PROCESS DEVELOPMENT FOR A SHEEP WHEY BEVERAGE

A Thesis presented in

partial fulfilment of the requirements for the degree of

Master of Food Technology at Massey University

Palmerston North New Zealand

Maheeka Isurinie Weerawarna N. R. P.

2017

ABSTRACT

The potential of New Zealand sheep whey as a stable base ingredient in beverage manufacturing was studied using 4 model whey and 7 commercial whey streams. In order for sheep whey containing beverages to have a commercially adequate shelf life they are required to undergo a heat treatment. Due to the artisan small scale nature of sheep cheese manufacture in New Zealand heat treatment using continuous processing was not deemed feasible. The most likely form of heat treatment would be in package batch heating to 90°C with the pH of the beverage less than pH 4.6. Thus this study investigated the variations in the physicochemical composition of sheep whey as a function of cheese manufacturing on heat treatment (90ºC/ 5min) under varying acidic pH conditions (pH 4.5 and pH 3.5).

The composition of sheep whey varied primarily based on the pH of the cheese curd formation and separation method. Additionally, the whey composition also differed with the type of cheese produced and quality control of the process by each manufacturer. Of particular importance is the curd separation technique as this can result in casein contamination of the whey. A model study containing 5% w/v of contaminating curd in whey showed that significant Ca and Mg migrated from the curd into the whey during overnight cold storage (5ºC) at pH 3.5 resulting in an overall increase in ionic strength of the whey.

The stability of sheep whey during heat treatment (90ºC for 5 min) at pH 4.5 and pH 3.5 was studied via sedimentation and colour (L, a, b) measurement. The L value of whey samples heated at pH 4.5 increased and the samples produced 13 – 40% (v/v) sedimentation after overnight storage at 5ºC. Comparatively, whey heated at pH 3.5 was generally stable with less than 1% (v/v) sedimentation and no significant changes in the L value. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed comparatively high loss of monomeric whey proteins in whey heated at pH 4.5 than at pH 3.5 and further confirmed the above results. However, a commercial whey with high ionic strength (conductivity 193.6 mS cm-1) was unstable at pH 3.5 heat treatment and produced about 40% (v/v) sedimentation and a prominent increase in the L value.

Consequently, an extended study on the effect of ionic strength, varied by the addition of NaCl, on heat stability (90ºC for 5 min at pH 3.5) of sheep whey was performed. Whey was stable with up to 0.1 mol L^{-1} added NaCl (conductivity 102.1 mS cm^{-1}) and produced 6% (v/v) sedimentation after the heat treatment followed by the overnight storage. In contrast, a dramatic

increase in sedimentation (about 60%, v/v) was evident at 0.15 mol L^{-1} NaCl with gradual reduction of sedimentation upon subsequent addition of NaCl. Transmission electron microscopy (TEM) images of the sediments showed hairy like aggregates at 0.15 mol L^{-1} NaCl which became dense with further increases in NaCl concentration and perhaps explain the sedimentation behaviour. Further, conductivity of whey increased linearly with increasing NaCl concentration (R^2 = 0.98 at a 95% level of confidence) and thus conductivity measurement could be used to predict the stability of whey during thermal processing.

Moreover, a prototype whey beverage was produced using the above established conditions (90^{o}C for 5 min heat treatment at pH 3.5 and conductivity below 102.1 mS cm^{-1}). However, the product, which was heated in 450 mL bottles (at 1.94°C/ min) rather than the test tubes (at 28°C/ min) of the earlier work, were found to be unstable. Further a rapid heat treatment (at 4.7°C/ min) of the beverage only produced floating aggregates. It was thought that the instability was due to extended heating and cooling rates. A subsequent study showed that holding time at the heat treatment affected the properties of whey protein aggregates and consequently produced either floating aggregates or sediments. Therefore, minimizing heating and cooling rates would be a significant consideration in commercial scale sheep whey processing.

Additionally, the research outcomes would assist on-site decision making for individual cheese manufacturers on the utilisation of different sheep whey streams as a stable base ingredient in beverage processing.

ACKNOWLEDGEMENTS

I would like to express my special thanks to my main supervisor Dr Alsitair Carr for his friendly guidance, encouragement and helps throughout the course of this study. Similarly, I greatly acknowledge support and encouragement given by my co-supervisors; Dr Abby Thompson, Dr Li Day and Dr Linda Samuelsson, throughout this project.

Financial assistance from Bioresource Processing Alliance on this project is gratefully acknowledged.

I would like to thank Kingsmeade Artisan Cheese, Masterton and Sentry Hill Organics, Waipukurau for providing commercial sheep whey for the project and also for their warm welcomes during our visits.

Further, I must mention the support given by Dr Patrick Edwardson on NMR spectrometer.

I would also like to thank all the staff of the Department especially;

Dr Michael Parker, the Postgraduate Advisor (Food Technology), for his co-ordinations during the course work.

All the technical assistance given by Steve Glasgow, Warwick Johnson, Michelle Tamehana, Garry Radford, Chris Hall, Jack Cui and Jordan Taylor.

Finally, never ending love and support given by my family (mum, dad, sister, brother and in-laws) and friends (specially Jay and Hao) are admired with great respect.

TABLE OF CONTENTS

1. INTRODUCTION

Whey is considered as a valuable byproduct of cheese and casein processing and as such is commonly processed in to different food ingredients (Fox *et al*., 2000 and Sanmartin *et al*., 2012). World manufacture of lactose and whey proteins from liquid whey accounts for 7 and 1 million T per annum, respectively (Fox *et al*., 2000). Improved technologies such as microfiltration, ultrafiltration, nanofiltration, reverse osmosis and centrifugal separation are used in fat removal, fractionation of whey proteins, lactose and desalting of cow whey. Diaz *et al*. (2004) and Mazedo *et al*. (2012) studied microfiltration, ultrafiltration, nanofiltration and their combinations in recovery of proteins and lactose in sheep whey. Regardless, this further processing of whey mentioned above requires significant investment and cost to implement. However, due to the high volume of cow whey produced economies of scale render the processing feasible. Conversely, sheep dairying only accounts a minor 1.3% of the world dairy production (Park and Haenlein, 2008) and so the production of sheep whey would be significantly lower than the cow whey. Therefore, the feasibility of the above techniques in sheep whey processing is likely challenging. The cost associated with such methods and their applicability for small scale sheep cheese producers in New Zealand are likely prohibitive.

Additionally, cow whey, either its fractions (whey protein extracts or deproteinised whey) or whole whey is processed into beverages. Primary unit operations in cow whey beverage production include ingredient blending, heat treatment and packaging (Jelan, 2009). Nevertheless, such heat treatments have resulted in aggregation of whey proteins and thus the sedimentation of whey beverages during storage (Koffi *et al.*, 2005; LaClarie and Etzel, 2009; Sady *et al.*, 2013; Baccouche *et al.*, 2013; Kannan *et al.,* 2015; Chavan *et al.*, 2015). In contrast, the commercial use of fresh sheep whey as a base ingredient in beverages is not well established. Sheep whey composition variables and their respective effects on the stability of whey during thermal processing are not extensively investigated as cow whey and perhaps limit its applicability in beverage manufacturing. However, the factors governing the changes of cow whey constituents could be related to the same in sheep whey. Regardless, sheep milk and its products are increasingly preferred by consumers due to its perception as being nutritionally superior to cow milk. In fact, fresh sheep whey beverages could gain a reasonable market share in the functional beverage market. As regards the composition of sheep whey; nearly 94 % water and 6% solids (primarily made of lactose, whey proteins, bioactive peptides,

fat and minerals) (Ledesma *et al.*, 2011; Correa, *et al.*, 2014), it could be a reasonable raw material in thirst quenching functional whey drinks.

The need for the present research results from the increasing consumer demand for sheep dairy products (Griffiths, 2015) and to further grow the sheep cheese industry it is desirable to add value to the main sheep cheese waste stream, i.e. sheep whey, by conversion into a valuable food ingredient/s which could also assist the profitability of small scale sheep dairy operations in New Zealand.

The focus of this study is to identify composition variables in sheep whey and to elucidate their respective effects on the thermal stability of whey. Consequently, the research outcomes could enable the use of sheep whey to be used as a stable raw material in beverage processing. The research criterion is based on the product concept for sheep whey beverages, detailed in Figure 1.1.

