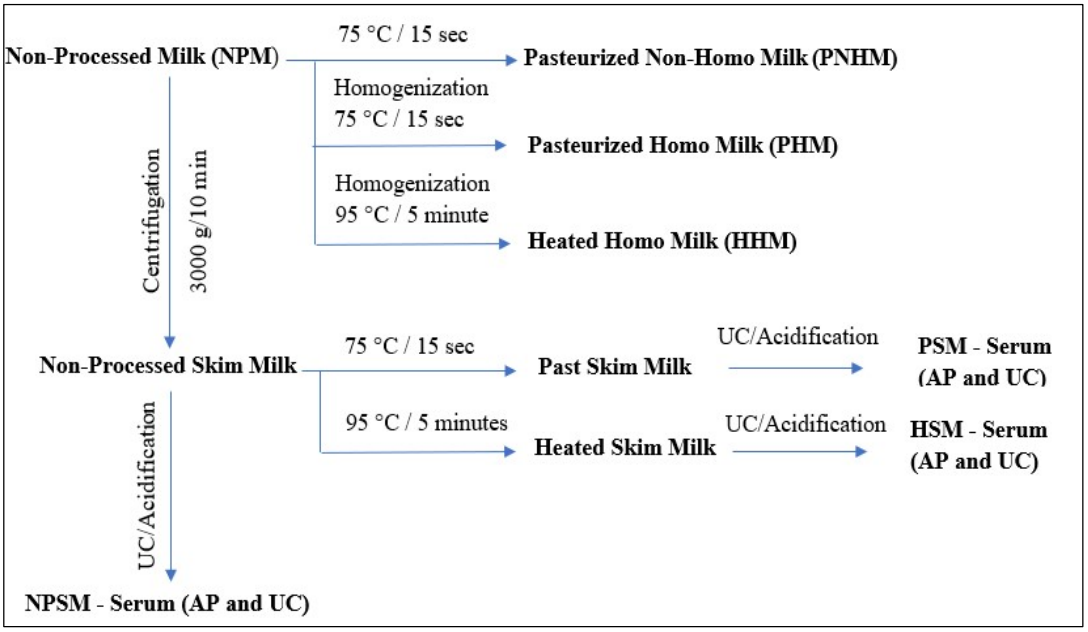

Figure 4.1: The schematic representation of processing and preparation of milk samples *AP: Acetic acid precipitation, *UC- Ultracentrifugation, *PSM: Pasteurized skim milk, *HSM: High heated skim milk.

Skimming of milk was done with centrifugation process (3000*g*/10 minutes at 10 °C) and skim milk samples were used for determination of protein composition and physicochemical analysis. Skim milk was treated with two different heat treatments (75 °C/15 s and 95 °C/5 minutes) to prepare the serum samples for the analysis of the heat-induced changes in deer milk protein with the RP-HPLC method. The preparation of serum samples was done with two different methods such as acetic acid precipitation and ultracentrifugation method as described in sections 3.2.1.1 and 3.2.1.2.

4.2.3 Milk analysis

To understand the impact of the lactation period on milk composition and mineral distribution in deer milk the compositional properties (fat, crude protein, and total solids contents) were measured by MilkoScan FT1 (Foss Electric, Denmark), and the mineral analysis was analysed in Hill Laboratories (Hamilton, New Zealand). To study the impact of processing conditions on physicochemical characteristics of deer milk different analysis was done using processed and non-processed deer milk samples as described in Figure 4.1. The physicochemical properties of deer milk include measurement of fat globules and casein micelles size, ethanol stability, Ca^{2+} concentration, pH, and buffering capacity. The protein composition and heat-induced changes in deer milk protein were determined by the RP-HPLC method using the skim and serum samples. Microscopy (TEM) of milk samples was also done to analyse the processing impact on the colloidal system and their distribution in different processed deer milk samples. To understand the impact of heat treatment and homogenization pressure on gelation properties of deer milk, two different gelation methods were used in this study, acid and rennet gelation. The detailed procedure and methods of each analysis are described in Chapter 3.

4.3 Results and discussion

4.3.1 Lactational variation in deer milk composition

The lactational variation in the proximate composition of deer milk is summarised in Table 4.1. The average fat, protein, and lactose contents of deer milk were 10.5%, 7.6%, and 5.4% respectively. The average value of solids not fat (SNF) and total solids of deer milk were measured as 13.6% and 23.9% respectively. These proximate compositions of deer milk were within the range of deer milk composition reported previously (Serrano et al., 2018; Y. Wang et al., 2017). In contrast, De la Vara et al. (2018) observed the lower content of fat and protein concentration of deer milk as compared to our results.

Table 4.1: Seasonal composition of deer milk (% w/w)

Components	Early-lactation	Mid-lactation	Late-lactation
Fat	8.2 ± 0.8^{c}	10.6 ± 0.6^{b}	12.6 ± 0.6^{a}
Protein	7.2 ± 0.2^{b}	7.3 ± 0.2^{b}	8.2 ± 0.5^{a}
Solids not fat	13.6 ± 0.1	13.4 ± 0.3	13.7 ± 0.5
Lactose	5.7 ± 0.2^{a}	5.5 ± 0.3^{a}	5.1 ± 0.1^{b}
Total solids	21.4 ± 1.1^{c}	23.9 ± 0.8^{b}	26.6 ± 1.2^{a}

[abc] Mean values (Mean ± SD) with different lowercase superscripts within the same row differ significantly ($P < 0.05$).
*Analyzed by one-way ANOVA test.

The fat content of deer milk increased significantly with the lactation stage from early to late ($P < 0.05$). The fat content of deer milk increased almost 50% in late lactation as compared with the early stage. The protein content in deer milk increased throughout the lactation period but the major variation happened during the late lactation period. Milk received in this period contained almost 14% higher protein content than milk received in the early and mid-stage of lactation. A similar trend of lactational variation in fat and protein content has been observed in sheep milk (Aganga, Amarteifio, & Nkile, 2002; Kuchtík, Sustova, Urban, & Zapletal, 2008). In contrast, Heck, Van Valenberg, Dijkstra, and Van Hooijdonk (2009); Mayer and Fiechter (2012) observed that the fat and protein content of cow and goat milk was lowest in mid-lactation and increased with advancing lactation. The lactose content reduced slightly over lactation and the lowest value of lactose in deer milk was measured in late lactation. This trend

agreed with other studies that reported on cow milk and goat milk in a lactational calving system. (Li et al., 2019; Mayer & Fiechter, 2012).

Lactational variation in the protein composition of deer milk – The fraction of individual proteins and their variation along with the lactation period is summarised in Table 4.2. The average value of total casein content in deer milk was measured at 62.1 g/L, which was around 82.02% of total milk protein. The casein content in deer milk increased over the lactation period and was highest in late-stage lactation (67.2 g/L). The casein of deer milk was distributed among four different types including κ-caseins, α_{s2}-, α_{s1}- and β-casein.

Table 4.2: Faction of major proteins present in deer milk

Proteins (g/L)	**Early lactation**	**Mid lactation**	**Late lactation**
Casein (g/L)	59.3 ± 0.02 [a]	59.8 ± 0.01 [b]	67.2 ± 0.01 [c]
κ-CN	4.0 ± 0.35 [c]	5.0 ± 0.34 [b]	5.8 ± 0.32 [a]
α_{s2}-CN	6.5 ± 0.97 [a]	4.8 ± 0.30 [b]	4.7 ± 0.06 [c]
α_{s1}-CN	2.4 ± 0.08 [a]	2.1 ± 0.22 [b]	2.0 ± 0.67 [c]
β-CN	46.4 ± 0.25 [a]	45.9 ± 0.80 [b]	45.6 ± 1.39 [b]
Whey protein (g/L)	12.7 ± 0.02 [c]	13.2 ± 0.01 [b]	14.8 ± 0.01 [a]
WP-1	6.4 ± 2.30 [c]	7.3 ± 0.25 [b]	9.3 ± 0.96 [a]
WP-2	1.9 ± 0.66 [a]	1.6 ± 0.23[c]	1.7 ± 0.09 [b]
WP-3	3.6 ± 0.56 [a]	3.5 ± 0.35[b]	3.4 ± 0.13[c]
WP-4	0.8 ± 0.30 [a]	0.6 ± 0.10 [b]	0.4 ± 0.07 [b]

Mean values (Mean ± SD) with different superscripts within the column differ significantly ($P < 0.05$), CN – Casein, WP – Whey protein. *Units used for all presented values: g/L, *Analysed by one-way ANOVA test.

The proportion of individual casein content (κ-caseins, α_{s2}-, α_{s1}- and β-casein) varied significantly with lactation of deer ($P < 0.05$). The concentration of κ-caseins increased whereas α_{s2}-, α_{s1}- and β-casein content in deer milk decreased over the lactation period. In deer milk, β-casein was determined as the dominant protein (average value of ~45.9 g/L), whereas the α_{s1}-casein was determined as the lowermost protein (average value of ~2.2 g/L) among all four caseins.

Wang et al. (2017) also reported the β-casein as the dominant protein in deer milk, however, the concentration was reported around ~34 g/L of total protein. Opatha Vithana (2012) also mentioned that β- casein is a dominant protein in most non-bovine milk such as goat milk (~26 g/L), sheep milk (~36 g/L), and deer milk (~32 g/L). In contrast, in cow milk α_{s1}-casein (~21 g/L) is considered the dominant protein, followed by β-casein (~15 g/L). The relative proportion of individual caseins in deer milk was different from other species' milk such as cow, goat, and sheep. These differences likely arise due to differences in species or genetic variants that may produce milk with different protein compositions.

The average value of total whey protein in deer milk was ~13.6 g/L which was approximately 17.98% of total milk protein. Four different types of whey protein have been observed in deer milk during the current study and the concentration of total whey protein increased over the lactation period. The WP in the current study was divided into 4 different categories, WP1, WP2, WP3, and WP4, due to limited available information regarding the individual and specific characterization of whey protein in deer milk. However, it has been observed that the two dominant whey proteins in deer milk were β-Lactoglobulin (~8.89 g/L) and α-Lactalbumin (~2.3 g/L) (Opatha Vithana, 2012; Wang et al., 2017). In the current study, the concentration of WP1 and WP3 were measured as the most dominant whey protein, 7.7 g/L, and 3.5 g/L respectively. Overall, the concentration of whey protein in deer milk increased significantly ($P < 0.05$) which is mainly driven by WP1, however, the concentration of the other three whey proteins decreased over the lactation period.

Lactational variation of mineral content in deer milk – The impact of lactation on the mineral distribution of deer milk is summarised in Table 4.3. Major minerals were calcium, magnesium, potassium, phosphorus, sodium, and chloride, whereas copper, iodine, selenium, and zinc were found at trace levels. The average contents of calcium, phosphorus, and potassium in fresh deer milk were 295 mg, 206 mg, and 134 mg (per 100 g of milk), respectively. The concentration of sodium and chloride increased, whereas calcium, phosphorous, and potassium content significantly ($P < 0.05$) decreased over the lactation period. The concentration of calcium and phosphorous decreased over lactation despite the increase in protein content of deer milk. The same variation of these minerals was observed in deer milk by (Gallego, Landete-Castillejos, Garcia, & Sánchez, 2006). Variation of some other minerals present at trace levels in deer milk was also observed throughout lactation. The

concentration of selenium increased, whereas iodine and copper decreased significantly ($P < 0.05$) over the lactation. The average concentration of Cu of fresh deer milk in late-lactation reduced drastically as compared to early-lactation.

Table 4.3: Mineral composition of fresh deer milk

Minerals	**Lactation**	**Total amount**
Calcium (mg/100g)	Early	300 [b]
	Mid	307 [a]
	Late	280 [c]
Magnesium (mg/100g)	Early	16.9 [b]
	Mid	17.1 [a]
	Late	16.4 [c]
Potassium (mg/100g)	Early	145 [a]
	Mid	135 [b]
	Late	122 [c]
Sodium (mg/100g)	Early	34.3 [c]
	Mid	37.3 [b]
	Late	44.0 [a]
Phosphorus (mg/100g)	Early	217 [a]
	Mid	207 [b]
	Late	198 [c]
Chloride (mg/100g)	Early	68.0 [c]
	Mid	75.7 [b]
	Late	91.5 [a]
Copper (mg/kg)	Early	0.31 [a]
	Mid	0.07 [b]
	Late	0.05 [c]
Iodine (mg/kg)	Early	0.72 [a]
	Mid	0.77 [a]
	Late	0.53 [b]
Selenium (mg/kg)	Early	0.01 [c]
	Mid	0.02 [b]
	Late	0.03 [a]
Zinc (mg/kg)	Early	10.3 [a]
	Mid	8.97 [c]
	Late	9.35 [b]

Figure 4.2 summarised the distribution of soluble and colloidal concentrations of Ca, P, and Mg in deer milk. The major concentrations of Ca and P were distributed in the colloidal phase, on the other hand, Mg was found mainly distributed in soluble form in deer milk. The trend of soluble Ca (21%), Mg (57%), and P (33%) in deer milk were aligned with other studies of cow and goat milk (Gallego et al., 2006). O'connor and Fox (1977) mentioned that the concentration

of soluble minerals mainly depends on the methods of determination as well as the composition of milk.

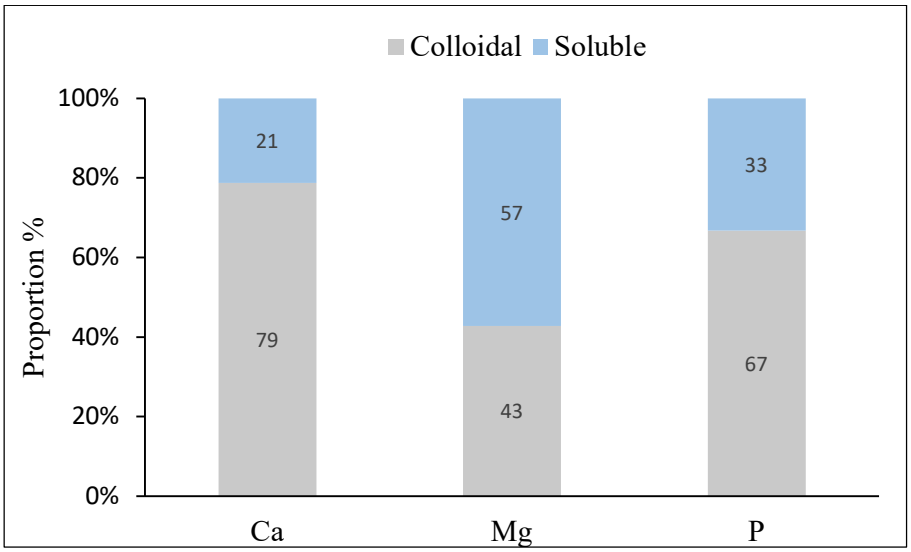

Figure 4.2: The schematic representation of soluble and colloidal phase of Ca, Mg, and P in deer milk

4.3.2 Effect of processing on physicochemical properties of deer milk

Table 4.4 shows the processing effects on the physicochemical properties of deer milk. Overall, the processing conditions affected the physicochemical properties except for the pH of the milk.

Table 4.4: Effect of processing conditions on physicochemical properties of deer milk

Properties	NPM	PNHM	PHM	HHM
pH	6.67 [a]	6.66 [a]	6.67 [a]	6.65 [a]
Buffer capacity (dB/dpH)	0.06 [a]	0.05 [b]	0.05[b]	0.04 [c]
Ionic Calcium (mM)	3.34 [a]	3.10 [b]	3.09 [b]	3.07 [c]
Ethanol Stability	57.5 ± 1.3	ND	ND	ND
Viscosity (cP)	6.1± 0.01 [c]	6.2 ± 0.01 [c]	110 ± 2.7 [a]	790 ± 16.2 [b]
Casein Micelles size (nm)	195 ± 4.30 [c]	199 ± 4.70 [b]	199 ± 4.70 [b]	244 ± 7.80 [a]
Fat globule size - D [4,3] (µm)	6.7 ± 0.19 [a]	6.6 ± 0.12 [a]	0.48 ± 0.05 [c]	0.61 ± 0.02 [b]

NPM – Non-processed deer milk, PNHM - Pasteurized (75 °C/15 sec) non-homogenized deer milk PHM – Pasteurized (75 °C/15 sec) homogenized (200/50 Bar) deer milk, HHM – High heated (95 °C/5 minutes) homogenized (250/50 Bar) deer milk (95 °C/5 minutes), D [4, 3] – Fat globule size or volume-weighted mean (µm), ND – Not determined.