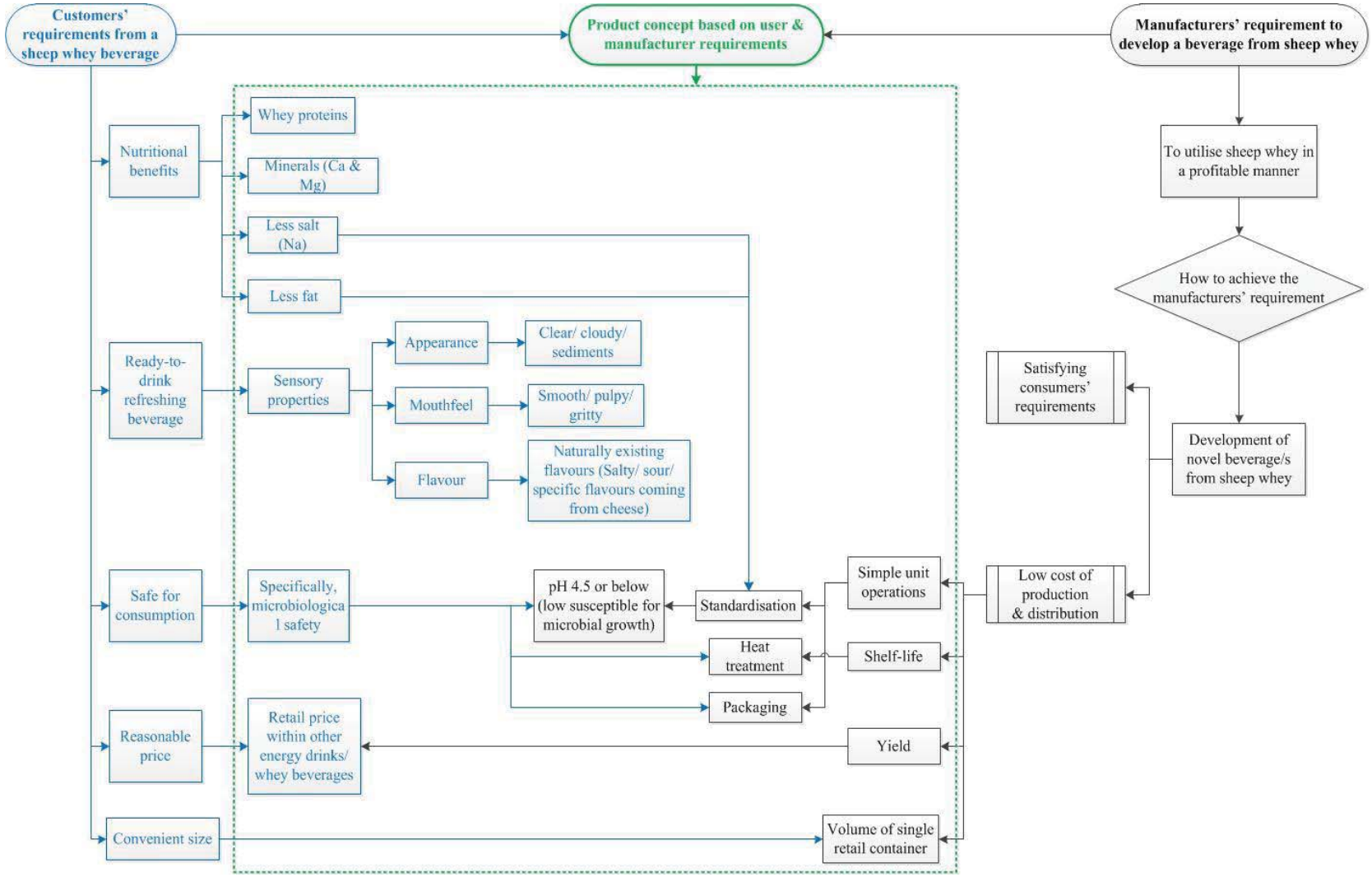


Figure 1.1: Product concept for sheep whey based beverages.

2. LITERATURE REVIEW

2.1. Whey based beverages

2.1.1. Types of whey beverages

Four main types of whey based beverages are frequently cited in literature namely; mixtures of whey with fruit/ vegetable juices, dairy type fermented/ unfermented beverages, carbonated beverages and alcoholic beverages. Further, Holsinger *et al*., (1974) has reviewed whey beverages under three main catergories such as; beverages from whole whey, non alcoholic beverages from deproteinised whey and alcoholic beverages. Many researchers have studied the potential of cow whey as an ingredient in such beverages (Djuric *et al*., 2004; Koffi *et al*., 2005; Childs *et al*., 2007; LaClarie and Etzel, 2009; Magalhaes, *et al*., 2010; Pescuma *et al*., 2010; Baccouche *et al*., 2013; Jain *et al*., 2013; Kannan *et al*., 2015; Chung *et al*., 2015; Kumar, 2015) but there has not been any studies on the use of sheep whey.

2.1.2 Ingredients and unit operations

Selection of whey, additional ingredients and unit operations in beverage processing are mainly determined based on the intended functional and physicochemical properties of the final product. Further, whole whey processing is considered as the cheapest method of whey beverage production: a whole whey beverage will typically comprise of flavour addition, pasteurisation and packaging. For an example, acid whey was mixed with fruit juices, particularly citrus juices (Holsinger *et al*., 1974 and Chavan *et al*., 2015), in development of acidic drinks based on their flavour compatibility. Acidic whey drinks were mostly within the pH 3.2 – 4.8. A benefit of manufacturing whey beverages under acidic conditions is that, aside from compatibility with popular fruit flavours, the acidic pH (i.e. below pH 4.0) hinders the growth of most food spoilage and pathogenic microorganisms (Jay *et al*., 2005). Due to the low pH (pHs below 4.6) it is possible to manufacture shelf stable beverages with relatively low heat treatments (less than 100°C) compared to UHT processes (about 140°C. For instance, Holsinger *et al*., (1974) reported that the manufacture of an acidic orange, lemon and grape fruit flavoured whey beverage heated at 90^{o}C had a shelf life of 6 months without refrigeration. From an engineering perspective the ability to heat treat at low temperatures (< boiling point) is relatively simple whereas high temperature processing requires more complicated and costly equipment that can handle the high pressures necessary to ensure that the beverages remain as fluids throughout processing. The low capital expenditure requirements for low temperature

acidic beverage processing are likely to be preferable for artisan sheep cheese producers (small scale industries) in New Zealand.

In contrast within the bovine dairy industry it is typical to fractionate whey to produce protein rich fractions and protein free fractions. Deproteinised whey has been used as a base for some carbonated and alcoholic beverages as it has the advantage of not possessing the foam stability characteristics of the protein (Dragone *et al.*, 2009; Jelan, 2009; Magalhaes *et al.*, 2010; Chavan *et al.*, 2015). Typically, such fractionation is achieved using ultrafiltration with a molecular cut-off of about 10,000 kDa. Deproteinisation of whey was achieved by heating at pH 7 and filtration or centrifugation to clarify heat-aggregated whey proteins (Holsinger *et al.*, 1974). Similarly, deoderisation and decolourisation of whey have been used to improve the sensory properties of specific whey beverages.

In addition to the more complex fractionation methods mentioned whey (acid whey derived from paneer manufacture, either whole or deproteinised) has been suggeted by Goyal and Gandhi (2009) as a suitable base ingredient in the preparation of electrolyte drinks due to its high amount of minerals.

Therefore, thorough understanding on different sheep whey streams and their respective physicochemical compositions would assist the selection of optimum unit operations to obtain specific properties in different whey beverages. This is discussed in detail under sections 2.2 and 2.3.

2.1.3 Heat treatment of whey beverages

2.1.3.1 Types of heat treatments

Heat treatment is essentially practiced as the primary method of ensuring microbial safety of food and beverages. Batch heat treatments are often used in small scale manufacturing of whey beverages. For an example, (Divya & Kumaria, 2009) studied different heat treatments, 60 - 70^{o}C for 15 - 35 min, on a whey – guava beverage. They reported that the heat treatment at 70^{o}C for 35 min provided the best sensory and physicochemical parameters after 45 days of storage (storage conditions were not specified). At higher heat treatment temperatures, the time required to render a product safe decreases: Chavan *et al.* (2015) reviewed development of dairy type strawberry and lemon whey drinks heat treated at 82^{o}C for 2 min while Pogon *et al.* (2015) heat treated a fermented orange – whey drink at 90^{o}C for 30 s, and Schlabitz *et al*.

(2015) used 90^{o}C for 5 min heat treatment in the production of fermented ricotta cheese whey drink.

Comparatively, continuous thermal processing such as ultra high temperature (UHT) treatments is seldom found in literature of whey beverages. Nevertheless, Koffi *et al*. (2005) investigated effects of storage temperature on the storage stability and sensory properties of UHT treated (140^{o}C for 5 – 7 s) whey – banana beverage formulations. However, the capital cost of installing UHT processing and the equipment necessary to aseptically bottle is such that it is likely to be economically unfeasible for small scale sheep dairy producers in New Zealand and thus in sheep whey processing.

2.1.3.2. Effects of heat treatments on particle size of whey beverages

Heat denaturation, aggregation and subsequent sedimentation of whey proteins during the heat treatments are undesirable in many whey beverages (Koffi *et al*., 2005; Jain *et al*., 2013; Chavan *et al*., 2015). Particle size of the denatured protein aggregates determines the physical stability of the beverage as particles below 1 µm remain in colloidal suspension whereas particles greater than 1 µm form aggregates (Damodaran *et al*., 2008). The aggregates are likely to sediment during the whey beverage storage. In addition to sediment, particle size contributes to the visual appearance of the colloidally stable portion of a beverage system by influencing the colour (Walstra and van Vliet, 2007). For an example, Baccouche *et al*. (2013) studied physicochemical parameters of prickly pear – whey beverage formulations developed using heat treated (80^{o}C for 30 min at pH 4.86) and unheated acid whey. Significant differences were evident in L, a and b colour parameters and turbidity of the two whey drinks.

Additionally, size, shape and texture of the suspended particles alter the tactile oral perception of liquid foods (Lawless and Heymann, 2010). Similarly, aggregated proteins in heated whey perhaps affect the mouthfeel of the beverage. For instance, whey protein aggregates in heated whey were reported to mimic the sensory perception of fruit pulps in fruit based whey beverages (Jelan, 2009). However, the particle size of the respective protein aggregates was not reported. Moreover, grittiness of soft – round suspended particles in a syrup was perceived when aggregates were in the range of 80 µm (Lawless and Heymann, 2010). Therefore, when studying heat treatment of a sheep whey beverage, it is important to characterize whey protein aggregation and their respective effects on the stability (sedimentation) and the sensory properties (colour, turbidity and mouthfeel). In fact, thorough knowledge on whey proteins and

the variables of whey protein aggregation during the heat treatments would provide a criterion to determine optimum processing conditions for sheep whey beverages and these aspects are reviewed in sections 2.4 and 2.5.

2.2 Origins of sheep whey streams

Sheep milk is often processed into cheese due to the unique composition; high in protein (4.5%), fat (7.4%) and total solids (19.3%) (Nudda, *et al.,* 2014 and Peterson and Prichard, 2015). Cheese is mainly produced by coagulation of casein using acids or rennet. Acidification of milk, up to the isoelectric pH of casein; (pH 4.6) or above, may be achieved through in situ fermentation by bacterial cultures resulting in the conversion of lactose to lactic acid, or directly by the addition of mineral acids or lactic acid. Direct chemical acidification is more easily controllable compared to fermentation (Fox *et al.*, 2000). By contrast, rennet coagulation is attained through cleavage of charged κ-casein fraction on link 105 – 106 (Park *et al.*, 2007). Nearly 75% of the cheese varieties are produced using rennet coagulation (Fox *et al.*, 2000). Casein coagulum entraps fat and a portion of the aqueous phase of the milk. The remaining non entrapped portion of the aqueous phase is drained off as whey upon curd cutting during cheese manufacturing (Figure 2.1). Whey essentially comprises of the water soluble fraction of milk. Typically, 10 L of milk could yield 1 kg of cheese and 9 L of whey (Britz & Robinson, 2008). Therefore, whey is the main by product in cheese processing (Durham and Hourigan, 2007).