[abc] Mean values (Mean ± SD) with different lowercase superscripts within the same row differ significantly ($P < 0.05$)
*Analysed by one-way ANOVA test.

pH and ionic calcium (Ca^{2+}) concentration - The average pH of deer milk was 6.67 which was unaffected by processing conditions. The mean value of Ca^{2+} for non-processed deer milk was 3.34 mM, and it was observed that heating intensity reduced the concentration of Ca^{2+} significantly ($P < 0.05$). The heating of fresh deer milk at 75 °C for 15 sec resulted in a reduction of almost 7% for both pasteurized samples (PNHM and PHM). A similar trend was observed when milk was heated at 95 °C for 5 minutes and resulted in the reduction of around 8% in Ca^{2+} concentration in deer milk. The same impact of heat treatment on Ca^{2+} concentration in different species (including cow, sheep, and goat) has been reported previously. It has been reported that high-temperature short time (HTST) pasteurization and

ultra-high temperature (UHT) of cow milk reduced the Ca^{2+} concentration between 5.5 % to 10 % (May & Smith, 1998). Li et al. (2019) also observed a similar reduction in Ca^{2+} concentration for cow milk after heating at 90°C for 6 min. The heating of milk at different temperature conditions leads to the reduction of solubility of calcium phosphate, and this might cause a change in Ca^{2+} concentration. (Omoarukhe & Lewis, 2010).

Buffering capacity (BC) - The average buffering capacity of fresh deer milk was 0.063 dB/dpH before any processing. It was observed that the BC of deer milk was consistently reduced by heating intensity ($P < 0.05$). The heating of fresh deer milk at 75 °C for 15 sec resulted in a reduction of almost 11% BC for both pasteurized samples (PNHM and PHM), on the other hand, the heating of fresh deer milk at 95 °C for 5 minutes reduced the BC by 22%. Wang and Ma (2020) also proposed that heating above 90 °C could affect the buffering capacity of cow milk. Wang and Ma (2020) observed that as temperature increases the concentration of serum Ca, inorganic phosphate (Pi) and citrate decreases. Calcium and phosphate precipitation is an important factor to decide the BC of milk. The buffering capacity of milk is mainly affected by the colloidal calcium phosphate, soluble phosphate, citrate, bicarbonate, caseins, and whey proteins (Salaün et al., 2005).

Ethanol stability - Ethanol stability of fresh deer milk was 57.5% (v/v) at a natural pH of milk and it was observed that the ethanol stability of deer milk was lower than cow milk (around 75%). De la Vara et al. (2018) also mentioned that the ethanol stability of deer milk was around 62 % (v/v) which slightly resembled sheep's milk (63%, v/v), marginally higher than that of goat's milk (50%, v/v) but far lower than bovine milk (73 to 83 % v/v). It is suggested that a higher concentration of proteins (particularly casein) leads to lower ethanol stability of milk by promoting aggregation between casein micelles (Li et al., 2019).

Casein micelles size - The average value of casein micelles size measured in fresh deer milk was around 195 nm (Table 4.4), which was larger than the size of casein micelles as observed by Hovjecki, Miloradovic, Rac, Pudja, and Miocinovic (2020) in sheep milk (~179 nm) but smaller than goat milk (~253 nm). Roy, Ye, Moughan, and Singh (2020b) also observed the same results regarding the size of casein micelles in deer milk and proposed that casein micelles were the smallest in cattle milk (~165 nm), whereas sheep milk (~188 nm) and deer milk (~190

nm), which were similar, had the largest casein micelle diameters, followed by goat milk (~182 nm) and buffalo milk (~176 nm). The average casein micelles size of fresh deer milk was around 195 nm before any processing conditions. The impact on casein micelles sizes consistently increased with high-intensity heating. The heating of deer milk at 75 °C for 15 sec leads to a slight increase (~5 nm) in the size of casein micelles, on the other hand, the heating of milk at 95 °C for 5 minutes resulted in an almost 25 % increase in size (~244 nm) as compared to fresh deer milk (~195 nm) (Table 4.4). Hovjecki et al. (2020) also reported a similar impact of heating on casein micelles size in goat milk. It was observed that pasteurization (75°C/30s) did not affect the micelle size in goat milk, but a higher degree of heat treatments (90°C/1 min) increased the micelle size from 253 nm to 321 nm. The extent of increase in micelle size at comparable heating conditions (95 °C) was larger in deer milk than cow milk (~ 5.6% increase in size) (Li et al., 2019). The heating of milk at high temperature (above 90 °C) resulted in denaturation of whey protein and association of denatured whey protein (especially β-LG) with casein micelles. The denatured whey protein-micelles association, as well as micelles-aggregation, have been considered the main reasons for the increase in casein micelles size (Donato, Alexander, & Dalgleish, 2007; Li et al., 2019).

Fat globule size - The average diameter of fat globules in fresh deer milk was measured at 6.7 µm. Homogenization reduced the size of fat globules by 12-14 times, as expected. The slight variation of fat globule size of deer milk was observed with heating effect, the size of fat globules of HHM was marginally higher as compared with PHM, but was not significantly different ($P > 0.05$) as compared with the effect of homogenization. The size of fat globules present in deer milk was almost double that observed in different species, including cow milk (~3.69 µm), goat milk (~3.49 µm), sheep milk (~3.30 µm). The size of deer milk fat globules was similar to that of buffalo milk fat globules (~5.92 µm) (El-Zeini, 2006; Park, 2010).

Viscosity - The viscosity of fresh deer milk (NPM) was 6 cP (Table 4.4) as an average of all milk samples, which is higher than the viscosity of cow milk (~ 4.76 cP) and goat milk (~ 4.71 cP) as observed by Ibrahim, Naufalin, Muryatmo, and Dwiyanti (2021). Variation of viscosity in deer milk with processing conditions was observed during this study. The heating of milk at 75 °C for 15 sec (PNHM) without homogenization did not affect the viscosity of deer milk but when the pasteurized milk was processed with homogenization conditions (250/50 bar) the

viscosity of deer milk increased spontaneously (6 cP to 110 cP). High-intensity heat treatment of deer milk at 95 °C for 5 minutes (HHM) further increased the viscosity (110 cP to 790 cP). Y. Li et al. (2018) proposed a larger number of fat globules present in higher fat milk resulted in the increase of viscosity with homogenization and heating conditions because of the resistance of fat particles to the flow. The size of casein micelles also increases with the processing conditions, and the viscosity of deer milk was correlated with the micelle size ($P<0.05$, $r = 0.72$). The results of TEM (shown below), as well as HPLC, also indicated similar changes in whey proteins and casein micelles of deer milk with processing conditions. It has been suggested that the heating of skim milk at high temperature (at 85 °C) resulted in the association of denatured whey protein with casein micelles, which leads to increases in particle size. This impact of heat causes a change in the structure of micelles and increases the volume fraction, which would further result in a higher viscosity of milk (Skelte G Anema & Li, 2003b; Jeurnink & De Kruif, 1993).

4.3.3 Heat-induced changes in deer milk protein

Figure 4.3 shows the Transmission electron microscopy (TEM) images of pasteurized and high heated homogenized deer skim milk. The casein micelles of pasteurized milk appeared relatively uniform and small with a smooth surface, as compared with the casein micelles in heated milk. The image of high heated milk clearly shows larger sized casein micelles caused by high heat treatment (95 °C for 5 minutes). In pasteurized milk, most of the casein micelles were distributed uniformly without any aggregation (Figure 4.2A), whereas in the high heat deer milk, aggregation of casein micelles can be seen clearly, and micelles are bigger, as highlighted with the yellow circle (Figure 4.3B). It was also observed that Figure 4.3B contains a more hairy/stringy structure as compared to 4.3A, which indicates denatured whey protein aggregation in high heated deer milk, as highlighted with the red circle. The TEM images as well as the size of casein micelles provided supporting evidence that the size of casein micelles increased along with the heating effect (mainly heating at 95 °C for 5 minutes). The association of whey proteins and casein during heat treatment of milk dramatically affects the protein organization in both colloidal and serum of milk. The heat treatment and subsequent formation of denatured whey protein/ κ-casein complexes on the surface of casein micelles resulted in the aggregation of casein micelles and bigger micelles (Donato & Guyomarc'h, 2009; Li et al., 2019).

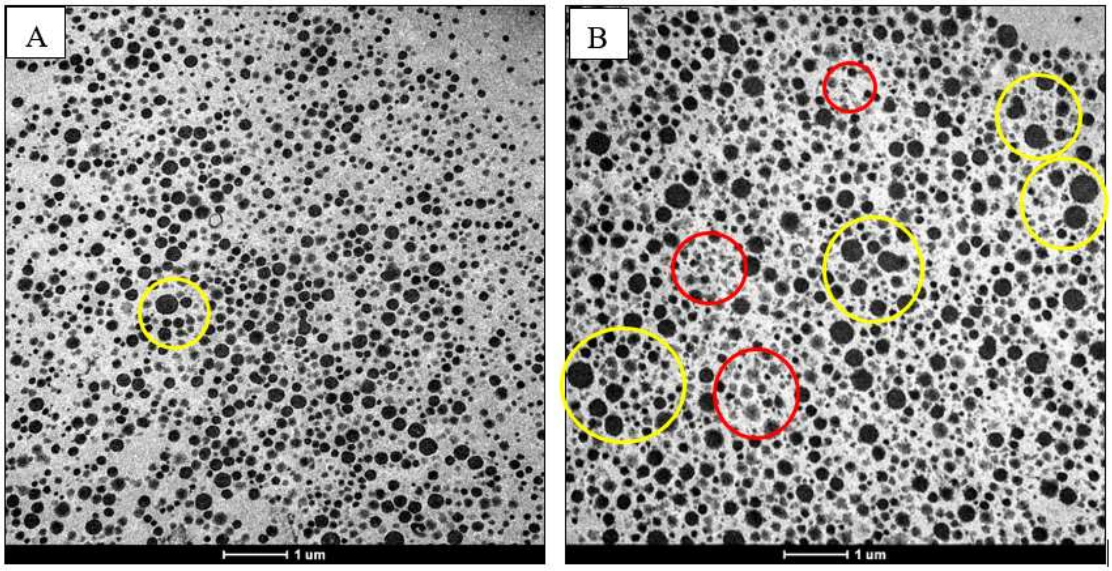

Figure 4.3: Transmission electron microscopy (TEM) images at 11500*X* magnification of (A) Pasteurized homogenized (75 °C/15 sec) and (B) high heated homogenized (95 °C/5 minutes) deer skim milk. Yellow circles represent the aggregation of casein micelles and red circles represent the aggregation of denatured whey protein with casein micelles. The scale for both images was used as 1 micrometer in size.

Figure 4.4 shows the denaturation level of individual whey protein in deer milk at two different heating conditions. The heating of deer milk at 95 °C for 5 minutes resulted in a higher extent of whey protein denaturation, as compared to milk heated at 75 °C for 15 sec. The average denaturation of total WP in high-heated deer milk was almost 95%, whereas the denaturation level of WP in pasteurized milk was around 65%. The denaturation level of all four WP varied according to the heating conditions. The maximum level of denaturation was observed for WP4 (~97%) and WP2 (~96%) at high heating conditions, whereas the variation of denaturation level at pasteurized conditions varied from 59 to 69%.

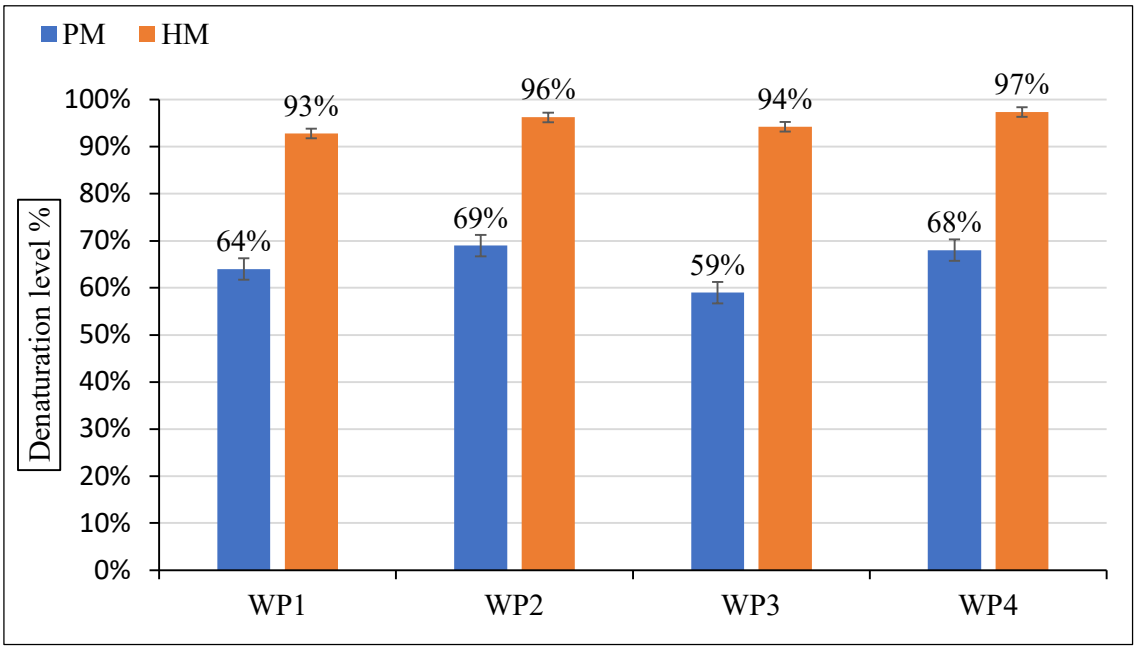

Figure 4.4: Denaturation level of various whey proteins of pasteurized deer milk (PM: 75 °C for15 sec) and high heated deer milk (HM: 95 °C for 5 minutes). Error bar represents the standard deviation errors (±).

Whey protein distribution in pasteurized deer milk (75 °C for 15 sec) is shown in Figure 4.5A. The distribution of pasteurized deer milk was determined as native protein WP (~36%), micelles associated WP (~45%) and serum aggregates WP (~36). On the other hand, when deer milk was heated at 95 °C for 5 minutes (Figure 4.5B) the concentration of micelles associated WP was determined ~87%, and the remaining 7% sustained as serum aggregates and 6% recovered as a native whey protein. The fraction of micelle-bound whey protein increased significantly more ($P < 0.01$) in high heated milk than the pasteurized milk. The difference in heating rate must be associated with a different extent of the whey protein-casein micelles association (Li et al., 2019). Oldfield, Singh, and Taylor (1998) also reported that WP-casein micelle association was more favored during slower and high heating conditions, such as the high heating (95 °C for 5 minutes) used in this study, as compared to rapid heating or lower heating temperature. The higher extent of whey protein denaturation as described in Figure 4.2 for high heated deer milk also favored the association of whey protein and casein micelles.

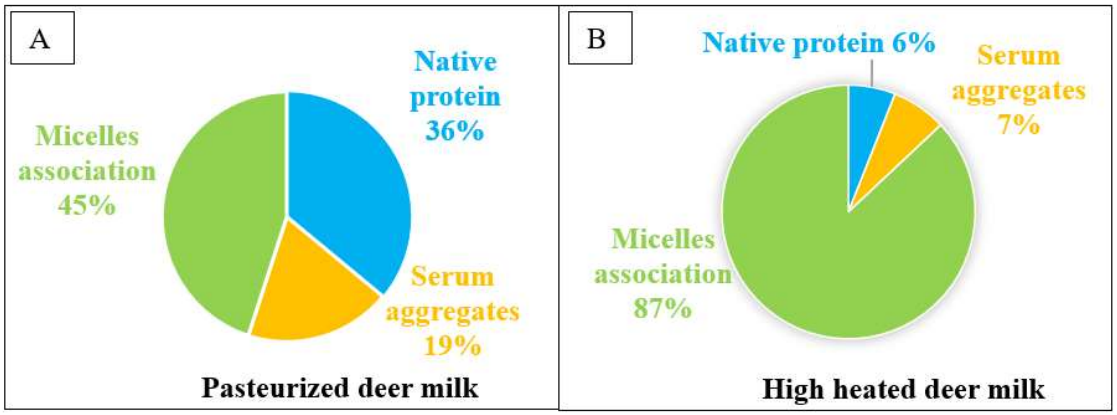

Figure 4.5: The fraction of whey protein distribution in (A) pasteurized deer milk (PM: 75 °C for15 sec) and (B) high heated deer milk (HM: 95 °C for 5 minutes) as micelle association, native protein, and serum aggregates.

The fraction of individual whey protein in high heated deer milk (95 °C for 5 minutes) is summarized in Figure 4.6, showing distribution in micelle-association, serum aggregates, and native protein. Among all whey proteins, WP3 was determined to be least associated with casein micelles (76%) compared with the other three whey proteins (~90%). The results supported the assumption that the WP3 was *α*-La, which tends to associate with the casein micelles at a lower extent during heating than *β-lg* (Dannenberg & Kessler, 1988).

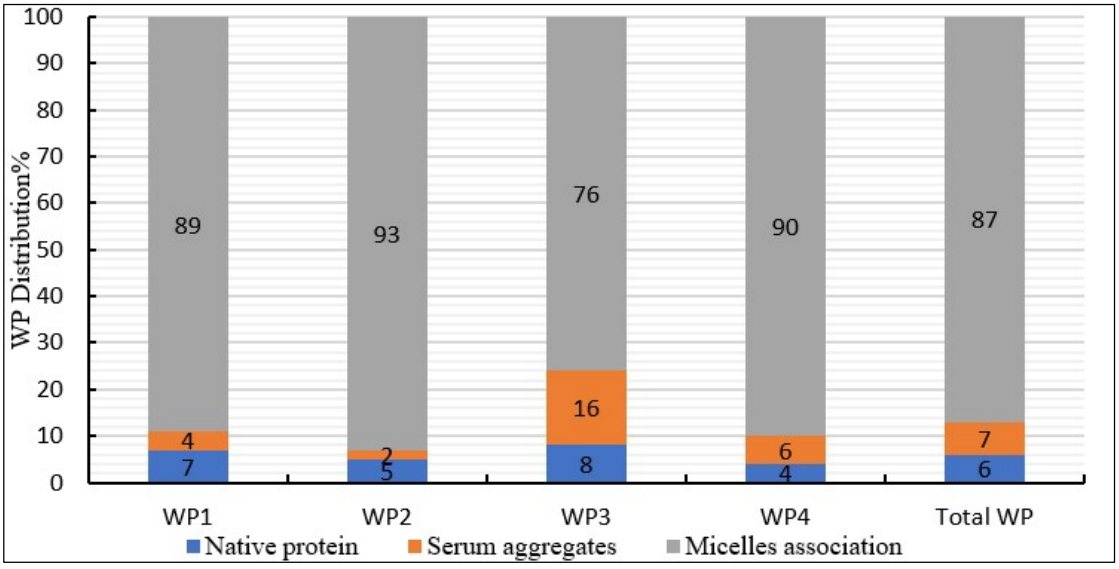

Figure 4.6: The fraction of whey protein distribution in high heated deer milk (95 °C for 5 minutes) as a micelle's association, native protein, and serum aggregates.

4.3.4 Effect of processing conditions on gelation properties of fresh deer milk

Acid gelation - Figure 4.7 represented the overall effect of processing conditions on acid gelation properties (*G* and pH profile) of deer milk. Table 4.5 shows the impact of processing conditions on individual acid gelation properties (*G,* tan delta, gelation time, and pH at different stages of acid gelation) of deer milk. The acid gel made from high heated deer milk (95 °C for 5 minutes) had overall better gelation properties as compared to those made from other milk. Although the other processing conditions, pasteurization, and homogenization, also improved the acid gelation properties of deer milk.

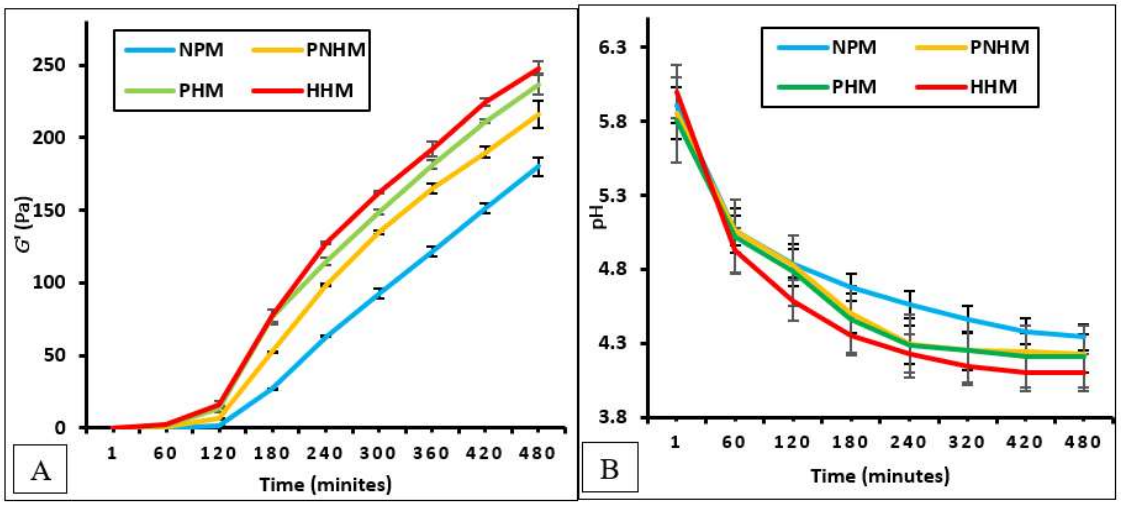

Figure 4.7: Processing effect on acid gelation properties G′ (A) and pH profile (B) of deer milk. NPM – Non-processed deer milk, PNHM - Pasteurized (75 °C/15 sec) non-homogenized deer milk - PHM – Pasteurized (75 °C/15 sec) homogenized (20 MPa/5 MPa) deer milk, HHM – High heated (95 °C/5 min) homogenized (20 MPa/5 MPa) deer milk (95 °C/5 min), Error bar represents the standard deviation errors (±).

Table 4.6 shows that the gel made from non-processed deer milk had the longest gelation time as compared with the acid gels made from processed deer milk. The acid gelation time was reduced by almost 50% when deer milk was heated at 75 °C for 15 sec without homogenization. In addition, homogenization, and high heating conditions (95 °C for 5 minutes) further enhanced the acid gelation properties and reduced the acid gelation time of deer milk. The acid gel made from high-heated homogenized deer milk had a shorter gelation time (30.8 minutes), which was almost 76% lower as compared with the acid gel made from non-processed deer milk (130.7 minutes). The mean values of final *G′* and tan delta of acid gels were also affected due to different processing conditions and improved along with the intensity of heat treatment.

The acid gel made from HHM had the highest final G and lowest Tan δ values as compared with acid gels made from other processed and non-processed milk. The final Tan δ was significantly lower for processed milk indicating the more elastic behavior or "solid-like" properties of the acid gels. The pH results of acid gels also echoed the variation of gelation properties in deer milk due to different processing conditions. The non-processed milk (NPM) was gelled at a lower pH of 4.8 than the other processed milk (approximately between 5.12 to 5.18). It is expected that milk with lower gelation pH would take a longer time to form the gelation and also form a stronger gel (Li, Ye, & Singh, 2020a).

Table 4.5: The impact of processing conditions on rennet gelation properties of deer milk

Acid Gelation parameters	**NPM**	**PNHM**	**PHM**	**HHM**
Gelation time (minute)	130.7 ± 4.12 [a]	64.9 ± 5.09 [b]	43.8 ± 4.80 [c]	30.8 ± 3.49 [d]
Tan δ at gelation time	0.35 ± 0.01 [a]	0.33 ± 0.02 [b]	0.31 ± 0.01 [c]	0.24 ± 0.18 [d]
Gelation pH	4.81 ± 0.05 [b]	5.12 ± 0.03 [a]	5.15 ± 0.04 [a]	5.18 ± 0.02 [a]
Final G' (Pa)	180 ± 5.10 [d]	216 ± 4.30 [c]	237 ± 5.12 [b]	243 ± 1.01 [a]
Tan δ	0.32 ± 0.02 [a]	0.27 ± 0.01 [b]	0.26 ± 0.02 [b]	0.26 ± 0.01 [b]
Gelation pH	4.32 ± 0.02 [a]	4.19 ± 0.03 [b]	4.22 ± 0.04 [b]	4.13 ± 0.01 [c]

[abcd] Mean values (Mean ± SD) with different lowercase superscripts within the same row differ significantly ($P < 0.05$), Tan δ – Tangent delta.
*Analysed by one-way ANOVA test.

Overall, heating of deer milk at 95°C for 5 minutes resulted in a shorter gelation time, higher gelation pH, and stronger gel formation. These results were broadly consistent or agreed with other studies regarding the impact of processing conditions on acid gelation properties of cow milk (Skelte G. Anema et al., 2004). The heating of milk (above 80 °C) is attributed to the heat-induced denaturation and aggregation of whey proteins (especially β-LG) and their interaction with casein micelles. The casein micelles of heated milk begin to aggregate at higher pH than

those from unheated milk leading to shorter gelation time. (del Angel & Dalgleish, 2006). Skelte G. Anema et al. (2004) mentioned that the gel made from heated milk had a significant correlation of soluble denatured whey proteins on the final G' value. The denatured whey proteins in the serum phase of milk support the aggregation of particles during acidification of milk, which potentially would be involved in the gel matrix. The same impact of heating on acid gel has been discussed by Lucey et al. (1997), who suggested that the mechanism behind the improvement of acid gel properties of heated milk could be due to the increase in participation of whey protein in gel network formation. The high heating conditions (HHM) favour more association of denatured whey protein, as well as casein micelles, which resulted in the increase in micelles size, which provides a compact structure during gel formation and better gelling properties. The increase in viscosity of PHM and HHM may also have played a part to impact the gelling properties of milk.

Rennet gelation - Figure 4.8 represented the variation of G′ value along with the time of acid gels of processed and non-processed deer milk. Table 4.6 states the average values of rennet gelation properties with standard deviation (G', final G', tan delta, gelation time) of deer milk as affected by different processing conditions. Overall, the processing conditions did not cause a consistent change in gelation properties of deer milk rennet gel.

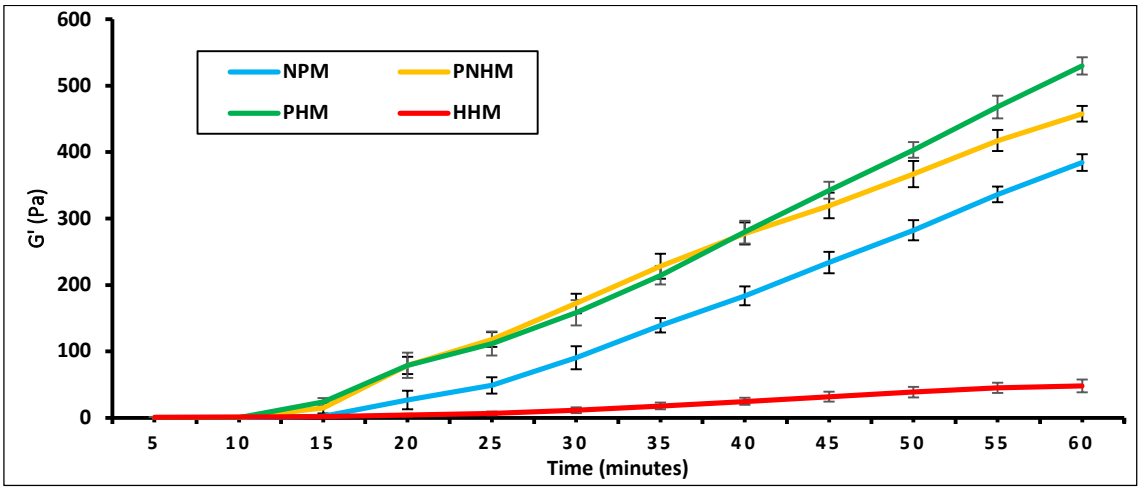

Figure 4.8: Processing effect on rennet gelation properties (final G′) of reconstituted deer milk. Processing conditions: NPM – Non-processed deer milk, PNHM – Pasteurized (75 °C/15 sec) non-homogenized deer milk PHM – Pasteurized (75 °C/15 sec) homogenized (20 MPa/5 MPa) deer milk, HHM – High heated homogenized (20 MPa/5 MPa) deer milk (95 °C/5 min). Error bar represents the standard deviation errors (±).

The gel made from pasteurized deer milk (PNHM and PHM) had better gelation properties than those made from non-processed and high heated deer milk, as shown in Figure 4.8. The mean values of rennet gelation properties (gelation time, final G′, and Tan δ) of processed and non-processed reconstituted deer milk are summarised in Table 4.8. The gelation time of rennet gel made from non-processed deer milk was higher than the gel made from processed deer milk. The gelation time reduced uniformly with increasing heating intensity and homogenization conditions. The gelation time of gel made from PHM and HHM was reduced by almost 22.5 % as compared to the NPM. The slight reduction in the mean values of Tan δ (lower value signifies the more elastic behaviour or "solid-like" properties of the gel) was also observed with different processing conditions of deer milk. The heating of milk at 75 °C for 15 seconds increased the final *G′* value of rennet gel almost 19%, as compared with a gel made from non-processed deer milk (NPM), in addition to heating, the homogenization conditions (PHM) also increased firmness of gel as determined with the final G′ (increased 37.7% of npm) value of rennet gel. But when the milk was heated at high heat intensity (95 °C/ 5 min), it resulted in a negative impact on the rennet gelation properties of deer milk. The gel made from HHM had the lowest final *G′* value as compared with other milk samples, which indicated that rennet gelation properties of deer milk reduced when processed with high heat treatment.

Table 4.6: The impact of processing conditions on rennet gelation properties of deer milk

Rennet gelation parameters	**NPM**	**PNHM**	**PHM**	**HHM**
Gelation time (minute)	19.1 ± 1.55 [a]	16.6 ± 0.07 [b]	14.9 ± 1.74 [b]	14.8 ± 0.53 [b]
Final Tan δ	0.31 ± 0.01 [a]	0.28 ± 0.01 [b]	0.26 ± 0.01 [b]	0.27 ± 0.01 [b]
Final G' (Pa)	384 ± 7.14 [b]	457 ± 7.21 [a]	529 ± 8.12 [a]	50 ± 3.5 [c]

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

*Analysed by one-way ANOVA test

It is well established that the rennet gelation properties of reconstituted milk can depend on various factors, including milk composition, the interaction of whey proteins, and casein micelles during processing conditions. The interaction of whey proteins provided both positive and negative impacts on rennet gelling properties during cheese manufacturing. On the one hand, denatured whey proteins could be incorporated into cheese curd and lead to higher yield from a given quantity of milk, while on the other hand, the interaction of whey proteins with casein interferes with the rennet coagulation process and leads to long coagulation time or weak curd structures (Logan et al., 2015). Heating conditions that induced extensive whey protein denaturation (HHM) reduced the rennet gelation time of deer milk, but also led to a marked decrease in the final *G'* of the gel. Heating conditions also don't affect the rennet gelation of sheep or goat milk (Montilla, Balcones, Olano, & Calvo, 1995; Raynal-Ljutovac et al., 2007). Further investigations are needed to confirm the exact impact of processing conditions on the rennet properties of reconstituted deer milk.