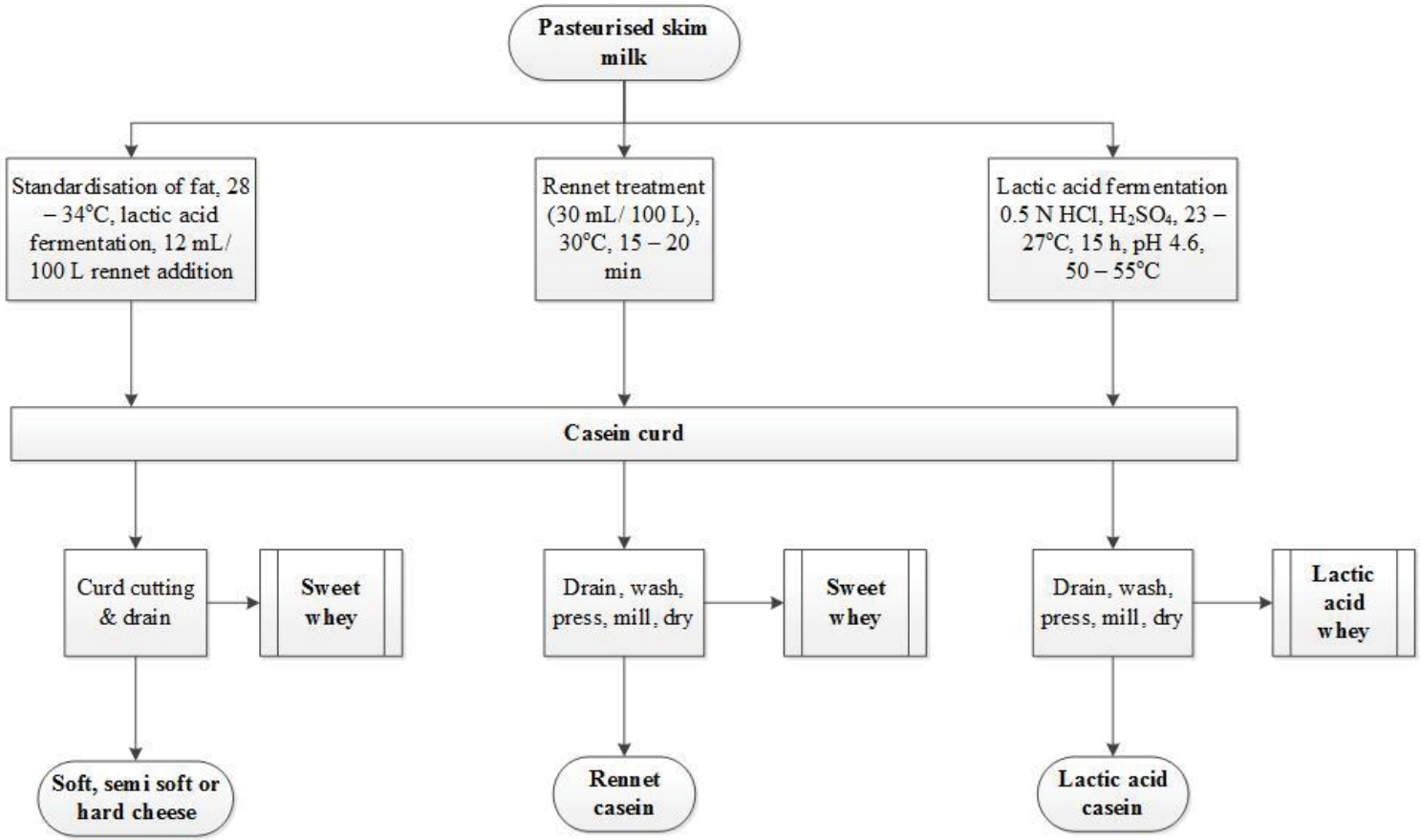


Figure 2.1: Origins of different whey streams

Source: Sienkiewicz and Riedel, 1990.

2.3 Composition of sheep cheese whey and its variables

Sheep whey is a source of proteins, fat, lactose and minerals (Park & Haenlein, 2008). Average compositions of cow and ewe milk and whey from different sources are presented in Table 2.1. Whey composition however may differ based on the composition of whole milk and cheese processing conditions (Ledesma *et al.*, 2011). The composition of whole milk varies according to sheep breed, ewe nutrition and the stage of lactation (Park & Haenlein, 2008). For instance, increase of fat, protein, mineral and total solids contents and lower lactose amounts would exist in milk towards the end of lactation period (Park *et al.,* 2007; Hejtmankova *et al.,* 2012). As a result, the composition of whey also can differ with reference to the composition of whole milk. Further, the composition of whey is largely dependent on the cheese processing conditions and some of those conditions and respective outcome are discussed hereafter.

2.3.1 Effect of heat treatments

Thermal treatments in cheese processing for instance, can determine the amount of total solids in whey. Sheep whey may contain nearly a half of the solids in whole milk when whey proteins remain unaffected by thermal treatments prior coagulation during cheese production. Additionally, thermal treatments in cheese processing can affect both colloidal (casein) and serum phase proteins (whey proteins) in milk. This could result in unfolding of whey proteins followed by denaturation, aggregation and formation of whey protein/κ-casein complexes (Donato & Guyomarc, 2009). For an example, heating of milk at 95^{o}C for 10 min is reported to denature nearly 80% of whey proteins followed by co-precipitation with casein upon acidification to pH 4.6 (O'Mahony & Fox, 2013). Hence, cheese produced under high temperature heat treatments probably produce whey with fewer proteins. These modifications could alter the functional properties of different whey streams.

Table 2.1**:** Average composition (%) of cow and sheep milk and whey

Component	Cow milk	Sheep milk	Cow Cheddar cheese whey	Sheep Manchego cheese whey
Total solids	12.7	19.3	6.7	7.46
[1]Protein	2.9	4.5	0.65	1.05
Lactose	4.1	4.8	5.2	5.16
Fat	4.5	7.4	0.3	0.82
Minerals (ash)	0.5	1.0	0.52	0.43
pH	6.7	6.8	5.9	-

[1]Protein = N% x 6.38.

Compiled from (Fox *et al.*, 2000; Park and Haenlein, 2008; O'Mahony and Fox, 2013).

2.3.2 Effect of casein coagulation method

2.3.2.1 Protein composition

The whey composition is essentially affected by method of casein coagulation. Cheese manufactured by rennet or acid precipitation produces either rennet (sweet) whey or acid whey, respectively. Further, the amounts of milk fat, uncoagulated casein fines and curd particles in sweet whey vary with quality controlling at curd cutting (Britz & Robinson, 2008). Degree of casein hydrolysis (Bonzanic *et al.*, 2014) and or the extent of acidification (Fox *et al.*, 2000) prior to whey separation also can modify the whey composition. Bonzanic *et al.* (2014) reported that acid and sweet whey contain nearly the same amount of total proteins. Regardless, sweet whey contains 4 folds higher concentration of free amino acids than acid whey. Additionally, sweet whey carries caseino-macropeptides (CMP), which are produced through the chymosin activity on casein during coagulation (Park *et al.*, 2007).

2.3.2.2. Mineral composition

Level of acidification in casein precipitation consequently determines the pH of fresh acid and sweet whey. In detail, pH of the former can vary around pH 4.6 and the latter around pH 6.6 – 5.8, comparatively (Fox *et al.*, 2000). Additionally, the pH of casein curd formation and rate of acidification affect the solvation of colloidal minerals in casein micelles. Solubilisation of colloidal calcium phosphate in the micelles increases upon acidification of milk up to the isoelectric pH of casein, i.e. pH 4.6. Subsequently, the dissolved calcium phosphate drains along with whey (McMahon and Oommen, 2013). Acid whey, for an instance, contains higher amounts of Ca (6.0 – 8.0 g/L) and P (2.0 – 4.5 g/L) in comparison to Ca (0.4 – 0.6 g/L) and P (1.0 – 3.0 g/L) in sweet whey (Bonzanic *et al.*, 2014). Further, Wong *et al.* (1978) reported three times higher Ca content in cottage cheese acid whey compared to cheddar cheese sweet whey. Moreover, use of mineral acids in acidification of cheese milk potentially increase the mineral contents in respective whey streams.

Accordingly, such deviations of the whey composition, specifically proteins and minerals, probably alter the behaviour and functionality of whey as a raw material in beverages. In addition, selection of whey streams for whey beverage manufacturing and their respective composition variations would be a concern in nutrition information panels (NIP) on the final product labels.

2.4 Whey proteins

Whey proteins are comparatively soluble in aqueous phase and heat sensitive. The main whey proteins include of β-lactoglobulin (BLG), α-lactalbumin (ALA), serum albumin (SA), immunoglobulin (Ig), lactoferrin, proteose-peptones (PP) and few other minor protein fractions. BLG accounts for nearly 50% of the total whey proteins and its properties are well defined. Whey proteins are characterised by different amino acid compositions and structures (Table 2.2), and consequent functionalities (Fox and McSweeney, 2003; Swaisgood, 2007 and Guinee and O'Brien, 2010).

Table 2.2**:** Characteristics of whey proteins in cow milk

Characteristics of whey proteins		**BLG**	**ALA**	**SA**	**Ig**
Concentration in milk (g/ kg)		3.3	1.2	0.4	0.7
Molecular weight (Da)		18, 362	14, 174	66, 267	$(1.5 – 10) \times 10^5$
Isoelectric pH in milk		5.13	4.4	4.8	-
Amino acids mole $^{-1}$		162	123	34	-
-SH groups mole $^{-1}$		1	0	1	-
S-S bonds mole $^{-1}$		2	4	17	-
[1]Secondary structure (%)	α-helix	6.8	26.0	67.0	2.5
	β -sheet	51.2	14.0	0.0	67.2
	β-turns	10.5	0.0	0.0	17.8
Native configuration				Globular	

Abbreviations: BLG - β-lactoglobulin, ALA – α-lactalbumin, SA – serum albumin, Ig – Immunoglobulin. [1]Secondary structure % of total amino acid residues.

Compiled from (Singh and Havea, 2003; Damodaran, 2008 and Guinee and O'Brien, 2010).

2.4.1 Forces governing the stability of whey protein structure

Native whey protein structure is stabilised by intramolecular bonds and remains thermodynamically stable (Monahan *et al.*, 2010). The intramolecular bonds dominate protein folding via intrinsic forces of the proteins (steric interactions and van der Waals forces) and extrinsic forces emanating from the dispersed solvent (H bonds, hydrophobic interactions and electrostatic forces). Steric interactions hinder the dihedral angles of N – C_α (φ) and C_α – C (ψ) bonds and thus the rotational freedom of the peptide chain. Consequently, the specific segment in the peptide chain limits the configurations of the protein molecule. Similarly, van der Waals interactions can be either attractive or repulsive and exists among induced dipoles or dipole – induced dipole of neutral atoms in proteins. Conversely, H bonds are created in between an electronegative atom and a H atom attached to another electronegative atom. H bonds are presumed as the impetus of protein folding and controls their native state stability. Additionally, hydrophobic interactions (among non-polar groups) and disulfide bonds (formed upon oxidation of sulfhydryl groups) are also known to dominate the protein folding and stabilising the folded structure. Moreover, electrostatic forces are primarily determined by the number of negatively and positively charged groups in a protein molecule and hence its net charge. Subsequently, the net charge would assist stabilisation or destabilisation of the protein structure (Damodaran, 2008).