4.4 Conclusion

The composition of deer milk was investigated over three milking lactation stages early, mid, and late. The fat and protein content of deer milk was majorly affected during the different stages of lactation and increased from the early to the late stage of lactation. The impact of the lactation period was also observed for the distribution of minerals in deer milk, particularly the concentration of sodium and chloride increased, whereas calcium, phosphorous, and potassium content significantly ($P < 0.05$) decreased over the lactation period. Overall, the physiochemical and gelation properties of deer milk were affected by processing conditions, heat treatment, and homogenization. The high heat treatment (95 °C /5 min) of deer milk impacted the casein micelles and whey protein structure, which resulted in bigger micelles and more association of whey protein with casein micelles. The current study observed that homogenization of deer milk (25/5 MPa) greatly increased the viscosity of milk and high heating intensity further increased the viscosity of deer milk. Based on consistent literature reports, as well as consistent results of the current study it can be proposed that overall acid gelation properties of deer milk improved with heating intensity, and it can be suitable for yogurt production with superior gel firmness. It has been reported that milk composition (fat and protein content) and physicochemical (viscosity, casein micelles size, and whey protein denaturation) play an important role in curd formation during the gelation process. The impact of processing conditions on rennet gelation properties of deer milk was not consistent with

heating intensity, it was observed that the rennet gelation time for deer milk reduced with heating intensity, but the impact on *G'* was not consistent. Hence, the overall result doesn't lead to a solid conclusion about the processing effects on the rennet gelation properties of deer milk. The impact of homogenization and heating conditions on the viscosity of deer milk was noticeable in this study, which is not commonly observed with other ruminant milk. Further investigation to understand the factors influencing the viscosity of deer milk during homogenization or heating conditions and give more clarity on the colloidal stability of deer milk is provided in "Chapter 5".

5. Impact of processing treatment on the colloidal stability of deer milk

5.1 Introduction

The processing impacts on the physiochemical and gelation properties of deer milk were studied in Chapter 4 and it was observed that homogenization and high heating conditions (95 °C for 5 minutes) impacted the viscosity of deer milk. The viscosity of milk has been correlated with various factors including milk composition, size of fat globules and casein micelles, and association of casein micelles with denatured whey protein. The detailed investigations were conducted in this chapter to understand the factors influencing the viscosity of deer milk during homogenization and heating. The heated deer skim milk samples were used to understand the impact of different heating conditions on casein micelle size, viscosity, and distribution of whey protein in the absence of fat. The homogenized whole milk samples were used to determine viscosity and fat globule size variation with different homogenizing conditions to understand the impact of milk fat on viscosity.

5.2 Material and methods

5.2.1 Milk sampling and preparation

Deer milk samples were supplied by "Pamu Deer Milk" from a farm located in Taupo, New Zealand. The study has been conducted in two phases including skim milk processing and whole milk processing. Skimming and processing of whole milk described in Chapter 3 with detailed methods.

5.2.2 Milk processing and flow diagram

The whole milk samples were processed with two different processing conditions, homogenization, and heating treatment. Milk was homogenized at different homogenization pressures in the first stage, 10, 15, 20, 25, and 35 MPa, and the second stage was done at 5 MPa for all samples. Homogenized samples were treated with two different heating conditions, pasteurization (75 °C/15 s) and high heating (95 °C/5 min). The skim milk set of samples were centrifuged at 3000 *g* for 10 minutes to obtain skimmed milk and then processed with two different heating treatments, 75 °C and 95 °C with different holding times (1, 5, 10, and 30

min). The heat treatment of milk samples was followed by immediate cooling and storage at 4 °C for further analysis.

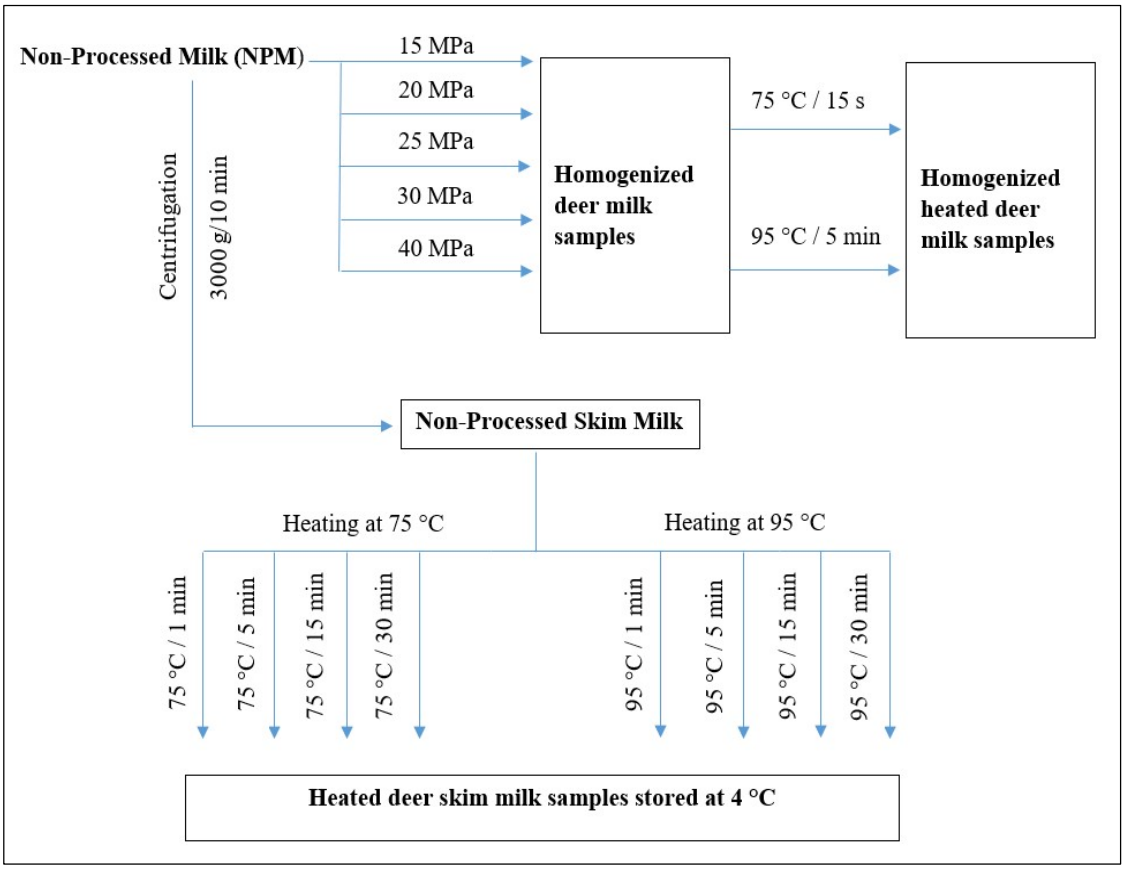

Figure 5.1: The schematic representation of processing and preparation of milk samples

5.2.3 Analysis

To understand the composition properties (fat, crude protein, and total solids contents) deer whole milk and skim milk samples were analysed by MilkoScan FT1 (Foss Electric, Denmark). The viscosity, fat globules, and casein micelle size were determined to understand the impact of heating conditions and homogenization on the colloidal stability of deer whole and skim milk. The protein composition and heat-induced changes in deer milk different proteins were determined by the RP-HPLC method using skim milk and serum samples. Sediment was measured using centrifugation, slightly modifying the method as described by (Boumpa, Tsioulpas, Grandison, & Lewis, 2008). The skim milk was mixed properly in a container and transferred to centrifuge tubes to make the total weight up to 55 ± 0.025 g along with tubes. These tubes were centrifuged at 3000 *g* at 20 °C for 15 min. The milk was drained from the tubes and wet weight was measured along with tubes. The tubes were then dried in a hot air

oven at 105 °C for 12 hours. The tubes with the dry sediment were weighed again to get the dry sediment weight and the sediment weight was reported as grams of sediment. Microscopy (TEM) of skim milk samples was also done to study the processing impact on the colloidal system and their distribution in different processed deer milk samples. The heated deer skim milk samples were used to understand the impact of different heating conditions on casein micelle size, viscosity, and distribution of whey protein. The homogenized whole milk samples were used to determine viscosity and fat globule size variation with different homogenizing conditions. The detailed procedure and methods of each analysis are described in Chapter 3.

5.3 Results

5.3.1 Milk composition of deer whole and skim milk

The milk composition of deer whole milk and skim milk is shown in table 5.1. The average fat, protein, and lactose content of milk was around 11.4%, 8.5%, and 5.4% respectively. The composition of milk samples used in this study was in the range observed in Chapter 4. Skim milk composition was also observed in this study and the average fat, protein, and lactose content were around 0.4%, 9.6%, and 6.2% respectively.

Table 5.1: Milk composition of whole and skim deer milk (% w/w)

Components	Whole milk	Skim milk
Fat (%)	11.4 ± 0.48	0.4 ± 0.02
Protein (%)	8.5 ± 0.2	9.6 ± 0.1
Lactose (%)	5.4 ± 0.14	6.2 ± 0.05

5.3.2 Impact of heating conditions on the colloidal stability of deer skim milk

Table 5.2 shows the impact of different heating temperatures and holding times on the casein micelle's size, viscosity, and sedimentation of deer skim milk.

Table 5.2: Impact of heating conditions on the colloidal stability of pasteurized and high heated deer skim milk

Heating temperature (°C)	Holding time (min)	Casein micelles size(nm)	Sediments (g)	Viscosity (cP)
Raw skim milk	N/A	196	0.11	5.6 ± 0.32
75	1	197 ^{d}	0.12 ^{a}	5.8 ± 0.46 ^{c}
	5	201 ^{c}	0.12 ^{a}	5.8 ± 1.01 ^{c}
	10	203 ^{b}	0.12 ^{a}	6.3 ± 0.89 ^{b}
	30	209 ^{a}	0.12 ^{a}	7.1 ± 0.91 ^{a}
95	1	236 ^{d}	0.12 ^{d}	17.4 ± 4.12 ^{d}
	5	239 ^{c}	0.13 ^{c}	23.2 ± 2.30 ^{c}
	10	245 ^{b}	0.14 ^{b}	26.4 ± 2.08 ^{b}
	30	246 ^{a}	0.15 ^{a}	33.4 ± 4.35 ^{a}

Mean values (Mean ± SD) with different lowercase superscripts within the same column differ significantly ($P < 0.05$)
*Analysed by one-way ANOVA test.

The casein micelles size - The average casein micelles size of fresh deer skim milk was around ~196 nm before any heating conditions. The heating of deer milk at 75 °C for 30 min led to a slight increase (~6%) in the size of casein micelles, on the other hand, the heating of milk at 95 °C for 1 minute resulted in the 23.4 % increase in casein micelles size as compared to fresh deer milk. (Table 5.2). The heating of deer milk at 95 °C for 30 min further increased the casein micelles size by around 30% as compared to non-processed casein micelles. Overall, the size of casein micelles consistently increased with the intensity of heating.

Sediment test - Table 5.2 shows the amounts of milk solid sediment level under mild centrifugation of different processed and non-processed deer skim milk samples. The sediment level for non-processed samples was around 0.11 g, which was similar to samples heated at

75°C, but the intensity of heating increased the level of sediment by 36% when the skim milk was heated at 95 °C/30 minutes.

Viscosity - The viscosity of non-processed deer skim milk was around 5.64 cP, this increased with heating intensity and holding time. The viscosity of deer skim milk increased slightly when milk was heated at 75°C but a major increase in viscosity of deer milk was observed when milk was heated at 95 °C. The heating of deer skims milk at 95 °C /1 min resulted in almost 3 times higher viscosity as compared to non-processed milk samples. Longer holding time further increased the viscosity of deer skim milk and it was observed that heating conditions of 95 °C /30 min increased viscosity around 6 times that of non-processed skim milk. Figure 5.2 shows the variation of casein micelles size and viscosity with different heating conditions. Both viscosity and casein micelle's size increased with higher intensity of heat and more changes were observed when milk was heated at 95 °C than at 75°C.

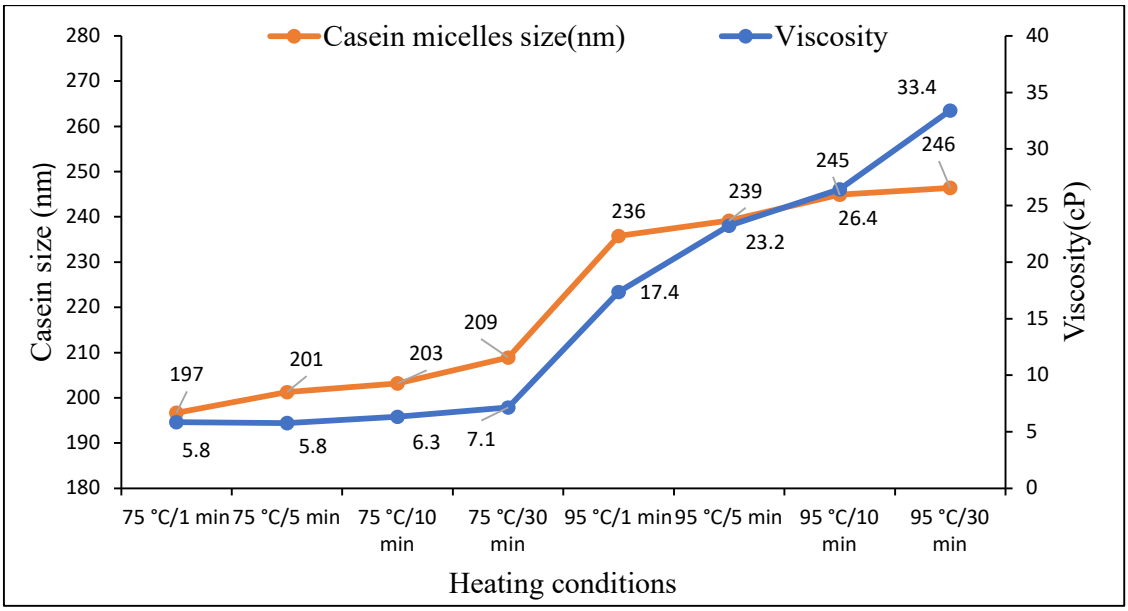

Figure 5.2: Impact of different heating conditions on the casein micelles size and viscosity of deer skim milk

Heat-induced changes in deer skim milk - Figure 5.3 shows the impact of different heating conditions on the distribution of whey protein in deer milk. The whey protein distributions among native, serum aggregates, and micelle associations varied along with heating intensity. The concentration of native protein decreased, whereas the association of whey protein with

casein micelles increased with heating intensity. There was a variation in the denaturation of whey protein in deer milk with different heating conditions. As expected, the denaturation level of WP increased significantly ($P < 0.05$) with heating intensity, and the maximum denaturation level of WP was observed when deer milk was heated at 95°C for 30 minutes (around 98% of total WP).