Alterations in the physicochemical environment of proteins such as; temperature, pH and ionic strength (Sakurai *et al.* 2009; Sawyer, 2013), could induce changes in intramolecular forces and thus impose structural modifications on the native state of the proteins. Conformational adaptability of proteins allows subtle modifications and compensates minor changes in its environment. Regardless, proteins undergo dramatic structural changes of its higher order state, other than cleavage of backbone peptide chain, during denaturation. Denatured proteins eventually aggregate and attain new equilibrium states (Damodaran *et al.*, 2008). Heat induced structural changes of cow whey proteins in varying physicochemical conditions have been broadly studied (Singh and Havea, 2003; Havea *et al*., 2002; Bryant and McClements, 2000; Havea *et al*., 1998; Gezimati *et al*., 1997 and Gezimati *et al*., 1996).

2.5 Heat induced interactions of whey proteins

2.5.1 Effect of temperature

Whey proteins undergo varying reactions upon thermal treatments. Individual whey proteins demonstrate different rates of thermal aggregation reactions. The phenomenon can be a dependent of different thermal transition enthalpies, thiol – disulfide interchanges, inter – protein interactions of whey proteins (Gezimati *et al.*, 1997) and thus the varying denaturation temperatures (Table 2.3) (Singh and Havea, 2003 and Damodaran, 2008). BLG, for example, exists as dimers in its native state. The BLG dimers dissociate during increasing temperature and denature at 78°C (Singh and Havea, 2003). Further, Gezimati *et al.*, (1996) studied thermal behavior of BLG and SA (when heated at 70 and 75^{o}C for 60 min) in a simulated whey protein concentrate (WPC) buffer. Native and sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) (which the method employs separation of the proteins based on molecular weight (Shi and Jackowski, 1998), revealed rapid aggregation and polymerization of SA than those in BLG. The high amount of disulfide bonds in SA, comparative to BLG (Table 2.2), perhaps provide more reaction sites and hence the rapid polymerisation during heating.

Disulfide linked polymerisation of the proteins were presumed to proceed via intermediate products formed with hydrophobic interactions. Similarly, BLG and ALA produced intermediate aggregates followed by polymerisation during heating at 75 and 80^{o}C for 60 min (Gezimati *et al.*, 1997). Moreover, the proportion of homo and hetero polymers produced in a WPC solution vary with respect to the ratio of whey proteins and heat treatment (Gezimati *et al.,* 1996). This is important in the current study as sheep whey composition (refer section 2.3) varies as a result of cheese manufacturing method and so the ratio of whey proteins in different whey streams possibly vary with respect to their origins. Consequently, each whey stream would demonstrate specific polymerised products during heat treatments.

Table 2.3**:** Thermal denaturation temperatures and enthalpies of whey proteins

Whey proteins	**Initial denaturation temperature / ^{o}C**	**ΔH_D / kJ mol^{-1}**
BLG	78	311
ALA	62	253
BSA	64	803
Ig	72	500

Abbreviations: BLG - β-lactoglobulin, ALA – α-lactalbumin, BSA – serum albumin, Ig – Immunoglobulin. ΔH_D – enthalpy of denaturation.
Adapted from (Singh and Havea, 2003).

2.5.2 Effect of protein concentration

Havea *et al.* (1998) described concentration dependent protein aggregation during heating. These researchers reported that 120 g/ kg WPC solution heated at 75^{o}C for 30 min showed comparatively high amounts of disulfide linked BLG dimers than those in a 10 g/ kg WPC solution treated the same. The effect of increasing whey protein concentration on loss of native and monomer states of the proteins followed first or second order reaction kinetics. Moreover, the reaction rate constant depended on protein concentration. However, the research outcomes would require specific extrapolations based on the whey protein concentrations and physicochemical properties (pH and minerals) of fresh sheep whey. Specifically, protein concentrations in fresh sheep whey is nearly 1 % (Table 2.1) and unlikely to have broad deviations as the concentrations studied by Havea *et al*. (1998).

2.5.3 Effect of pH

Interactions of whey proteins also depend on the pH of the heat treatment. Intermolecular bonds of whey proteins are affected by the pH of the medium and thus the subsequent aggregations. For an example, Monahan *et al.* (1995) studied the polymerisation of whey protein in 1% and 13% whey protein isolate (WPI) gels, using SDS-PAGE. Irreversible unfolding of the proteins and subsequent polymerisation was evident within pH 9 – 11 at 22^{o}C. By contrast, WPI gels at pH 3, 5 and 7 polymerised only upon heating up to 85, 75 and 70^{o}C, respectively. In addition, notable conformational variations of BLG occur within the pH range of 2 to 9. Native BLG

exists as dimers between pH 5 and 8 and rearranges as octomers at pHs between 3 to 5 (Damodaran, 2008). Further, BLG shows reversible structural modifications at pH 7 and denaturation accompanied aggregation above pH 8 (Sawyer, 2013). Furthermore, electrostatic repulsions among protein molecules can become significant below pH 3, and thus prevents protein – protein interactions. Therefore, the electrostatic repulsions dissociate BLG complexes into monomers below pH 3 (Hamada, *et al.,* 2009). Additionally, whey proteins can be resistant to heat induced coagulation below pH 3.6 (Jelan, 2009) and hence unlikely to form sedimentable aggregates during heating below that pH. However, ionic strength of the medium at heat treatment alter the aggregation of whey proteins (Damodaran, 2008) and thus the above findings would demonstrate deviations depending on the salt concentration of the whey protein solution at its heat treatment.

2.5.4 Effect of minerals

Minerals interfere with protein structure (specifically through altering the net charge) and consequently modify the behavior of proteins during heating. Varunsatian *et al.* (1983) and Zhu and Damodaran (1994) showed the effect of Ca, Mg and Na on heat denaturation of whey proteins at alkaline pH (> pH 5.5). Ca and Mg showed much greater impact than Na. Further, Parris *et al.* (1993) reported Ca assisted whey protein aggregation upon heating of dialysed sweet whey. Increasing Ca concentration from 0 to 9 mM reported three-fold reduction of soluble aggregate formation during heating at 85^{o}C for 30 min. Further, addition of NaCl or lowering the pH about pH 5.1 (i.e. isoelectric pH) of β-lactoglobulin solution produced large aggregates and thus turbid dispersions. The aggregation was a result of both physical (electrostatic, hydrophobic and hydrogen bonds) and chemical (disulfide exchange) interactions (Mulvihill and Donovan, 1987). Moreover, Verheul *et al.*, (1998) studied thermal aggregation of β-lactoglobulin (9 g L^{-1} dispersion) as affected by heat treatment temperature, pH and added NaCl concentration. The aggregation followed two consecutive reaction phases such as unfolding (first-order reaction) and subsequent aggregation (second-order reaction).

Havea *et al.* (2002) studied two commercial WPCs heat treated at 75^{o}C in 12 % (w/w) solutions at pH 6.9. Acid WPC initially contained Ca, Mg, K and Na at 40, 3, 424, and 30 mmol/ kg dry weight respectively and the same minerals in sweet whey at 136, 21, 193 and 91 mmol/ kg dry weight, respectively. The acid WPC reported comparatively soluble aggregates linked via disulfide bonds. Further the sweet WPC showed relatively large aggregates mainly associated with noncovalent interactions. Moreover, aggregation of BLG and ALA were rapid in the sweet

WPC over the other. Further studies on the same, yet with cross dialysis of the WPC solutions reversed the thermal aggregation properties. The findings were consistent with the work published by Hollar *et al.* (1995).

It is suggested that this effect is due to Ca^{2+} binding, neutralisation of net negative charge and successive isoelectric precipitation of whey proteins (Varunsatian *et al.,* 1983). Likewise, other positively charged mineral ions such as Mg^{2+}, Na^{+} and K^{+} probably assist isoelectric precipitation of whey proteins. Thus, fresh acid and sweet whey, possessing varying mineral compositions, may result different protein aggregates during thermal processing. Similar characteristics would exist in heated acid and sweet sheep whey. Whey contains naturally existing minerals originating from the milk source. In addition, sweet whey may contain salts added during cheese processing where salting is done in the cheese milk or calcium is added to enhance coagulation. Additionally, casein micelles in sheep milk are highly mineralized (about 3.7 g minerals / 100 g casein) (Park *et al.*, 2007) and thus perhaps produce whey with high mineral contents than cow whey. Therefore, further studies are required on the mineral composition of sheep whey samples and thus the effect of minerals on protein aggregation during thermal treatments. The outcomes would predict suitability of an individual sheep whey sample as an ingredient in beverage processing.

2.6 Concluding remarks

Whey contains valuable constituents. The composition of whole cow whey, whey extracts and their functional properties have been extensively studied. Therefore, cow whey (produced in large volume) is often processed into different food ingredients. By contrast, commercial utilisation of sheep whey in New Zealand is rather rare. The total volume of sheep whey produced by individual sheep cheese produces is significantly lower the volumes produced of cow whey at commercial factories (in the order of 20 to 200 L of liquid whey compared to 10s of thousands of metric tonnes of dry whey powder) and thus expensive processing is not economically viable. However, sheep whey contains nutritionally beneficial functional compounds which are dissolved in an aqueous medium. Hence, sheep whey could be a reasonable raw material for beverage production in which minimal pre-processing of the whey is necessary. The main processing step in beverage processing is likely to be heat treatment to ensure microbial safety of the beverage. However, knowledge on the influence of sheep whey composition on thermal processing is scarce. There are however published work in the similar

field, using cow whey, which will be useful on which to design experiments and assist with interpretation of data.

2.7 Aim and objectives of the research

The aim of this research was to study the potential of New Zealand sheep milk whey as a stable raw material in beverage manufacturing. This primarily requires establishing knowledge of the composition variables of sheep whey and their influence on the behavior of whey proteins during thermal processing. Consequently, the findings would assist the decision making on suitability of different sheep whey streams as a stable base ingredient for beverages and or required standardisation which would enable specific whey streams to be suitable for such processing.