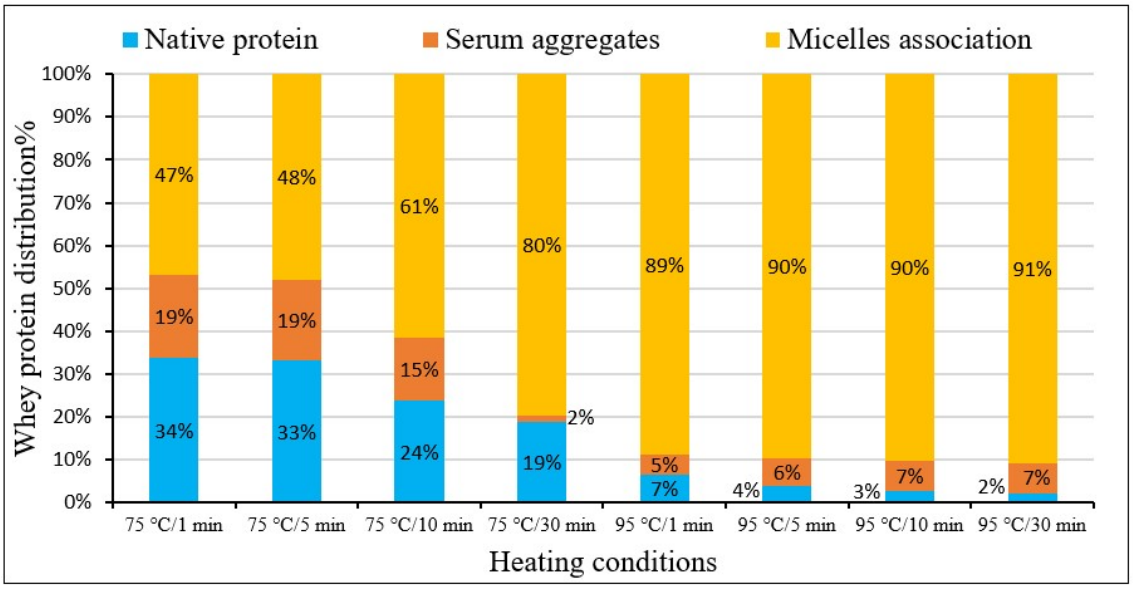

Figure 5.3: The impact of different heating conditions on the distribution of whey protein in deer milk

The distribution of native whey protein in heated deer milk at 75 °C/1 min was around 34% of total whey protein, which decreased consistently with the heating intensity. The maximum variation of the native protein in deer milk was observed when the milk was heated at 95°C, but it was observed that holding time 1-30 minutes at 95°C had minimal impact on the variation of the native protein in deer milk. The heating intensity also affected the distribution of serum aggregated and micelle-associated WP. The level of micelle association in heated deer milk at 75 °C/1 min was around 47% of total whey protein, which further increased to around 80% when milk was heated for 30 minutes at the same temperature. The maximum concentration of casein associated (89 - 91%) WP was observed when milk was heated at 95 °C but holding time at this temperature had minimal impact on the variation of casein associated WP.

TEM deer milk - Figure 5.4 shows the Transmission electron microscopy (TEM) images of deer skim milk processed with different heating conditions. The casein micelles can be seen in the images as a dark black sphere, and small particles of whey protein can be seen in the background. The casein micelles of non-processed milk (Figure 5.4A) appeared relatively smooth on the surface and uniformly distributed as compared to other processed samples. Heating deer milk at 75 °C (1-30 minutes) resulted in bigger-sized and hairy surfaced casein micelles (Figure 5.4B and 5.4C). On the other hand, the milk heated at 95 °C for 1 minute produced aggregated casein micelles, as shown with the red circle in Figure 5.4(D) and this impact further increased when the milk was heated at 95 °C for 30 minutes, which resulted in a large aggregated structure of casein micelles, as highlighted in Figure 5.4(E). Overall, the size of casein micelles, as well as hairy/stringy structure (indicating the denatured whey protein aggregation) increased with heating intensity as expected.

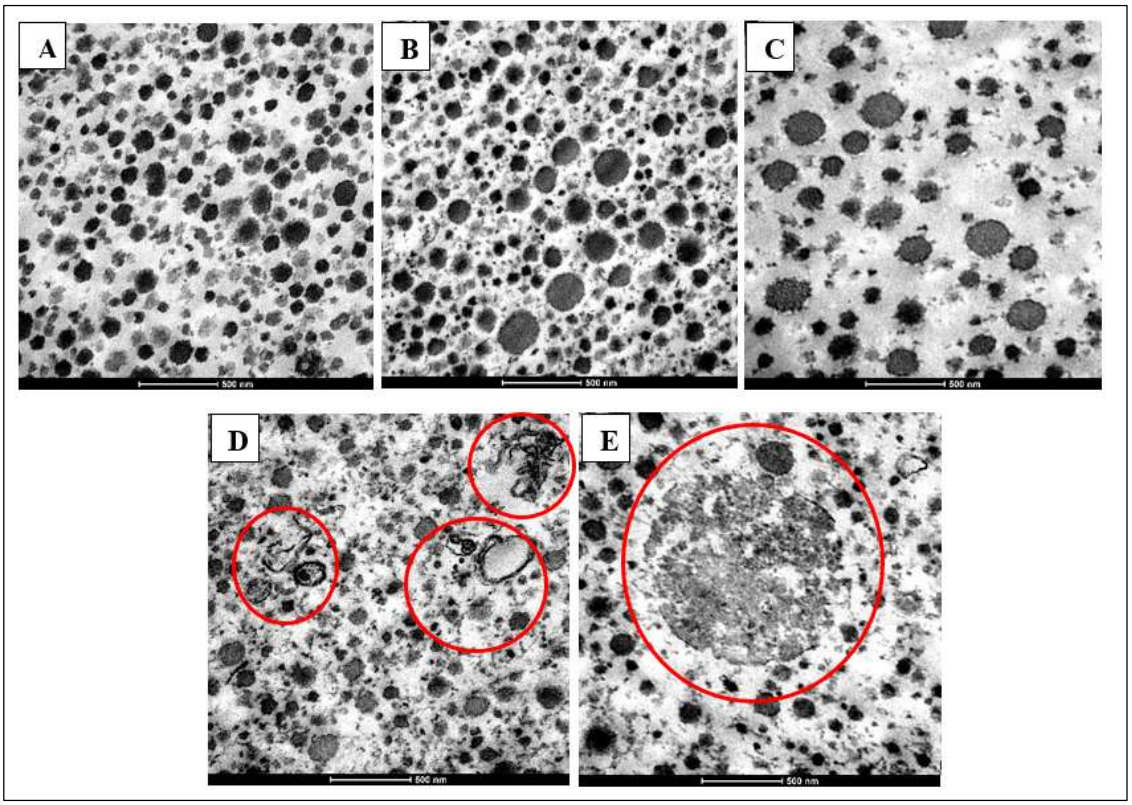

Figure 5.4: Transmission electron microscopy (TEM) images at 45000X magnification of (A) Non processed deer milk, (B) heated at 75 °C for 1 minute, (C) heated at 75 °C for 30 minutes, (D) heated at 95 °C for 1 minute and (E) heated at 95 °C for 30 minutes. Red circles represent the aggregation of denatured whey protein with casein micelles or casein-casein micelles. The scale for images used: 500 nm in size.

5.3.3 Impact of homogenization on fat globules size and viscosity of deer whole milk

Table 5.3 shows the impact of different homogenization conditions on fat globules size and viscosity of deer whole milk. The average size of fat globules in non-processed deer milk was determined as approximately 6.3 µm. Overall, the fat globule size was reduced with the homogenization pressure as expected. It was observed that fat globules heated at different temperatures but homogenized at the same pressure resulted in different fat globule sizes. The difference was small, but the size of globules was statistically significantly different from each other ($P < 0.05$). The fat globules of high heated milk samples were bigger than pasteurized milk globules at the same homogenization pressure. The fat globules of pasteurized milk samples were around 1.4 µm, whereas the size of fat globules was higher for high heated milk samples, around 1.6 µm (12.5 % bigger in size), at the same homogenization pressure (15 MPa). The same observation was determined for fat globules of pasteurized milk samples, 0.5 µm, whereas the size of fat globules was higher for high heated milk samples, around 0.9 µm at the same homogenization pressure (40 MPa).

Table 5.3: Impact of homogenization on the colloidal stability of pasteurized and high heated deer milk

Heating conditions	Homogenization pressure (MPa)	Fat globule size ($D_{4,3}$) (µm)	Viscosity (cP)
Non processed deer milk	-	6.3 ± 0.89	6.2 ± 0.9
Pasteurized milk (75 °C /15 sec)	15	1.4 ± 0.15 ^{a}	8.1 ± 1.1 ^{c}
	20	0.8 ± 0.01 ^{b}	8.1 ± 0.7 ^{c}
	25	0.7 ± 0.06 ^{c}	8.5 ± 0.8 ^{c}
	30	0.6 ± 0.04 ^{cd}	12.2 ± 1.6 ^{b}
	40	0.5 ± 0.13 ^{d}	17.9 ± 0.4 ^{a}
High heated milk (95 °C /5 min)	15	1.6 ± 0.23 ^{a}	51.6 ± 2.6 ^{e}
	20	1.1 ± 0.35 ^{b}	79.5 ± 7.4 ^{d}
	25	1.0 ± 0.1 ^{bc}	103.3 ± 3.9 ^{c}
	30	1.0 ± 0.1 ^{bc}	139.0 ± 11.7 ^{b}
	40	0.9 ± 0.02 ^{c}	282.1 ± 4.2 ^{a}

Mean values (Mean ± SD) with different lowercase superscripts within the same row differ significantly ($P < 0.05$)
*Analysed by one-way ANOVA test.

The mean diameter of fat globules in high heated milk was around 14% bigger than the fat globules of pasteurized deer milk at 15 MPa homogenization pressure. In addition to this, the difference in the size of fat globules between the two heat treatments increased along with homogenization pressure. The size of fat globules of high heated milk was 37%, 42%, 66%, and 80% bigger than the size of fat globules of pasteurized deer milk at homogenization pressure 20 MPa, 25MPa, 30 MPa, and 40 MPa respectively.

Table 5.3 and Figure 5.5 show the variation of viscosity of pasteurized and high heated deer milk with different homogenization pressures. The viscosity of non-processed deer milk was determined at approximately 6.2 cP, but pasteurization at 75°C for 15 s increased the viscosity to 8.1-8.5 cP for deer milk homogenized at 15-25 MPa. At higher homogenization pressures of 30 and 40 MPa, the viscosity of pasteurized deer milk further increased to 12.2 and 17.9 MPa, respectively. The greater increase in viscosity of homogenized deer milk was observed after high heating conditions and it was observed that milk processed at 40 MPa homogenization pressure and heated at 95 °C/ 5 minutes resulted in the maximum increase in viscosity. The viscosity of deer milk increased with homogenization pressure and heating intensity further increased the viscosity of homogenized deer milk. It was observed that the combination of high heating intensity and high homogenizing pressure resulted in the maximum increase in viscosity of deer milk. The viscosity of high heated deer milk was around 6 times higher than the viscosity of pasteurized milk at 15MPa homogenization pressure. This difference further increased with homogenization pressure, and it was observed that the viscosity of high heated deer milk was almost 16 times higher than the viscosity of pasteurized deer milk at 40 MPa homogenization pressure.

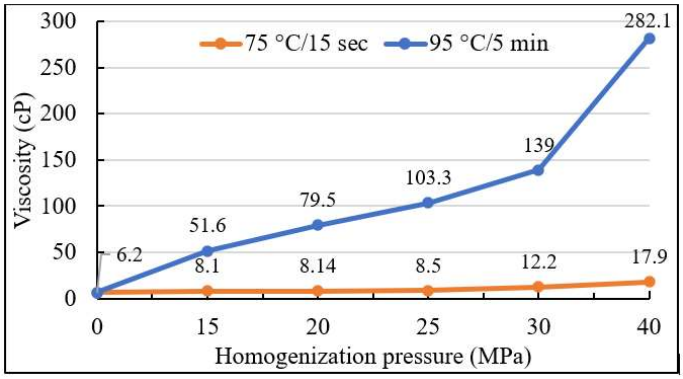

Figure 5.5: The impact of different homogenization pressures on the viscosity of pasteurized (75 °C/15 sec) and high heated (95 °C/5 min) deer milk.

*Viscosity for control samples is considered as a starting point of this graph.

5.4 Discussion

Overall, the size of deer milk casein micelles consistently increased with heat intensity and greater effects were observed when the milk was heated at 95 °C as compared to 75°C temperature, holding time further increased the size of micelles. The same impact of different heating conditions on casein micelles size of deer milk was observed in Chapter 4th (Table 4.4). The sediment level in milk samples also increased with the heating intensity and holding time. The percentage of whey protein and the association of whey protein with casein micelles increased with heating intensity. The heating of milk at high temperature (above 90 °C) resulted in the rapid denaturation of whey protein and association of denatured whey protein (especially β-LG) with casein micelles. The proportion of serum-phase WP and micelle-associated WP was higher in high heated milk as compared to pasteurized milk, which was correlated with the dissociation of κ -CN and WP-casein micelle associations (Skelte G Anema & Li, 2003a; Singh, 2004).

Heating at 95°C for only 1 min resulted in the denaturation of > 90% of the whey proteins, most of which were associated with the casein micelles. Little further change was observed in the level of whey protein/casein micelle association when prolonging the holding time at 95°C to 30 min. This indicated that whey protein association did not play a major role in the further increases in micelle size, viscosity, and sediment formation during prolonged high heating. The increase in the size of casein micelles with heating intensity has been correlated with minor aggregation of micelles, whereas the sedimentation represented the formation of large aggregates in casein micelles (Donato, Guyomarc'h, Amiot, & Dalgleish, 2007).

The fat globule size reduces with the homogenization pressure as expected, but it was observed that heating intensity slightly increases the fat globule size and higher homogenization pressure further increases the size of fat globules. It was observed that heating conditions consistently impacted the viscosity of deer skim and whole milk, and the homogenization pressure further increased in viscosity of deer milk. A similar variation of viscosity of deer milk was also observed in Chapter 4. Y. Li et al. (2018) proposed that the presence of a larger number of fat globules in higher fat milk resulted in the increase of viscosity with homogenization and heating conditions because of the resistance of fat particles to the flow. It is reported that the heating of skim milk at high temperature (85 °C/5 min) resulted in the association of denatured whey protein with casein micelles which leads to increases in the particle size. Heat-induced association between the casein micelles and with the denatured whey proteins likely changed

the structure of the micelles and increases their volume fraction, which would further result in a higher viscosity of milk (Skelte G Anema & Li, 2003b; Jeurnink & De Kruif, 1993).

5.5 Conclusion

Deer milk was investigated at different homogenizing pressure and heating conditions to understand the colloidal stability of deer milk. Overall, the viscosity increased with high heat treatment and homogenizing pressure. Heat intensity greatly increased the viscosity of deer milk, especially after homogenization. The impact of heating on casein micelles and whey protein was consistent, and the size of casein micelles was majorly increased at a higher rate when deer milk was heated at 95 °C. The interactions between casein micelles and denatured whey protein-casein micelles are considered the prime reason for the large size of casein micelles in heated milk. So, based on the size of the fat globules, viscosity/ TEM imaging, and casein micelles size results, it can be hypothesized that higher homogenization pressure (above 20 MPa) and higher heating conditions (95 °C) resulted in micelle-micelle aggregation and micelle-fat aggregation, which may cause the observed physicochemical changes in deer milk properties.

6. Effect of processing on characteristics of reconstituted deer milk

6.1 Introduction

Deer milk has been characterized for its nutritional aspects and processibility for cheese production (Opatha Vithana, 2012). Fresh deer milk composition and its physiochemical properties have been discussed and reviewed previously (Gallego et al., 2006; Malacarne et al., 2015; Y. Wang et al., 2017). In the dairy industry, milk powder and reconstituted milk are essential ingredients for many dairy products and the properties of deer milk powder may affect the physicochemical characteristics of reconstituted milk. In addition, the understanding of reconstituted deer milk and milk powder has not been explored. Therefore, understanding the impact of processing conditions on reconstituted deer milk characteristics is also essential for the development of dairy products, improving the product quality, and optimization of industrial processing. The objective of this study was to explore the effect of processing conditions on the characteristics of reconstituted deer milk.

6.2 Material and methods

Deer whole milk powder was supplied by Pamu Deer Milk (Wellington, New Zealand). Three different batches of milk powder were used for this study and all three batches were processed and analysed to understand the effect of processing on characteristics of reconstituted deer milk. The detailed procedure regarding the respective methods of analysis is described in Chapter 3.

6.3 Preparation of reconstituted deer milk

The reconstituted deer milk was prepared following the manufacturer's instructions. A measured quantity of milk powder (21.4% w/w) and preheated water (50 °C) was mixed with a high-speed mixer (Silverson mixer of PD lab, Massey University) at 6000 RPM for 2 minutes. After mixing the reconstituted milk was kept at refrigerated condition (4 °C for 12 hours) to optimize the hydration of milk powder. Filtration was done before processing with the help of a fine sieve (60 μm) and the filtration resulted in the retention of undissolved powder particles. The filtrate was used for analyses and any particles retained on the sieve were discarded.