Specific objectives of the study were;

- To identify key factors affecting the composition of sheep whey
- To study the effects of casein coagulation method and whey separation pH on the composition of sheep whey
- To investigate the effect of storage pH on mineral (Ca and Mg) migration from contaminating curd in sheep whey
- To study the stability of sheep whey as affected by the physicochemical composition at its heat treatment
- To relate the composition and behaviour of model whey streams upon heat treatment and to understand the deviations of commercial sheep whey streams
- To establish knowledge on the behaviour of sheep whey upon heat treatments which could predict necessary processing parameters, and thus the on-site decision making for individual sheep cheese producers in using whey as a stable base ingredient in beverages

3. MATERIALS AND METHODS

3.1. Materials

3.1.1 Sheep milk and commercial sheep whey samples

Pasteurised sheep milk was obtained from Kingsmeade Artisan Cheese, Masterton, New Zealand, and was used to produce laboratory whey samples for model studies. Seven commercial sheep whey streams were collected from two cheese producers in New Zelanad (Kingsmeade Artisan Cheese, Masterton and Sentry Hill Organics, Waipukurau). Collected whey samples were stored at – 18^{o}C till further analyses and defrosted overnight at 5^{o}C prior to experiments.

3.1.2 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) reagents

SDS-PAGE analyses were performed using Mini-PROTEAN® TGX™ precast gels, broad range molecular weight standard and Dual Xtra standard (Bio-Rad Laboratories, USA). Electrophoresis buffers were prepared using analytical grade chemicals (Sigma-Aldrich, St. Louis, Missouri, USA). MilliQ – water (Milli-Q PLUS 185 Ultra-Pure Water System) was used in preparation of the buffers.

3.2 Analytical methods

All the measurements were performed in triplicate unless otherwise specified.

3.2.1 Total soluble solids and pH

Total soluble solids and pH of the samples were measured using a refractometer (Atago, Japan) and pH meter (Orion 3 Star pH Benchtop, Thermo Electron Corporation, Singapore), respectively.

3.2.2 Ionic strength

Ionic strength of the sheep milk and whey samples were determined using conductivity, measured by conductivity meter (SC82, Personal SC meter) at 20^{o}C. The conductivity meter was calibrated using a 0.1 mol L^{-1} NaCl standard solution.

3.2.3 Moisture

The moisture content of the sheep milk and whey samples was analysed using an air oven method. Pre-weighed samples were dried at 105^{o}C for 4 h in an oven (Contherm, New Zealand) and transferred to a desiccator at room temperature. Samples were reweighed after 2 h (AOAC, 2005).

3.2.4 Fat

Fat content of the samples was quantified using Mojonnier method. Fat in the samples was sequentially extracted using ethanol, diethyl ether and petroleum ether as described in official methods of analysis (AOAC, 2005).

3.2.5 Protein

Total N in the samples was determined using the Kjeldahl method (AOAC, 2005) with a blank estimation. Kjeltabs (1000 Kjeltabs S/3,5, Foss Analytical, Hilleroed) were used in sample digestion, followed by distillation (Kjeltec™ 2100 distillation unit, Tecator™, Sweden) and titration using 0.1 M HCl. A N conversion factor of 6.38 (Fox and McSweeney, 2003) was used to calculate the total protein content of the samples.

3.2.6 Minerals (ash)

Minerals were determined by dry ashing of the samples at 550^{o}C for 4 h in a muffle furnace (Carbolite BMF 1100, Hopevally, England).

3.2.7 Ca and Mg

Ca and Mg content in the sheep milk and whey samples were analysed by the Nutrition Laboratory (Riddet Innovation, Massey Institute of Food Science and Technology, Palmerston North, New Zealand) using the *O*-cresolphthalein complexone and xylidyl-blue colorimetric methods, respectively.

3.2.8 Individual proteins

Proteins in the samples were separated by SDS-PAGE using Mini – Protein II dual cell system (Bio-Rad Laboratories, USA). Mini-PROTEAN® TGX™ precast gels were used in the analyses. Stock running buffer (250 mM Tris, 1.92 M glycine, 1% SDS, pH 8.3) and stock sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue)

were diluted 1:9 and 1:1 (Bio-Rad, 2011) with Milli-Q water in use. Sample volume of 12 µL were loaded on gels and electrophoresis was performed at 70 mA current and 280 V voltage for 35 min. Each gel was stained using 50 ml of Coomassie blue solution (0.5% Coomassie blue R-250, 25% isopropanol, 10% acetic acid) for 1 h and destained overnight using 100 ml of destaining solution (10% isopropanol, 10% acetic acid). Molecular weight of the protein bands on the destained gels were identified by simultaneous electrophoresis of broad range molecular weight standard and Dual Xtra™ standard (Bio-Rad Laboratories, USA). Thereafter, the gels were scanned using Molecular Imager® Gel Doc™ XR+ imaging system and analysed using Image Lab™ software to obtain relative intensities of the protein bands. The peak area was reported as a percentage of respective protein band in the control sample.

3.2.9 Particle size distribution

Particle size distribution of the whey samples were analysed using Mastersizer (Malvern 2000) based on laser light scattering. D (0.1), D (0.5) and D (0.9) values were obtained for each whey sample.

3.2.10 Turbidity

Turbidity of the samples was measured as % transmittance at 500 nm using UV/Visible spectrophotometer (Pharmscis LKB Ultrospec II). Distilled water was used as the reference of 0 turbidity and thus 100 % transmittance (Banavara *et al.*, 2003).

3.2.11 Colour

Colour of the whey was measured using a colourimeter (Minolta Chroma meter CR – 200, Japan). The colourimeter was calibrated using a calibration tile (Konica Minolta Sensing Inc, Japan) for L = 95.48, a = -0.07 and b = 2.39. Aliquots (10 mL) of the samples were transferred into 60×14 mm polystyrene petri dishes (Thermo Fisher Scientific, New Zealand) and presented to the colourimeter. L, a and b parameters were obtained for each sample.

3.2.12 Transmission electron microscopy (TEM)

Aggregates in heated whey were analysed by the Manawatu Microscopy and Imaging Centre, Massey University, Palmerston North, New Zealand, according to the method cited in Havea *et al.* (2002).

3.2.13 Small molecular metabolites

Whey samples were centrifuged (3000×g / 30 min) to remove fat and prepared as described in Figure 3.1 with trimethylsilyl propionate-d9 (TSP) as an internal chemical shift standard. 1H nuclear magnetic resonance (NMR) spectroscopy was performed using Bruker 700 MHz Ultrashield spectrometer. 1-dimentional 1H spectra were acquired for individual whey samples using the standard Bruker "noesygppr1d" pulse sequence while suppressing the large water signal. Spectra were phased and baseline correction was performed using MestReNova 8.1.2. Metabolites were putatively identified using the Chenomx NMR Suite 7.0 software and database.

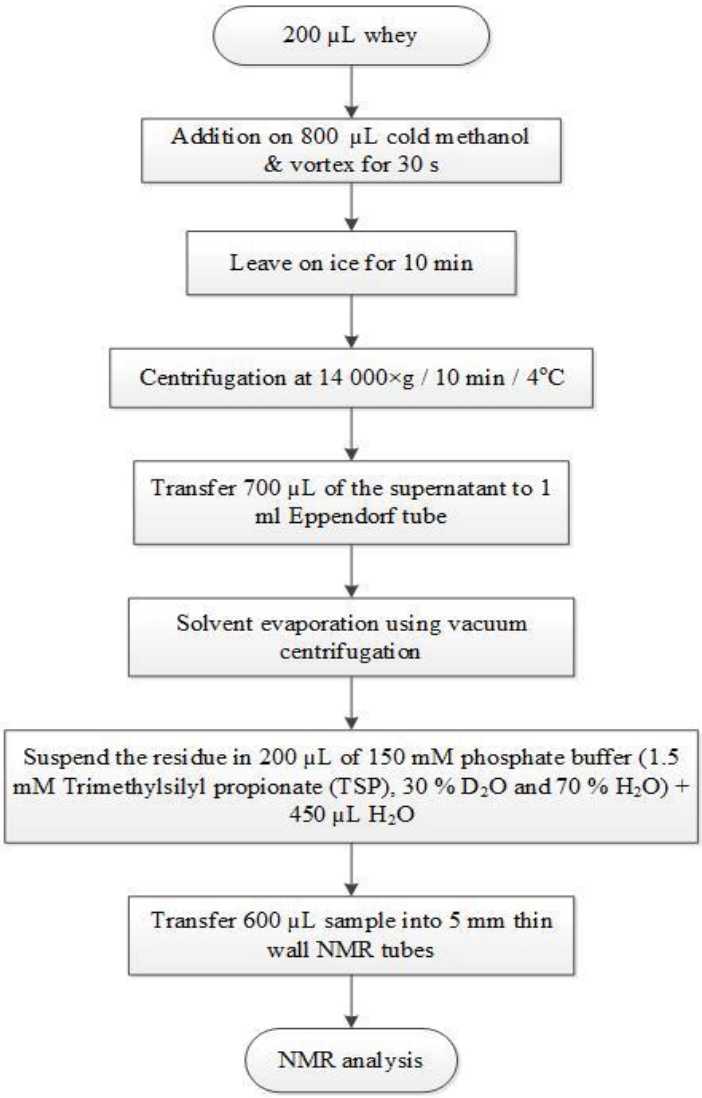


Figure 3.1: Sample preparation for NMR analyses

3.3 Preliminary experiments

3.3.1 Composition analysis

A commercial sweet whey sample was filtered through a cheese cloth to remove contaminating curd particles. Contaminating curd weight was obtained after 1 h of draining from cheesecloth filtration. Total soluble solids, pH (section 3.2.1), conductivity (section 3.2.2) and proximate composition; moisture (section 3.2.3), fat (section 3.2.4), protein (section 3.2.5) and ash (section 3.2.6), of the whey sample were analysed.

3.3.2 Effect of pH and heat treatment on the turbidity of sheep whey

pH of the sheep whey sample was adjusted to pH 3.5, pH 4.5 (via dropwise addition of 20% w/v lactic acid) and pH 5.9 (i.e. the natural pH of the whey) and subjected to two heat treatments: 72^{o}C for 15 s and 90^{o}C for 5 min. Turbidity changes were visually observed and compared with unheated whey samples at respective pH levels. Findings of the experiment were used to determine the pH range and the heat treatment for following studies.

3.4 Preparation of acid whey and rennet whey for model studies

One type of acid whey and three types of rennet whey were prepared using the pasteurised sheep milk (Figure 3.2).