6.3.1 Processing of reconstituted deer milk

The reconstituted milk samples were processed at the Food Pilot^{TM}, Massey University Palmerston North, New Zealand. The reconstituted milk was homogenized using a two-stage homogenizer (20 MPa in the first stage and 5 MPa in the second stage at 65°C) and milk was heated with an indirect UHT system with two different heat treatments, pasteurization (75°C/15 s) and high heating (95°C/5 min). A preheated water bath (95°C) was used to complete the holding process for the milk heated at 95°C in the UHT. All three processed milk samples were followed by immediate cooling (20°C) using ice water and stored at 4 °C with the addition of sodium azide (0.02% w/w) for further analysis within 72 hours, as schematically described in Figure 6.1.

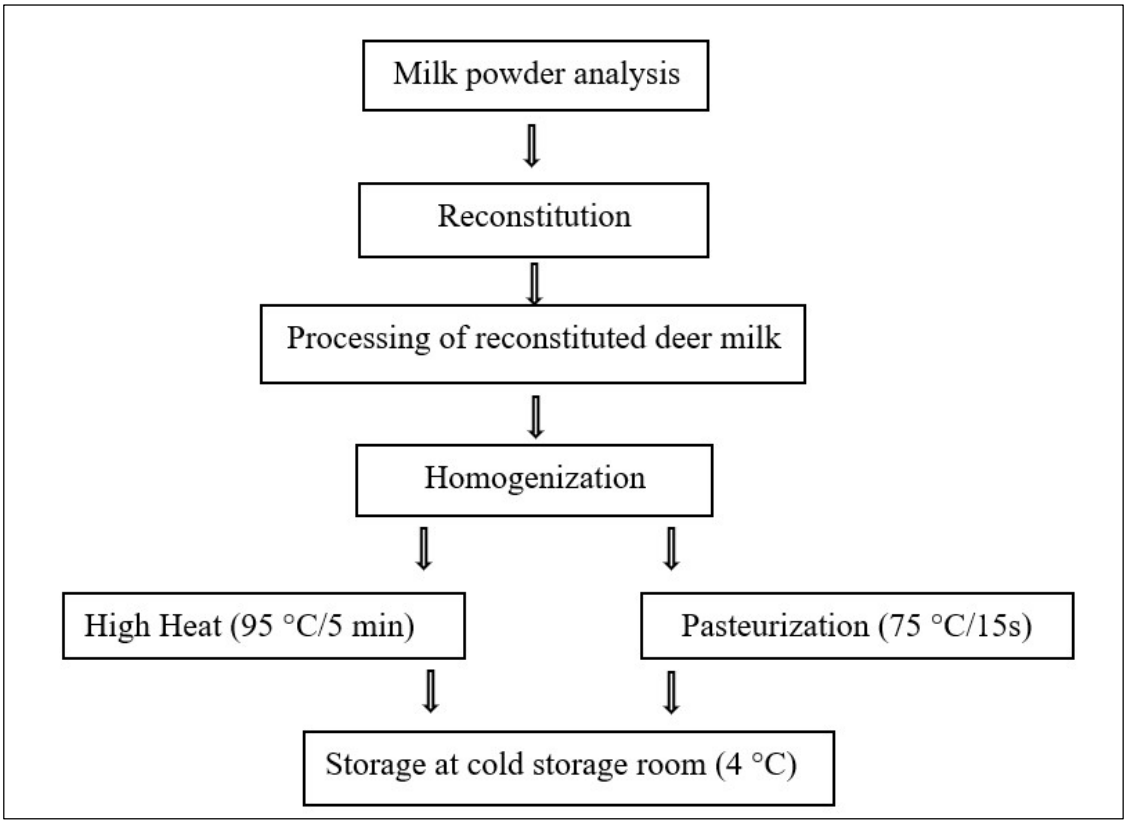

Figure 6.1: Process flow diagram for processing of reconstituted deer milk

6.3.2 Analysis of reconstituted deer milk

To understand the reconstitution properties of deer whole milk powder was analysed for different properties such as moisture, wettability, flowability, bulk density, and particle size distribution. The wettability, flowability, and bulk density of milk powder were determined by using IDF protocols, whereas the particle size distribution was determined by laser light scattering using a Malvern Master-Sizer 2000 (Malvern Instruments Ltd, Malvern, UK) as mentioned in Section 3.2.6. To study the impact of processing conditions on physicochemical characteristics of reconstituted deer milk, different analysis was done in this study using processed and non-processed deer milk samples. The physicochemical analysis included measurement of pH, fat globules, casein micelles size, Ca^{2+} concentration. Microscopy (TEM) of milk samples was also done to study the processing impact on the colloidal system and their distribution in different processed deer milk samples. The impact of heat treatment and homogenization pressure on acid and rennet gelation was investigated. The detailed procedures and methods of each analysis are described in Chapter 3.

6.4 Results and discussion

6.4.1 Composition of whole deer milk powder

Table 6.1 shows the details of compositional details of reconstituted deer milk which was used for this study. The total solid of reconstituted milk was around 19.4 % of total milk whereas fat, protein, and lactose content of reconstituted milk were measured around 7.3%, 8.2%, and 3.9% respectively. The compositional values of reconstituted deer milk were within the range observed for fresh deer milk (Chapter 4).

Table 6.1: Milk composition of reconstituted deer milk

Composition	Quantity (%)
Protein	7.3
Fat	8.2
Lactose	3.9
Total solid	19.4

6.4.2 Reconstitution properties analysis of deer whole milk powder

Table 6.2 shows the properties of deer whole milk powder used for the reconstitution process. The reconstitution properties of milk powder were estimated before the reconstitution process to understand the quality of milk powder. The moisture content of deer milk powder was determined to be 3.1 ± 0.0%. The average values of wettability and flowability of deer whole milk powder were determined as 62 and 150 seconds respectively, whereas the bulk density was measured around 0.7 g/cm^3. The moisture content of deer whole milk powder was similar to instant cow whole milk powder, but the other properties such as bulk density, wettability, and flowability were higher for deer whole milk powder than instant cow whole milk powder (Bulk density: 0.45 g/cm^3, Wettability: max 15 seconds and flowability: max 50 seconds (Niro, 2005).

Table 6.2: Reconstitution properties of deer whole milk powder

Properties	**Mean ± SD**
Moisture (%)	3. ± 0.0
Wettability (s)	62 ± 6
Flowability (s)	150 ± 2
Bulk density (g/cm^3)	0.7 ± 0.0
Average particle size D [4, 3] (μm)	132 ± 4

The reconstitution properties of milk powders depend on various factors including the processing conditions, milk composition, and storage. The particle size, wettability, and flowability are important properties for the reconstitution process which define the mixing and hydration ability of milk powders (Y. Lin, Kelly, O'Mahony, & Guinee, 2018). Fitzpatrick, Iqbal, Delaney, Twomey, & Keogh, (2004) suggested that the milk powder with particle size around 250 μm and wettability (max 15 s) or flowability (max 50 s) provide the optimum reconstitution properties. The reconstitution properties of milk powder provided the essential information which was considered during the reconstitution process for further trials and sample preparation.

6.4.3 Physicochemical properties

Table 6.3 shows the processing impact on physicochemical properties of reconstituted deer milk. Processing conditions majorly impacted the concentration of ionic calcium in reconstituted deer milk.

Table 6.3: Effect of processing conditions on physiochemical characteristics of reconstituted deer milk

Properties	NPRM	PHRM	HHRM
pH	6.74 ± 0.02	6.72 ± 0.03	6.73 ± 0.02
Buffering capacity (dB/dpH)	0.065 ± 0.01[a]	0.055 ± 0.02[b]	0.058 ± 0.02 [b]
Ionic Calcium (mM)	3.85 ± 0.01 [a]	3.65 ± 0.01 [b]	3.56 ± 0.01 [b]
Average fat globules diameter D [4, 3] (µm)	8.80 [a]	0.51 [b]	0.56 [b]

NPRM – Non-processed reconstituted deer milk, PHRM – Pasteurized (75 °C/15 s) homogenized (250/50 Bar) reconstituted deer milk, HHRM – High heated (95 °C/5 min) homogenized (250/50 Bar) reconstituted deer milk (95 °C/5 min), Ca^{2+} – Ionic calcium, D [4, 3] – Fat globule size or volume-weighted mean (µm).

*Mean values with different lowercase superscripts within the same row differ significantly ($P < 0.05$, one-way ANOVA).

pH and Ionic calcium (Ca^{2+}) concentration - The average pH of reconstituted deer milk was measured at around 6.73, which was unaffected by different processing conditions. The concentration of Ca^{2+} significantly decreased with heating. The average Ca^{2+} concentration of non-processed reconstituted deer milk was 3.85 mM, which is higher than that of fresh deer milk (3.34 mM). The heating of reconstituted deer milk at 75 °C for 15 s resulted in a reduction of almost 5% in Ca^{2+} concentration, as compared to non-processed milk. The heating of reconstituted deer milk at 95 °C for 5 minutes resulted in a further reduction of approximately 7% of Ca^{2+} concentration as compared to non-processed milk (Table 5.3). Overall, heating significantly reduced the Ca^{2+} concentration ($P < 0.05$). It has been reported that high-temperature short time (HTST) pasteurization and ultra-high temperature (UHT) of cow milk reduced the Ca^{2+} concentration between approximately 5 % to 10 % (May & Smith, 1998).

Li et al. (2019) also observed a similar reduction in Ca^{2+} concentration for cow milk after heating at 90°C for 6 min. The heating of milk at different heat treatments leads to the reduction of solubility of calcium phosphate, and this might impact Ca^{2+} concentrations (Lewis, 2011) (Omoarukhe & Lewis, 2010).

Buffering Capacity (BC) – The buffering capacity of non-processed reconstituted deer milk was determined to be around 0.065 dB/dpH and the impact of heating on it was observed. The heating of milk (75 °C for 15 s) caused an approximately 15% reduction in BC (0.055 dB/dpH). Higher heat treatment (95 °C/5 min) caused a slight increase in BC (0.058 dB/dpH). Salaün (2005) proposed that buffering capacity of milk is mainly affected by the colloidal calcium phosphate, soluble phosphate, citrate, bicarbonate, caseins, and whey proteins (Salaün et al., 2005). It has been observed that as temperature increased the concentration of serum Ca, inorganic phosphate (Pi) and citrate decreased. Calcium and phosphate precipitation is an important factors to decide the BC of milk. (Wang & Ma, 2020) mentioned that the equilibria of calcium and phosphate were disturbed under high heat treatment (above 90 °C), which led to the increase in the buffering capacity of milk.

The fat globule size - The average diameter of fat globules in reconstituted deer milk was 8.8 µm. Homogenization reduced the size of fat globules by approximately 12-14 times, as expected. Slight variation of fat globule size was observed with heating, the fat globules of HHM were marginally larger as compared with PHM. A similar impact of heating on fat globule size was observed in Chapter 4 for fresh deer milk.

Heat-induced changes in reconstituted deer milk - Figure 6.2 shows the heat-induced changes in reconstituted deer milk in transmission electronic microscopy (TEM) images. Overall association of casein micelles together and with fat globules increased with heating intensity and after homogenization. The TEM images in Figure 6.2 shows the distribution of fat globules (highlighted with yellow circle), casein micelles (highlighted with red circles), and whey proteins (small particles distributed around the casein micelles and fat globules). The TEM image of NPRM (Figure 6.2A) shows fat globules with an average size of more than 500 nm, whereas the casein micelles are distributed uniformly with minor signs of aggregation with

the fat globules. The TEM images of PHRM (6.2B) and HHRM (6.2C) show the distribution of the smaller fat globules with an average size of less than 500 nm, as compared to NPRM. Similar behaviour of casein micelles has been observed for high heated and pasteurized fresh deer milk samples, whereas non-processed reconstituted deer milk shows a slight aggregation of casein micelles, which was not observed for fresh deer milk. The heat treatment and subsequent formation of denatured whey protein/ κ-casein complexes on the surface of casein micelles resulted in the aggregation of casein micelles, and larger micelles (Donato & Guyomarc'h, 2009; Li et al., 2019), which may have occurred during the processing of the milk powder.

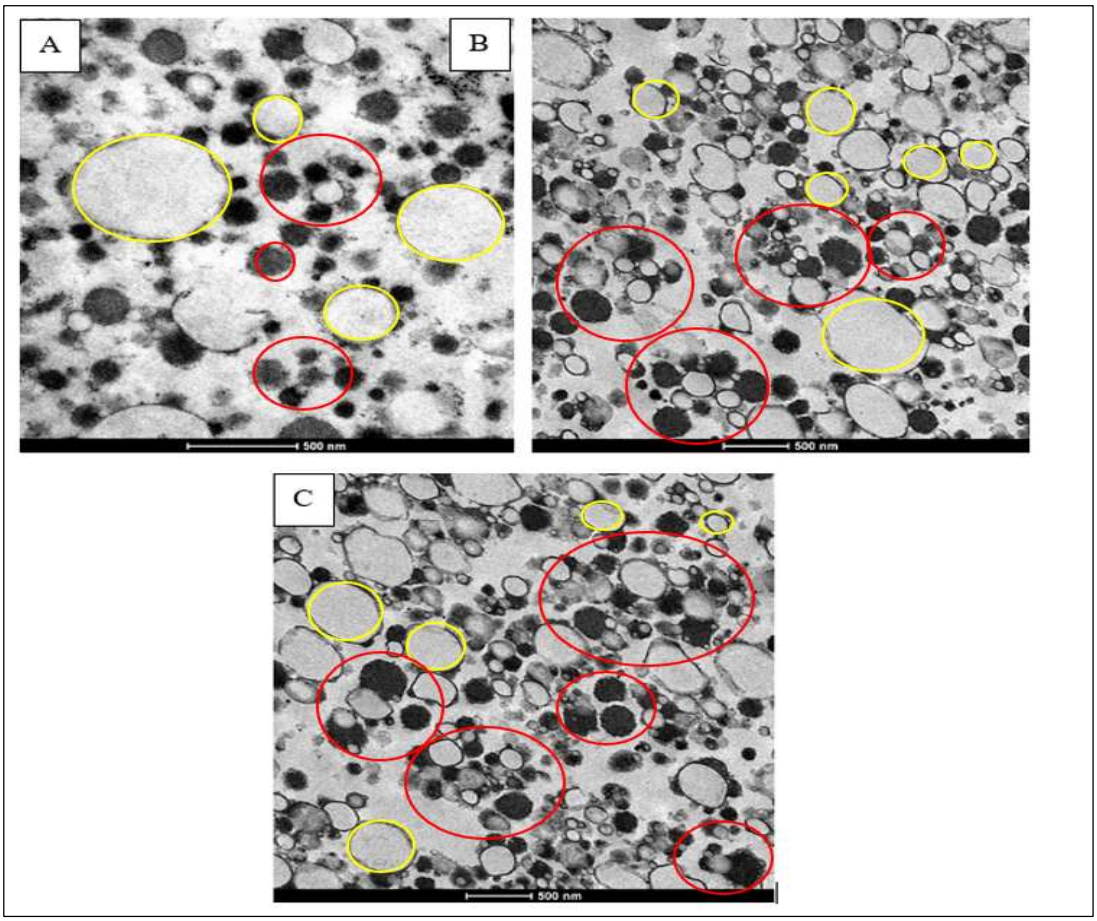

Figure 6.2: Transmission electronic microscopy (TEM) for heat induces changes of reconstituted deer milk. (A): Non-processed reconstituted deer milk (NPRM), (B): Pasteurized (75 °C/15 s) homogenized (25/5 MPa) reconstituted deer milk (PHRM), (C): High heated (95 °C/5 min) homogenized (25/5 MPa) reconstituted deer milk (HHRM).

6.4.4 Effect of processing conditions on gelation properties of reconstituted deer milk

Acid gelation - Table 6.4 and Figure 6.3 summarise the impact of processing on acid gelation characteristics of reconstituted deer milk. The reconstituted deer milk without processing showed a longer gelation time (approximately 100 min) and lowest final G' (approximately 208 Pa) as compared to processed deer milk samples. The HHRM had the shortest gelation time (approximately 31 min) and highest final G' (approximately 409 Pa), followed by PHRM with gelation time of approximately 50 min and final G' of approximately 326 Pa. The average values of Tan δ were not impacted much by different heating conditions. The different gelation pH also showed similar changes in gelling properties in reconstituted deer milk due to different processing conditions, it was observed that the HHRM and PHRM gelled at a significantly higher pH, above 5.0 as compared to NPRM (pH 4.91).