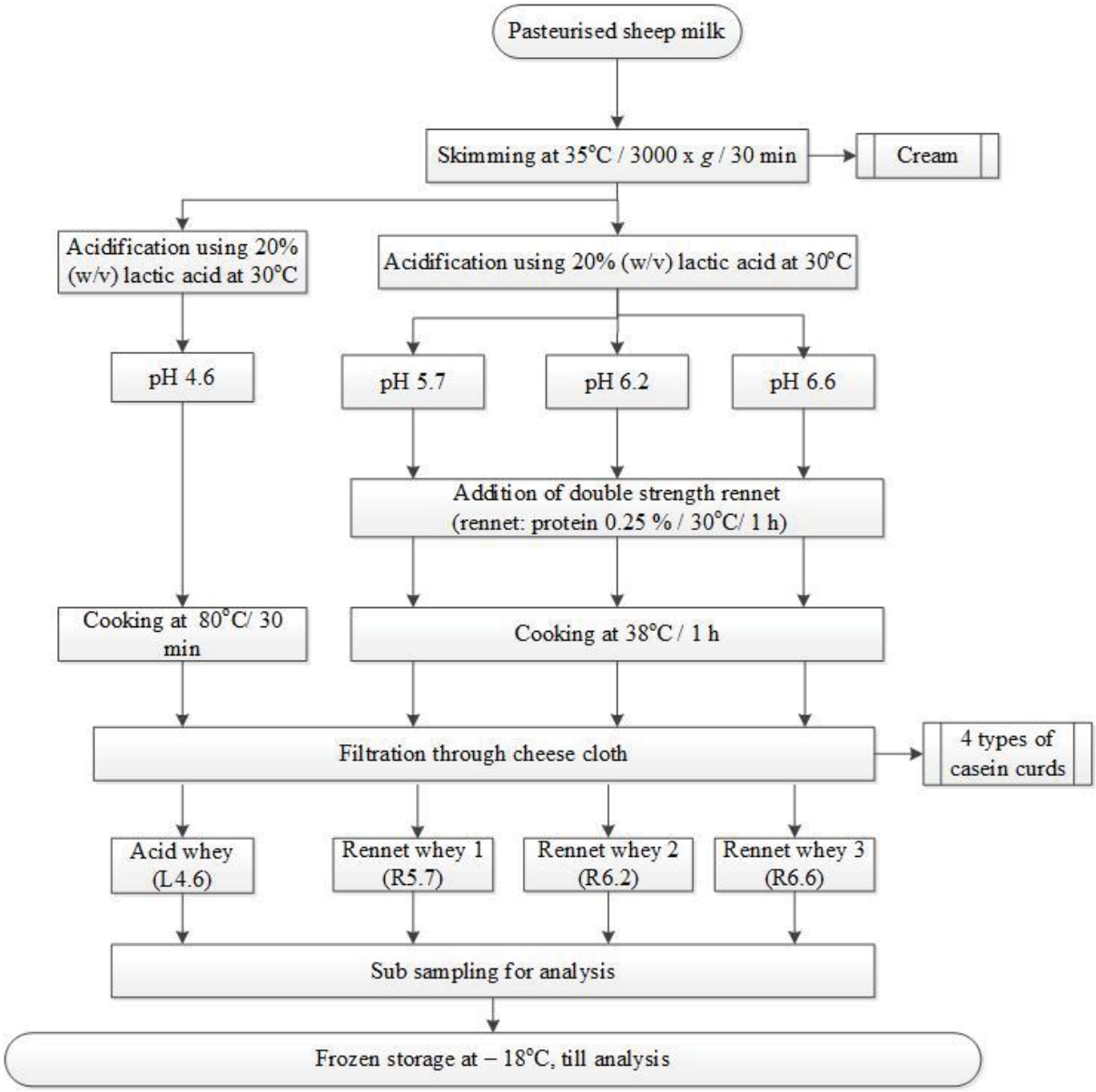


Figure 3.2: Production steps of model acid whey and rennet whey

3.5 Compositional analysis of sheep milk, model whey and commercial whey streams

Physicochemical composition (total soluble solids, pH, ionic strength, moisture, protein, fat, ash, Ca and Mg) of sheep milk (which was used to prepare the model whey), four types of model whey and seven commercial whey streams were analysed by the methods described in sections 3.2.1 to 3.2.7. Effects of the casein coagulation method and dewheying pH on the composition of sheep whey were studied using the four model whey samples. The results were used to validate composition variations of the seven commercial whey streams based on their respective casein coagulation method and dewheying pH.

3.6 Effect of storage pH on and Ca and Mg migration from contaminating curd in whey

The whey produced at pH 6.6 (Figure 3.2) was contaminated with 5% (w/v) curd from the same process. Contaminating curd % was determined based on the results obtained from the preliminary experiment in section 3.3.1. The pH of the samples was adjusted to pH 3.5, 4.0 and 4.5, using 20% (w/v) lactic acid, and held overnight at 5^{o}C. The added curd was removed through cheese cloth filtration. The ionic strength, Ca and Mg concentrations of the filtered whey were measured as described in section 3.2.2 and 3.2.7.

3.7 Effect of pH at heat treatment on the stability of whey

The pH of the model whey and commercial whey samples was adjusted to pH 3.5 and 4.5 (using 20% (w/v) lactic acid), and heated in a water bath at 90^{o}C for 5 min. The samples were immediately immersed in ice after the heat treatment.

Particle size distribution (section 3.2.9), turbidity (section 3.2.10), colour (section 3.2.11), and residual protein % (section 3.2.8) of the heated whey were measured.

Sedimentation of the heated whey was measured after standing overnight at 5^{o}C. The resulting whey samples were centrifuged for 5 min each at 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000×g, and sediment heights were measured after centrifugation.

3.8 Effect of added NaCl on the stability of whey during heat treatment

NaCl was added to whey (model whey produced at pH 6.6) at 0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 and 0.35 mol L^{-1}, and heat treated at 90 ^{o}C for 5 min at pH 3.5.

Ionic strength (section 3.2.2), particle size (section 3.2.9) and turbidity (section 3.2.10) of the heated whey were measured. Sedimentation of the whey was analysed after standing overnight at 5^{o}C. The resulting whey samples were centrifuged for 5 min at different speeds from 100×g to 1000×g (as described in section 3.7) and sediment heights were measured after each centrifugation. The resulting supernatant from the centrifugation was analysed using SDS-PAGE (section 3.2.8) to study the residual whey proteins and thus the effect of NaCl concentration on heat induced whey protein aggregation.

Transmission electron microscopic (TEM) images (section 3.2.12) were used to compare the structure of the sediments produced at each treatment.

3.9 Statistical analysis

Results were analysed using one-way ANOVA and Tukey difference test on Minitab statistical software (Minitab 17.0). The level of significance was determined at $P < 0.05$.

3.10 Prototype sheep whey beverage development

A prototype beverage was developed according to Figure 3.3. A lemon juice concentrate used in the beverage formulation was at pH 2.0, with a conductivity of 4.21 mS cm^{-1} and total soluble solids 42.7% (Brix). The physicochemical composition (moisture, protein, fat, ash, total soluble solids, pH and ionic strength) of the beverage was determined as detailed in sections 3.2.1 to 3.2.6.

The ionic strength (section 3.2.2) of different commercial products (a carbonated soft drink, orange juice, soup stocks) was used as a guideline for sheep whey beverage development (Appendix 8).

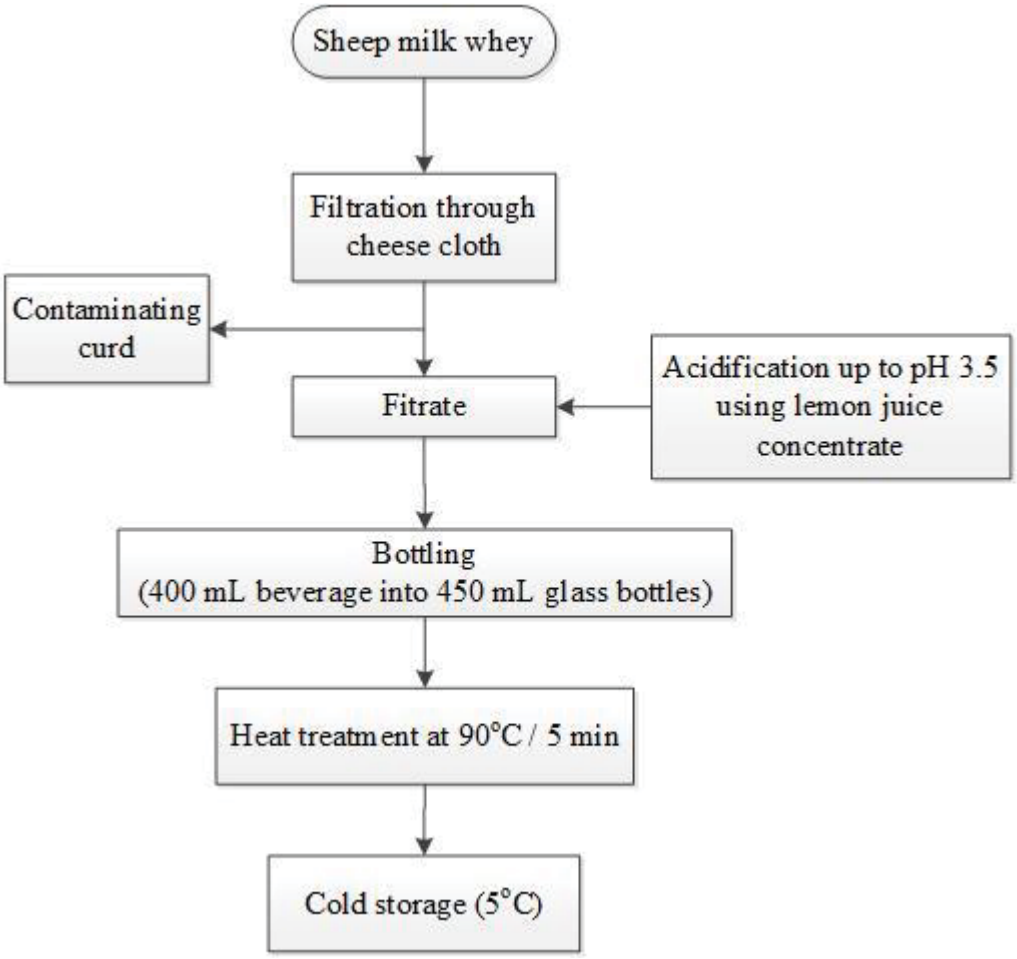


Figure 3.3: Sheep whey beverage – prototype product development

3.10.1 Stability of the whey beverage during cold storage

3.10.1.1. Laboratory scale study

Aliquots (15 mL) of the beverage at 20C were transferred into 20 mL glass tubes (with screw caps) and heat treated at 90°C for 5 min. The resulting sample tubes were immersed in ice water for 1 h immediately after the heat treatment. Temperature was measured, using a K-type thermocouple, at 30 s intervals in triplicate throughout the heating and cooling time. Then the beverage was stored overnight at 5°C and the stability of the beverage was studied based on sedimentation (by comparing the photographs taken after the immersion in ice and overnight cold storage, Figure 4.20).