Table 6.4: The impact of processing conditions on acid gel properties of reconstituted deer milk

Gelation parameters	NPRM	PHRM	HHRM
Gelation time (minute)	99.8 [a]	50.2 [b]	31.2 [c]
Final G' (Pa)	208.7 [a]	326.5 [b]	408.9 [c]
Tan δ	0.24 [a]	0.22 [b]	0.22 [b]
Gelation pH	4.91 [a]	5.13 [b]	5.20 [c]

NPRM – Non-processed reconstituted deer milk, PHRM – Pasteurized (75 °C/15 s) homogenized (25/5 MPa) reconstituted deer milk, HHRM – High heated (95 °C/5 min) homogenized (25/5 MPa) reconstituted deer milk (95 °C/5 min).

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

*Analysed by one-way ANOVA test.

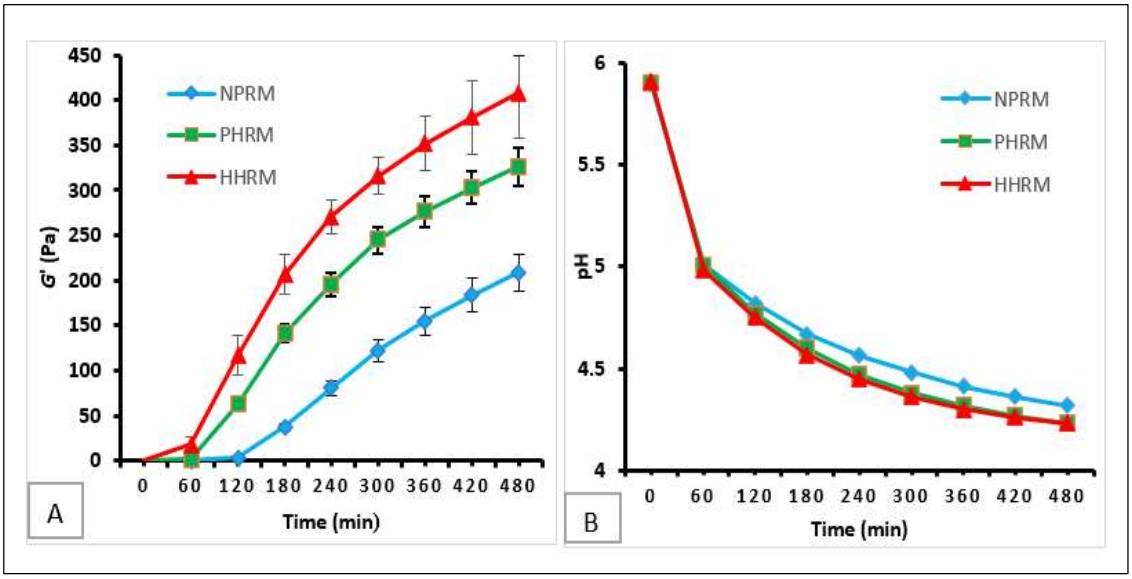

Figure 6.3: Processing effect on acid gelation properties G′ (A) and pH profile (B) of reconstituted deer milk. NPRM – Non-processed reconstituted deer milk, PHRM – Pasteurized (75 °C/15 s) homogenized (250/50 Bar) reconstituted deer milk, HHRM – High heated (95°C/5 min) homogenized (250/50 Bar) reconstituted deer milk (95 °C/5 min).

Figure 6.3 represents the gelling properties, including *G′* values and pH profile of acid milk gel with different processing conditions. The acid gelation properties of reconstituted deer milk improved with processing conditions (especially heating). The HHRM had better acid gelling properties, as compared to PHRM and NPRM. The non-processed reconstituted deer milk resulted in a longer gelation time and lower final *G′* as compared to PHRM and HHRM. Heating significantly reduced the gelation time for reconstituted deer milk ($P < 0.05$). These results were consistent with fresh deer milk acid gelation properties in Chapter 4, and those reported in previous studies investigating the impact of processing conditions on acid gelation properties of cow milk (Skelte G. Anema et al., 2004). It is expected that the milk with lower gelation pH would take longer to form a gel during acidification and our results regarding gelation time and pH profile of gelled milk also showed the same trends. The extent of variation in final Tan δ also significantly changed with different processing conditions. The final Tan δ is mainly depending on the gelation properties of milk gel, it decreased consistently with heating (Table 5.4), indicating higher elasticity. Overall, heating of reconstituted deer milk at 95°C for 5 minutes resulted in a shorter gelation time, higher gelation pH, and stronger gel formation which was shown by final *G′* and Tan δ values.

Rennet gelation - The mean values of rennet gelation properties (gelation time, final *G′*, and Tan δ) of reconstituted deer milk samples are summarised in Table 6.5 and Figure 6.4. The rennet gelation time of NPRM was 15.2 minutes, which was reduced significantly ($P < 0.05$) with homogenization and heating intensity. The homogenization and pasteurization of milk at 75 °C for 15 seconds (PHRM) resulted in the reduction of gelation time by approximately 50%, whereas the more intense heating of reconstituted deer milk (HHRM) at 95 °C reduced the gelation time by approximately 56%. The heating of milk at 75 °C for 15 seconds increased the final *G′* (115.6 Pa) of rennet gel, as compared with a gel made from non-processed deer milk (97.3 Pa). But heating milk at high heat intensity (95 °C/ 5 min) resulted in a negative impact on the final *G'* value (102.3 Pa) as compared to PHRM. The impact of heating intensity on the final Tan δ on rennet gel of reconstituted deer milk was non-significant ($P < 0.05$).

Table 6.5: The impact of processing conditions on rennet gelation properties of reconstituted deer milk

Gelation parameters	**NPRM**	**PHRM**	**HHRM**
Gelation time (minute)	15.2 [a]	7.5 [b]	6.6 [c]
Final *G′* (Pa)	97.3 [c]	115.6 [a]	102.3 [b]
Final Tan δ	0.28 [a]	0.27 [b]	0.27 [b]

NPRM – Non-processed reconstituted deer milk, PHRM – Pasteurized (75 °C/15 sec) homogenized (250/50 Bar) reconstituted deer milk, HHRM – High heated (95 °C/5 min) homogenized (250/50 Bar) reconstituted deer milk (95 °C/5 min).

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

*Analysed by one-way ANOVA test

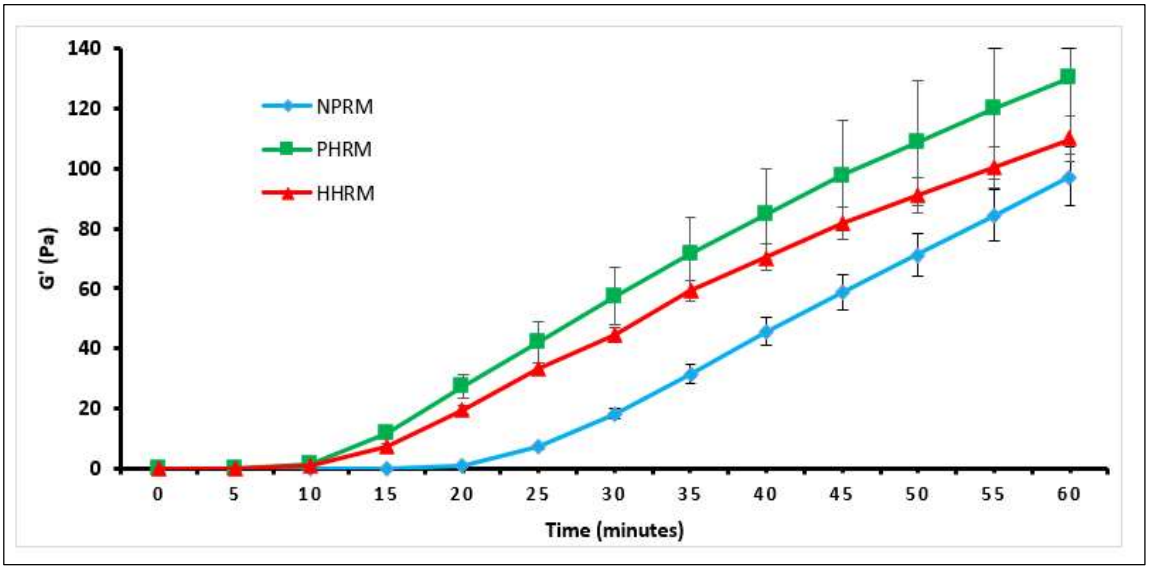

Figure 6.4: Processing effect on rennet gelation properties (final G′) of reconstituted deer milk. Processing conditions: NPRM – Non-processed reconstituted deer milk, PHRM – Pasteurized (75 °C/15 s) homogenized (250/50 Bar) reconstituted deer milk, HHRM – High heated homogenized (250/50 Bar) reconstituted deer milk (95 °C/5 min). All samples were coagulated with rennet at pH 6.5 at 32 °C.

The gelation time was reduced when reconstituted deer milk was processed with heat treatment and homogenization. It is well established that the rennet gelling properties of reconstituted milk can depend on various factors, including milk composition, the interaction of whey proteins and casein micelles during spray-drying of milk powder, and the reconstitution conditions. The interaction of whey proteins provided both positive and negative impacts on rennet gelling properties during cheese manufacturing. On the one hand, denatured whey proteins could be incorporated into cheese curd and lead to higher yield from a given quantity of milk, while on the other hand the interaction of whey proteins with casein interferes with the rennet coagulation process and leads to long coagulation time or weak curd structures. (Logan et al., 2015). So further investigations are needed to confirm the exact impact of processing conditions on the rennet properties of reconstituted deer milk.

6.5 Conclusion

Overall, the physicochemical and gelation properties of reconstituted deer milk were affected by the processing conditions (especially heating temperature). The impact of processing conditions was significant on physicochemical properties, including fat globules size and the concentration of ionic calcium. Based on consistent literature reports as well as results of the current study it can be proposed that overall acid gelation properties of reconstituted deer milk improved with heating intensity and can provide a firm gel for yogurt production. However, based on the literature review, it has been reported that milk composition (fat and protein content) and physicochemical (viscosity, casein micelles size, and whey protein denaturation) play an important role during the gelation process. The impact of processing conditions on rennet gelation properties of reconstituted deer milk was different in reconstituted deer milk as observed for fresh deer milk. Hence, the overall result doesn't lead to a solid conclusion about the processing effects on the rennet gelation properties of reconstituted deer milk.

7. Conclusion and avenues for future work

7.1 General discussion

In this study, deer milk was investigated by focusing on the lactational variation of milk composition, physicochemical properties, processing-induced changes, and impact of processing on the gelation properties. Detailed information regarding the composition and properties of fresh deer milk was discussed in Chapter 4. Fresh deer milk was found to undergo different heat-induced changes, including variation in casein micelles structures and their aggregation, whey protein denaturation, salt balances, etc. The gelation characteristics (acid and rennet gel) of fresh deer milk were also discussed in this chapter and the impact of processing conditions (heating and homogenization) were observed on rennet and acid gel made from fresh deer milk. The processing-induced changes were studied further in Chapter 5, in which the colloidal instability of processed deer milk was also investigated at different heat and homogenization conditions. In Chapter 6, deer milk powder properties were investigated to understand the reconstitution properties of the powder, and reconstituted deer milk was studied to understand the physicochemical and gelation properties.

7.1.1 Composition and characteristics of cow and deer milk

Deer milk was found to have higher protein and fat contents compared to cow milk, as shown in Table 7.1. The average fat content in deer milk was determined to be approximately 10%, which was almost 2.5 times higher than cow milk fat content (~ 4%). Similarly, the protein content in deer milk was measured at around 8%, which was approximately 2 times higher than cow milk (~ 3.75%). The lactose contents of cow and deer milk were 4.4 % and 5% respectively. Deer milk contained similar ratios of casein and whey protein to cow milk, but the distribution of individual protein in deer milk was different from cow milk. As discussed in Chapter 4, the deer milk protein was dominated by β-casein (~78% of total casein content) whereas α_{s1}-casein (~4% of total casein content) was the lowest of the four caseins. The concentration of κ-casein and α_{s1}-casein also varied from 8 to 9% of the total concentration of casein in deer milk. In contrast, cow milk caseins are dominated by α_{s1}-casein (~ 44% of total casein content), the dominant protein, followed by β- casein, κ-casein and α_{s2}-casein. The caseins in deer milk are present as micelles, with an average diameter of approximately 190 nm, which is larger than the casein micelles of cow milk (approximately 165 nm), as shown in Table 7.1. Genotypic variants within species or different breeds can be a factor in the variation

in the proportion of individual protein and casein micelle size (Alice Pierre, Michel, Le Graët, & Zahoute, 1998). A Pierre, Michel, and Le Graet (1995) studied the casein micelle size in goat milk from animals with different α_{s1}-casein genotypes and mentioned that these genotypes may have a role in casein micelle size regulation. They found that the average diameter of casein micelles in goat milk increased with a reduction of α_{s1}-casein, and it was observed that the milk with lower and higher amounts of α_{s1}-casein had diameters of 237 and 199 nm, respectively. Similar results were also observed in this investigation, as deer milk showed a lower concentration of α_{s1}-casein (~4% of total casein content) and larger casein micelles (~190 nm), as compared to cow milk, which contains a higher concentration of α_{s1}-casein (~44% of total casein content) and smaller casein micelles (~162.5 nm) as shown in Table 7.1. The casein micelles size, as well as the difference in the concatenation of fat and protein content, could also be the reason for the difference in average viscosity of deer milk (~6 cP) and viscosity of cow milk (2 to 2.5 cP) as described in Chapter 4.

Table 7.1: Composition and characteristics of deer and cow milk

Properties	**Deer Milk**	**Cow Milk***
Fat (%)	8.0 - 12	3.5 - 4.5
Protein (%)	7.5 - 8.5	3.0 - 3.5
Casein: WP	~80:20	~80:20
Distribution of caseins (% of total Casein)		
α_{s1}-casein	3.7	43.6
α_{s2}-casein	9.1	5.3
β-casein	78.3	31
κ-casein	8.4	20.1
Lactose (%)	4.5 - 5.5	4.0 - 4.8
Total solid (%)	20 - 26	10-13
Casein micelles size (nm)	190	160-165
Heat induced increase in casein micelles size (%)	24	6
Viscosity (cP)	6 - 6.2	2 - 2.5

*Cow milk data was taken from (Ha et al., 2014; Li et al., 2019; Roy et al., 2020a).

7.1.2 Impact of lactation on cow and deer milk composition

The lactational variation in the proximate composition of fresh deer milk was investigated in Chapter 4 (Table 4.1). Consistent with that observed for cow milk (Li et al. (2019), the fat and protein contents in deer milk continuously increased, whereas the lactose content decreased over the lactational period. The main variation in fat content in deer milk was observed from early to mid-lactation (~29% increase in fat compared to early-stage) whereas in cow milk the major impact was observed in late lactation (~13% higher fat content compared to mid-stage). The variation in protein content for both types of milk was similar, and a major impact was observed at the end of the lactation stage (~12% increase in deer milk and ~23% increase in cow milk). Overall, the protein content in deer milk as well as the proportion of casein and whey protein increased over the lactational period. The distribution of individual casein was also observed, and it was found that the proportion of κ-, α_{s2}-, α_{s1}- and β-casein varied significantly with lactation of deer ($P < 0.05$). The concentration of κ-caseins increased, whereas α_{s2}, $\alpha_{s1,}$ and β-casein content decreased in deer milk over the lactation period. The overall variation of total casein and whey protein content in deer milk over lactation was different from cow milk. Li et al. (2019) observed that the casein and whey protein proportions in cow milk did not vary significantly throughout the season. However, individual casein content (α_{s2}-casein and α_{s1}-casein) decreased with the lactational stage, whereas the κ-caseins and β-casein first reduced and then increased at the end of lactation. The information regarding the lactational variation of deer milk composition will contribute to an understanding of the processing properties of deer milk and the quality of dairy products throughout the lactation stages.