3.10.1.2. Pilot scale study (in-bottle heat treatment)

Similarly, the stability of the beverage was studied following in-bottle heat treatment (90ºC for 5 min) using retail size glass bottles (400 mL of the beverage was hot filled in to 450 mL glass bottles with lug caps, immediately after the heat treatment) and stored overnight at 5ºC. Sedimentation was monitored by comparing photographs before and after the cold storage.

3.10.1.3. Pilot scale study (rapid heat treatment)

Additionally, an alternative rapid method of heat treatment of the beverage was studied. The beverage was first heat treated (90ºC for 5 min) in a stainless-steel bowl, bottled (bottles were sterilised in a water bath at 100ºC for 5 min before hot filling of the beverage) and immersed in water at room temperature (15ºC) for 1 h and stored at 5ºC. The stability of the product was determined based on the sedimentation during a 20 day storage period by comparing photographs taken after 1 h of cooling and with photographs taken at different time points during the cold storage.

3.11 Effect of holding time at heat treatment on properties of whey protein aggregates

Aliquots of the beverage (15 mL each) at 20ºC were transferred into 20 mL glass tubes (with screw caps) and dipped in a water bath set to 90ºC. One tube was taken out from the water bath every 2 min interval (up to 30 min) and immersed in ice for 1 h and photographed to compare the aggregates.

4. RESULTS AND DISCUSSION

4.1. Preliminary experiments

The focus of this research was to elucidate the suitability of sheep whey as a base ingredient in beverages. The composition of sheep whey and its variables have not been as extensively studied as cow whey (section 2.3). Therefore, a thorough understanding of the composition of sheep whey was required.

The physicochemical parameters of a commercial sheep whey stream sample were initially studied (Table 4.1). The initial whey sample collected for this study was the entire whey stream from a commercial cheese batch (approximately 300 L). The whey was collected off the dewheying vat into 20 L containers. It was visually obvious that each 20 L aliquot of whey was contaminated with curd fines. Further, by inspection, the level of curd contamination varied between aliquots. In order to manufacture a beverage it would be necessary to remove/reduce the degree of contaminating curd from the whey. Cheese cloth was used in preference to other techniques as this method was more likely available, due to economic reasons, to the sheep cheese manufacturers in New Zealand. Thus bulk whey containing contaminating curd particles were removed by filtering through a cheese cloth to obtain clean whey. It was not possible to analyse the level of curd contamination for the entire batch. However, a mass balance was performed on one 20 L aliquot. Contaminating wet curd for this sample accounted for 5.12% (w/v) of whey. Further, it was noted that cheesecloth filtration reduced fat content of the whey by up to 0.3%.

Due to the variation between aliquots of whey it was decided to remove the curd contamination variation from the current study by working with a model whey (section 4.2). The physicochemical variation examined in the model system were determined from a survey of composition variations of 7 commercial sheep whey streams from two manufacturers.

Table 4.1: Composition of a commercial sweet whey sample (from sheep milk)

Physicochemical parameters	**Composition**
Moisture (%)	93.5 ± 0.3
[1]Protein (%)	1.05 ± 0.08
Ash (%)	0.45 ± 0.01
Fat (%)	0.80 ± 0.09
Total soluble solids (Brix %)	9.7 ± 0.2
pH	5.93 ± 0.01
Conductivity (mS cm^{-1})	46.3 ± 0.2

[1]Protein = N% x 6.38. Data are presented as mean ± standard deviation of triplicate.

Moreover, heat treatment is a primary unit operation in whey beverage processing which ensures food safety throughout the product's intended shelf life (section 2.1.3). Specifically, an extended shelf life could be achieved through intense heat treatments (such as 90^{o}C for 5 min) of acidic beverage formulations (section 2.1.2). Further, beverage pH during the heat treatment affects denaturation of whey proteins, their aggregation, subsequent sedimentation (section 2.5.3) and thus the beverage stability. Therefore, effects of heat treatment (90^{o}C for 5 min versus conventional pasteurisation 72^{o}C for 15 s) at pH 3.5 and pH 4.5 on the stability of sheep whey were studied. The stability was determined based on turbidity (visual observations) of heated whey.

Turbidity changes were not clearly evident upon pasteurisation of whey at the two pH levels (Figure 4.1) and presumably produced stable whey. However, the whey heated at 90^{o}C for 5 min at pH 4.5 (C2) and at the dewheying pH of 5.9 (C3) showed an increase in turbidity comparative to the control samples (A2 and A3). In contrast the turbidity of whey heated at pH 3.5 (C1) remained nearly unchanged with respect to unheated whey (A1). Therefore, further experiments were developed based on these observations, as such to investigate effects of pH (pH 3.5 and 4.5) at the heat treatment (90^{o}C for 5 min) on different physicochemical properties of whey (section 4.4). The findings were related to determine the stability and hence the applicability of sheep whey as a base ingredient in beverage production.

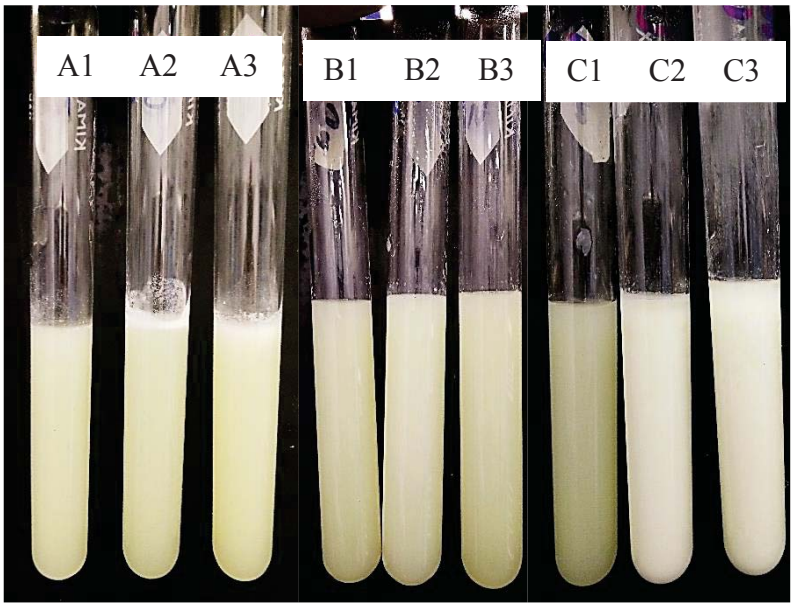


Figure 4.1: Effects of heat treatment and pH on turbidity of sheep whey. A – unheated whey, B – 72ºC for 15 s, C – 90ºC for 5 min, 1 – pH 3.5, 2 – pH 4.5 (pH adjusted via dropwise addition of 20% w/v lactic acid), 3 – pH 5.9 (natural pH of the whey)

4.2. Composition analysis of sheep milk, model whey and commercial whey streams

A main objective of this research was to identify key factors affecting the physicochemical composition of sheep whey. However, composition of cow whey is primarily determined by cheese processing conditions such as casein coagulation method and wheying pH (Fox *et al.*, 2000; McMahon and Oommen, 2013; Bonzanic *et al.*, 2014). Therefore, the effects of these two conditions on the composition of sheep whey were studied in the model systems. Four types of whey; one acid whey (at pH 4.6) and three rennet whey streams (at pH 5.7, 6.2 and 6.6), were produced using sheep milk (Figure 3.2). Composition of the sheep milk used in the model whey production is shown in Table 4.2. Sheep milk produced 5.2% (w/v) cream and 84.1% (v/v) skimmed milk upon skimming at 3000×g for 30 min at 35ºC. Casein coagulation, curd separation through cheesecloth filtration and subsequent draining for 1 h produced 77 – 81% (v/v) whey and 17 – 23% (w/v) wet curd (Figure 4.2). Dry weight of the curd was not considered as the curd production was not a concern in this study.

Table 4.2: Composition of whole sheep milk used in model whey preparation

Physicochemical parameters	Composition
Moisture (%)	85.49±0.06
[1]Protein (%)	5.03±0.02
Fat (%)	5.62±0.03
Minerals (ash) (%)	0.82±0.01
Ca (mmol L^{-1})	36.8±0.5
Mg (mmol L^{-1})	2.2±0.1
pH	6.6±0.0
Total soluble solids (Brix%)	7.1±0.0
Conductivity (mS cm^{-1})	25.6±0.4

[1]Protein = N% x 6.38. Data are presented as mean ± standard deviation of triplicate.

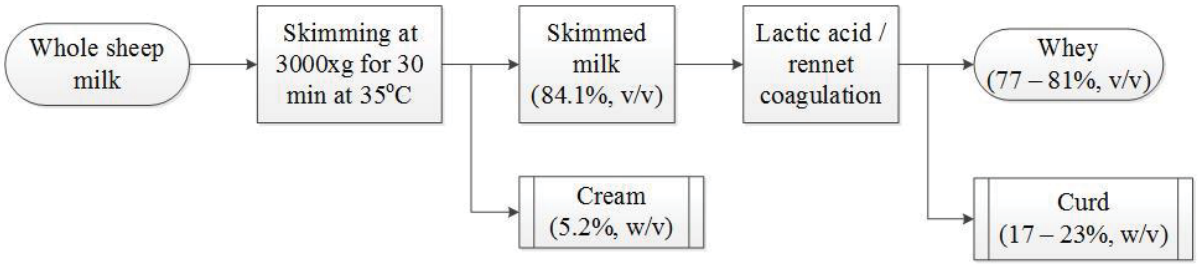


Figure 4.2: Sheep whey production process flow diagram

Physicochemical compositions of the model sheep whey streams are shown in Table 4.3. Protein contents of the three rennet whey samples were higher than the acid whey. The rennet whey produced at pH 6.6 had the highest mean protein content. Similar phenomenon in protein contents of cow whey have been reported by other researchers. For instance, Pesta *et al.,* (2007) reported 0.75% and 0.3% protein levels in rennet whey and acid whey, respectively. Further, comparatively higher protein content of rennet whey (1.0%) than acid whey (0.8%) was cited by Panesar *et al.* (2007). Rennet activity on κ-casein, i.e. cleavage of phenylalanine$_{105}$–methionine$_{106}$ peptide bond (O'Brien and Guinee, 2010), and thus the release of caseino-macropeptide (CMP) (C terminal 106 – 169 peptide residues) is evident in rennet coagulated cheese or casein production (Fox *et al.*, 2000; Panesar *et al.*, 2007; Ledesma *et al.,* 2011 and Bonzanic *et al*., 2014). Hydrophilic CMP (Neelima *et al.*, 2013) dissolves in the aqueous phase

of milk and consequently drains off with whey. Such peptides in rennet whey increases the total N content and hence the total protein content in the respective whey streams. Therefore, presence of CMP in rennet whey might be the primary reason for its higher total protein content. However, quantification of CMP in the sheep whey samples was not an objective of this experiment.