7.1.3 Heat-induced changes in deer and cow milk

Heat treatment results in several changes in deer milk, such as denaturation of whey proteins, association or aggregation of casein micelles, casein micelles size modification, and changes in salt equilibria, as discussed in earlier chapters (Chapter 4, 5, and 6). Here the main discussion will be on the denaturation of WP, and micelle association which causes subsequent changes in the milk, influencing the processing and functional properties of milk.

The extent of WP denaturation and their association with casein micelles in deer milk increased with heating intensity, like cow milk, but the heating kinetics were different in deer milk. Heating deer skim milk at 75°C for 1-5 min resulted in the denaturation of about 67% of whey proteins (Figure 7.1). In contrast, the denaturation levels of β-*lg* and α-*la* in bovine skim milk

heated at 75°C for 1 min were no greater than 5%, and they did not reach 60% until heating at 75°C for 30 min (Dannenberg & Kessler, 1988). Regarding the association between denatured whey proteins and casein micelles in cow milk, Oldfield et al. (1998) reported that the maximum level of association of *β-lg* with casein micelles was ~55% regardless of the temperature or holding time, whereas the maximum level of *α-la* varied with heating temperature, ~40% of the total when cow milk heated in between 95-130 °C and ~55% below 90 °C. In deer milk, the association level of whey protein with casein micelles was found to be higher. When deer skim milk was heated at 75°C for 1-30 minutes, the proportion of whey proteins associated with casein micelles varied from 50% to 80% of the total. When deer skim milk was heated at 95°C (1-30 min) whey protein association with casein micelles varied from 89 to 90%. The maximum change in whey protein and casein micelles association or denaturation of whey protein was observed when milk was heated at 95°C regardless of the time of heating. A simple explanation for the differences between cow milk and deer milk could be the higher concentration of proteins in deer milk, which facilitates protein-protein interactions during heating. Other factors such as distribution of casein and WP or ionic calcium concentration can also play important roles in the kinetics of heat-induced protein interactions (Law & Leaver, 2000; Li et al., 2019).

The heating kinetics of deer milk also highlighted the different behaviour of casein micelles' structure with different heat treatments, as compared to cow milk. The average size of casein micelles increased (~34%) at a higher rate when the deer milk was heated at 95°C for 5 min. On contrary, the heating of cow milk under similar conditions (90°C/ 6min) resulted in an approximately 6% increase in the micelle size (Li et al., 2019). The association of denatured whey proteins with the casein micelles is considered as the primary reason for the increase in micelles size upon heating, although some aggregation of casein micelles could also contribute to heat-induced changes in micelles size (Skelte G Anema & Li, 2003a; Li et al., 2019). According to Raynal and Remeuf (1998), the higher concentration of protein content and lower colloidal stability of milk could favour the aggregation of micelles, which further increases the population of larger micelles. This change in the structure of micelles increases the volume fraction, which would further result in a higher viscosity of milk (Skelte G Anema & Li, 2003a, 2003b; Jeurnink & De Kruif, 1993; Vasbinder & De Kruif, 2003). Therefore, it can be estimated that the major impact on the increase in casein micelles size of deer milk upon high heating (95 °C /5 min) is caused by aggregation of micelles, and the association of whey protein with casein micelles favours this process. These changes in the casein micelles likely further

led to the pronounced increase in viscosity of deer milk, particularly considering that deer milk has higher solids concentration than cow milk.

7.1.4 Gelation properties of deer and cow milk

Rennet gelation - Table 7.2 shows the rennet-induced gelation properties of deer and cow milk with different heating conditions. The gel made from fresh deer milk (final G' = 384 Pa) had higher firmness than fresh cow milk (final G' values = 58.5 Pa), presumably arising from the different protein contents of the two types of milk. The pasteurization coupled with homogenization improved the rennet gelation properties of deer milk, expressed as gelation time and final G' values, and the gel made from PHM shows better firmness compared to PHNM and NPM. The same impact of pasteurization was observed for cow milk with improved rennet-gel properties compared with NPM. Compared with PHM, high heat treatment (95°C/5 min) resulted in a sharp drop in the final G' of rennet gels made from both deer and cow milk. However, heating at 95°C significantly prolonged the rennet gelation time of cow milk but had no impact on the gelation time of deer milk. The detrimental effect of high-temperature heat treatment on the rennet gelation properties of cow milk has been widely reported. The rennet gel properties (extended gelation time and lower G') are mainly due to the formation of whey protein/casein complexes, which leads to the inhibition of κ-casein hydrolysis during the first stage of rennet coagulation which further explain why intense heating affects the gel rigidity G' of deer milk is similar to cow milk, but not the gelation time remains to be elucidated (Li et al., 2020a; Vasbinder & De Kruif, 2003)

Table 7.2: Rennet-induced gelation properties of deer and cow milk processed with different processing conditions

Milk	**Deer milk**				**Cow milk***			
Process	NPM	PNHM	PHM	HHM	NPM	PNHM	PHM	HHM
Gelation time (min)	19	16.6	14.9	14.8	13.3	ND	11.6	20.2
Final *G*' (Pa)	384	457	529	50	58.5	ND	95	14

NPRM – Non-processed deer milk, PNHM - Pasteurized (75 °C/15 sec) non-homogenized milk - PHRM – Pasteurized (75 °C/15 sec) homogenized (20 MPa/5 MPa) milk, HHRM – High heated (95 °C/5 min) homogenized (20 MPa/5 MPa) milk (95 °C/5 min), ND – Not determined.

*Data for cow milk taken from Delger (2018)

Acid gelation properties - Table 7.3 shows the acid-induced gelation properties of deer and cow milk with different heating conditions. Acid gel made from NPM shows a similar gelation time, approximately 130 min for both cow and deer milk, but the gel strength of fresh deer milk (final G' = 180 Pa) was higher than fresh cow milk which formed a weak gel (final G' = 29.4 Pa). Heat treatment combined with homogenization improved the acid gel firmness in both milks, but cow milk shows more improvement with high heating conditions (95°C/5 min), as expressed by final G' =519 Pa, compared to deer milk acid gel (final G' = 243 Pa). The gelation pH variation was similar for both types of milk, as milk starts to become gel at higher pH when heated. These findings indicated that homogenization and heat treatments had broadly similar impacts on the acid gelation properties of both deer milk and cow milk. However, the final G' of deer milk was lower than expected considering its solids content. Also, pasteurization caused a greater increase of gelation pH for deer milk than for cow milk (PNHM and PHM), which did not increase much further when heated more intensely at 95°C. This may explain the minimal increase in the final G' of HHM deer milk compared with the PNHM and PHM. The results suggested that deer milk possesses different functionalities, including gel strength and gelation time during acidification of heat-treated milk. The finding that deer milk whey proteins denatured faster when heated at 75°C than in cow milk may have played a part. Besides, the gelation behavior of deer milk might also be attributed to milk protein distribution, particularly the different levels and characteristics of α_{s1}-casein and β-casein in both milks (Li et al., 2019).

Table 7.3: Acid-induced gelation properties of deer and cow milk processed with different processing conditions

Milk	**Deer milk**				**Cow milk***			
Process	NPM	PNHM	PHM	HHM	NPM	PNHM	PHM	HHM
Gelation time (min)	130	64.9	43.8	30.8	133	ND	78.4	50.3
Final G' (Pa)	180	216	237	243	29.4	ND	126	519
Gelation pH	4.8	5.12	5.15	5.18	4.85	ND	5.05	5.24

NPRM – Non-processed deer milk, PNHM - Pasteurized (75 °C/15 sec) non-homogenized milk - PHRM – Pasteurized (75 °C/15 sec) homogenized (20 MPa/5 MPa) milk, HHRM – High heated (95 °C/5 min) homogenized (20 MPa/5 MPa) milk (95 °C/5 min), ND – Not determined.

*Data for cow milk taken from Delger (2018)

Comparison of fresh and reconstituted deer milk- In this study fresh and reconstituted deer milk physicochemical properties and gelation properties were investigated in Chapters 4 and 5. The fresh deer milk before processing had smaller fat globules (~7 µm) compared to reconstituted deer milk fat globules (~9 µm). The ionic calcium in fresh deer milk was also determined to be lower (~15%) than reconstituted deer milk. In both types of deer milk, acid gelation properties improved with the heating intensity. However, the value for final G' (~408 Pa) which shows the acid gel firmness of high heated (95°C/5 min) reconstituted deer milk improved at a higher rate than high heated (95°C/5 min) fresh deer milk (final G' = ~243 Pa). The rennet gelation properties for the two types of milk were different as non-processed reconstituted deer milk was determined to have lower final G' (~97 Pa) compared to non-processed fresh deer milk (final G' = 384 Pa). The processing history of deer milk powder and the reconstitution process is probably responsible for these differences. It should also be noted that the fresh and reconstituted deer milk was not produced in the same milking season.

7.2 Application and possible industrial outcomes for future work

7.2.1 New product development based on the functionality of deer milk

The current finding on protein characterization and process-induced changes in deer milk can be used for assessing its suitability for a specific type of dairy product or developing a new dairy product based on the technological functionality. Considering the lower production of deer milk, it is recommended for premium dairy products. For example, deer milk can be utilized for infant formulation as in this study it was found that deer milk contains a higher amount of β-casein and lower content of α_{s1}-casein.

7.2.2 Standardization and processing of products made from deer milk

The extensive recording of seasonal deer milk data (composition, physicochemical properties, heat-induced changes, and gelation properties) can help local manufacturers deal with product inconsistency associated with seasonality and processing conditions. The investigation regarding the colloidal stability of deer milk will provide knowledge to the dairy industry to optimize the processing parameters for deer milk.

7.2.3 Digestion behaviour of deer milk

Some non-bovine milk is considered to form softer curds during gastric digestion and is 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 (Wang et al., 2017). In this study, deer milk is well characterized regarding its physicochemical and gelation properties, but the digestion behaviour of deer milk still needs to be investigated. Further study is required to understand the digestion behaviour of deer milk concerning their physicochemical characteristics and the process applications.

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9. Appendix

Hill Laboratories
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Certificate of Analysis

Client:	Massey University	**Lab No:**	2609452 SPv1
Contact:	Siqi Li C/- The Registrar Private Bag 11222 Manawatu Mail Centre Palmerston North 4442	**Date Received:**	12-May-2021
		Date Reported:	26-May-2021
		Quote No:	101146
		Order No:	PN548493
		Client Reference:	
		Add. Client Ref:	0.02% Sodium Azide
		Submitted By:	Siqi Li

Sample Type: Dairy Products (liquid)

Sample Name:		Batch - 01 Deer Raw Skim Milk US	D2RMUC Mineral	D3 RMUC	D4 RMUC	
Lab Number:		2609452.9	2609452.10	2609452.11	2609452.12	
Calcium	g/100g as rcvd	0.062	0.059	0.061	0.067	-
Magnesium	g/100g as rcvd	0.0105	0.0094	0.0083	0.0103	-
Phosphorus	g/100g as rcvd	0.064	0.068	0.065	0.070	-

Sample Type: Milk (liquid)

Sample Name:		(1) Deer Raw Milk 1 19-Jan-2021	Raw Milk batch - 2 Deer	D4RM	D3 Rm Mineral	Frozen Mineral DF - 4186A
Lab Number:		2609452.1	2609452.2	2609452.3	2609452.4	2609452.5
Calcium	g/100g as rcvd	0.31	0.30	0.30	0.26	0.31
Magnesium	g/100g as rcvd	0.0176	0.0170	0.0177	0.0149	0.0159
Potassium	g/100g as rcvd	0.135	0.134	0.131	0.113	0.140
Sodium	g/100g as rcvd	0.037	0.041	0.045	0.043	0.033
Phosphorus	g/100g as rcvd	0.20	0.21	0.21	0.185	0.20
Copper	mg/kg as rcvd	0.067	0.054	0.055	0.052	0.177
Iodine	mg/kg as rcvd	1.44	0.34	0.62	0.45	1.09
Selenium	mg/kg as rcvd	0.009	0.041	0.024	0.037	0.009
Zinc	mg/kg as rcvd	8.3	9.0	10.1	8.6	9.1
Chloride*	g/100g as rcvd	0.075	0.085	0.097	0.086	0.069

Sample Name:		Frozen Mineral DF - 1st	Frozen Mineral DF - 4172	Frozen Mineral DF - 4200A		
Lab Number:		2609452.6	2609452.7	2609452.8		
Calcium	g/100g as rcvd	0.28	0.31	0.31	-	-
Magnesium	g/100g as rcvd	0.0175	0.0172	0.0168	-	-
Potassium	g/100g as rcvd	0.151	0.144	0.137	-	-
Sodium	g/100g as rcvd	0.034	0.036	0.035	-	-
Phosphorus	g/100g as rcvd	0.23	0.22	0.21	-	-
Copper	mg/kg as rcvd	0.48	0.28	0.096	-	-
Iodine	mg/kg as rcvd	0.53	0.55	0.53	-	-
Selenium	mg/kg as rcvd	0.014	0.011	0.010	-	-
Zinc	mg/kg as rcvd	12.0	9.8	9.6	-	-
Chloride*	g/100g as rcvd	0.069	0.066	0.067	-	-

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

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 * or any comments and interpretations, which are not accredited.

Sample Type: Milk (liquid)			
Test	**Method Description**	**Default Detection Limit**	**Sample No**
Biological Materials Digestion	Nitric and hydrochloric acid micro digestion, filtration.	-	1-12
TMAH Digestion	Tetramethylammonium hydroxide micro digestion, filtration. P.A.Fecher, I.Goldman and A.Nagengast. Journal of Analytical Atomic Spectrometry, 1998, **13**, 977-982.	-	1-8
Calcium	Biological materials digestion. Analysis by ICP-OES.	0.00010 g/100g as rcvd	1-12
Magnesium	Biological materials digestion. Analysis by ICP-OES.	0.00005 g/100g as rcvd	1-12
Potassium	Biological materials digestion. Analysis by ICP-OES.	0.0004 g/100g as rcvd	1-8
Sodium	Biological materials digestion. Analysis by ICP-OES.	0.0005 g/100g as rcvd	1-8
Phosphorus	Biological materials digestion. Analysis by ICP-OES.	0.0002 g/100g as rcvd	1-12
Copper	Biological materials digestion. Analysis by ICP-MS.	0.005 mg/kg as rcvd	1-8
Iodine	TMAH digestion. Analysis by ICP-MS. J. Anal. At. Spectrom., 1998, 13, 977 - 982.	0.0010 mg/kg as rcvd	1-8
Selenium	TMAH digestion. Analysis by ICP-MS.	0.002 mg/kg as rcvd	1-8
Zinc	Biological materials digestion. Analysis by ICP-MS.	0.10 mg/kg as rcvd	1-8
Chloride*	2% nitric acid extraction, potentiometric titration. AOAC (OMA) 971.27, 18th edition (modified).	0.00010 g/100g as rcvd	1-8

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Testing was completed between 17-May-2021 and 26-May-2021. For completion dates of individual analyses please contact the laboratory.

Samples are held at the laboratory after reporting for a length of time based on the stability of the samples and analytes being tested (considering any preservation used), and the storage space available. Once the storage period is completed, the samples are discarded unless otherwise agreed with the customer. Extended storage times may incur additional charges.

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CA Jones

Crystal Jones BSc
Client Services Manager - Food & Bioanalytical

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Title	Effect of lactation stage and processing on characteristics of deer milk
Institution name	Massey University, Palmerston North, New Zealand
Expected presentation date	May 2022
Order reference number	131
Portions	Figure 2.9: The schematic representation of the interaction of denatured whey protein and casein micelles at high intense heat treatment (90 °C for 10 minutes at pH 6.7) in cow milk (A) and goat milk (B)
Requestor Location	Massey University 12 B Lewis street AUCKLAND, AUCKLAND 0600 New Zealand Attn: Massey University
Total	Not Available