In contrast, acid whey was high in minerals (ash), total soluble solids and conductivity, comparative to the three rennet whey samples (Table 4.3). Likewise, Smithers (2015) reported ash contents in cow whey such as 0.8% in acid whey and 0.5% in sweet whey. Further, Bonzanic *et al.* (2014) revealed higher amounts of Ca (150.0 – 200.0 mmol L^{-1}) in acid whey with resepect to Ca (10.0 – 15.0 mmol L^{-1}) in sweet whey. Additionally, Ca (12.0 mmol L^{-1}) and Mg (2.4 mmol L^{-1}) in paneer whey (acid whey) were higher than the Ca (7.3 mmol L^{-1}) and Mg (1.5 mmol L^{-1}) in cheese whey (rennet whey) made from buffalo milk (Goyal and Gandhi, 2009). Regardless the source of milk, acid whey in both the studies showed characteristic high mineral contents with respect to rennet whey. This is perhaps due to increased solubility of colloidal minerals in casein micelles at acidic pH (about pH 4.6) (Park and Haenlein, 2008 and Bonzanic *et al.*, 2014). pH of the milk is reduced to the isoelectric pH of casein (i.e. pH 4.6) to induce acid coagulation of casein (Fox *et al.*, 2000; O'Mahony and Fox, 2013). Therefore, dissolved colloidal minerals at the acidic pH partitions into the whey and consequently produces an acid whey with higher mineral contents. Furthermore, high conductivity (ionic strength) of the model acid whey sample (Table 4.3) supports the above findings.

Table 4.3: Composition of the model sheep whey streams

Constituent/ parameter	Lactic acid coagulation	Rennet coagulation		
	pH 4.6	pH 5.7	pH 6.2	pH 6.6
Moisture (%)	91.74±0.01[a]	90.26±0.02[b]	91.19±0.01[c]	92.54±0.01[d]
[1]Protein (%)	0.92±0.01 [a]	1.26±0.01 [b]	1.29±0.01 [c]	1.33±0.01 [d]
Fat (%)	0.03±0.00 [a]	0.05±0.00 [b]	0.04±0.01 [b]	0.05±0.00 [b]
Minerals (ash) (%)	0.96±0.00 [a]	0.65±0.00 [b]	0.58±0.01 [c]	0.56±0.00 [c]
Ca (mmol L^{-1})	37.4±0.3 [a]	15.0±0.0 [b]	10.3±0.3 [c]	8.1±0.1 [d]
Mg (mmol L^{-1})	7.1±0.1 [a]	4.8±0.0 [b]	3.8±0.0 [c]	3.6±0.1 [d]
Total soluble solids (Brix%)	8.3±0.0 [a]	8.0±0.0 [b]	6.3±0.0 [c]	7.7±0.0 [d]
Conductivity (mS cm^{-1})	50.8±0.2 [a]	40.0±0.1 [b]	30.1±0.3 [c]	34.2±0.3 [d]

[1]Protein = N% x 6.38. Data are presented as mean ± standard deviation of triplicate. Values with different superscripts in the same row (a, b, c and d) differ significantly ($P < 0.05$). Model whey streams produced at pH 4.6, 5.7, 6.2 and 6.6 will be abbreviated as L4.6, R5.7, R6.2 and R6.6, respectively, here after.

Key composition differences between rennet whey and acid whey (high protein and low minerals in rennet whey with respect to acid whey) used in the model study are similar the published data of cow whey. However, the proteins, fat and mineral contents showed some deviations from the literature. This is perhaps due to the differences in milk composition (cow milk versus sheep milk) and cheese processing conditions (section 2.3) (Fox *et al.*, 2000; Park *et al.*, 2007; Britz and Robinson, 2008; Park and Haenlein, 2008; Ledesma *et al.*, 2011; Hejtmankova *et al.*, 2012; O'Mahony and Fox, 2013 and Bonzanic *et al.*, 2014).

The data on the composition of sheep whey from the commercial whey streams (seven types of whey collected from two sheep cheese producers) is shown in Table 4.4. The results show variations between the two sheep cheese manufacturers. The compositional differences in the commercial whey samples illustrate the variation that is possible and highlights the importance of understanding the impact of such variation on heat processing.

Table 4.4: Composition of commercial sheep whey streams

Constituent	Commercial whey samples						
	Manufacturer 1		Manufacturer 2				
	[2]A(R)	B(R)	C(R)	D(R)	E(L)	F(R)	G(L)
Moisture (%)	91.48±0.01	92.44±0.01	92.54±0.03	91.51±0.00	92.24±0.00	91.89±0.00	92.05±0.00
[1]Protein (%)	1.16±0.01	1.32±0.01	1.19±0.00	1.85±0.01	1.56±0.00	1.49±0.01	1.54±0.00
Fat (%)	0.31±0.00	0.31±0.00	0.32±0.00	0.62±0.01	0.14±0.00	0.66±0.00	0.45±0.00
Ash (%)	2.40±0.06	0.60±0.01	0.51±0.03	0.53±0.03	0.97±0.01	0.49±0.00	0.84±0.00
Ca (mmol L^{-1})	12.0±0.1	9.7±0.1	9.1±0.4	6.7±0.3	49.1±0.0	11.1±0.0	43.0±0.0
Mg (mmol L^{-1})	3.8±0.2	3.5±0.1	3.3±0.0	2.3±0.3	11.4±0.1	3.5±0.1	9.7±0.2
pH	6.16±0.00	6.25±0.01	6.19±0.01	6.57±0.01	4.32±0.00	4.48±0.02	4.41±0.02
[3]Wheying pH	6.30	6.48	6.58	6.83	4.8	6.65	4.73
Total soluble solids (Brix%)	9.9±0.0	7.6±0.0	8.3±0.0	7.1±0.0	8.1±0.0	8.4±0.0	8.7±0.0
Conductivity (mS cm^{-1})	193.6±0.8	32.9±0.3	33.6±0.0	32.5±0.0	55.3±0.4	36.5±0.1	56.8±0.1

[1]Protein = N% x 6.38. A – G: commercial whey. [2] salting milk before coagulation (details obtained from the manufacturer). [3]Wheying pH values as provided by respective manufacturer. R – rennet whey, L – acid whey. Data are presented as mean ± standard deviation of triplicate.

Understanding the relationship between composition data of model sheep whey streams (Table 4.3) and processability could enable manufacturers to either predict key cheese processing conditions to produce a whey stream suitable for beverage processing or to predict whether a given whey stream is suitable for beverage processing. For instance, rennet whey often contains comparatively high protein content compared to acid whey (Table 4.3 and Goyal and Gandhi, 2009). However, this was not found to be true for all of the commercial samples where the protein content of the rennet whey sample F obtained from the manufacturer 2 was lower than the acid whey samples E and G from the same manufacturer. High temperature heat treatment (at 90^{o}C for 2 min or greater holding time) of milk in cheese processing assists denaturation of whey proteins, complexation with casein and thus increase in yield (Jelen, 2011) and smooth texture of cheese (O'Brien and Guinee, 2010). Such a heat treatment of the cheese milk would, however, produce a whey with low whey protein content and could be a reason for the low protein content reported in whey sample F. Due to commercial sensitivity such details around processing were not available.

Interestingly the whey from manufacturer 2 contained a comparatively high amount of fat than the whey collected from manufacturer 1 with whey stream E containing the lowest fat content among the seven commercial whey samples. Fat content of whey is determined by quality control within cheese processing. In elaboration, heat treatment of milk at 82^{o}C for 26 s or greater denatures more than 25% proteins in milk and hinders its gelation upon renneting. The denaturation subsequently impairs the amount of fat that can get entrapped in casein curd (O'Brien and Guinee, 2010). In addition, curd cutting parameters such as curd particle size and strength of the curd at cutting also influence the fat loss from cheese and thus the fat content in whey. Syneresis (loss of whey) from smaller curd particles is comparatively higher and so is production of smaller curd particles is deliberately used in the production of low moisture cheese varieties. However, such small particles also enable high loss of fat from the curd (Park and Haenlein, 2008). Therefore, differences in heat treatment of cheese milk and curd cutting conditions might yield whey streams with varying fat contents.

Additionally, ash (0.49%), Ca (11.1 mmol L^{-1}) and Mg (3.5 mmol L^{-1}) of the sample F (rennet whey) were lower than those in samples E and G (two acid whey streams). Similar characteristics in mineral composition were observed in model sheep whey streams (Table 4.3 and Figure 4.3). Rennet coagulation of casein at higher pH results in whey with less minerals due to the reduced solubility of colloidal minerals at the respective pH range (section 2.3.2). It

is evident that the whey sample F has been produced at a higher pH and thus is probably a rennet whey. This was confirmed by the details obtained from the manufacturer on wheying pH which was pH 6.65. However, according to the physicochemical analysis, pH of whey stream F was pH 4.48 (Table 4.4) The discrepancy in pH is possibly caused by the activity of starter microorganisms in whey (which were used in the cheese processing and likely drained off with whey) along the prolonged standing at room temperature (or wheying temperature) before freezing thus enabling fermentation of lactose in the whey to lactic acid.

Furthermore, whey sample-A showed an exceptionally high amount of ash and conductivity. The reason for the high mineral content was that this whey had been produced from a cheese in which the cheese milk itself was salted rather than salting of the dewheyed curd. Thus the excess salts likely leached in to the whey stream and so explain the high ash content and high conductivity (ionic strength) in the sample. Ionic strength of the whey is important as this influences the properties of whey protein aggregates upon heat treatment (section 2.5.4), which is a key unit operation in whey beverage processing (sections 2.1.2 and 2.1.3). Hence, effects of added NaCl on the stability of whey was studied in the model system to understand the suitability of salty whey in beverage production (discussed in section 4.5).

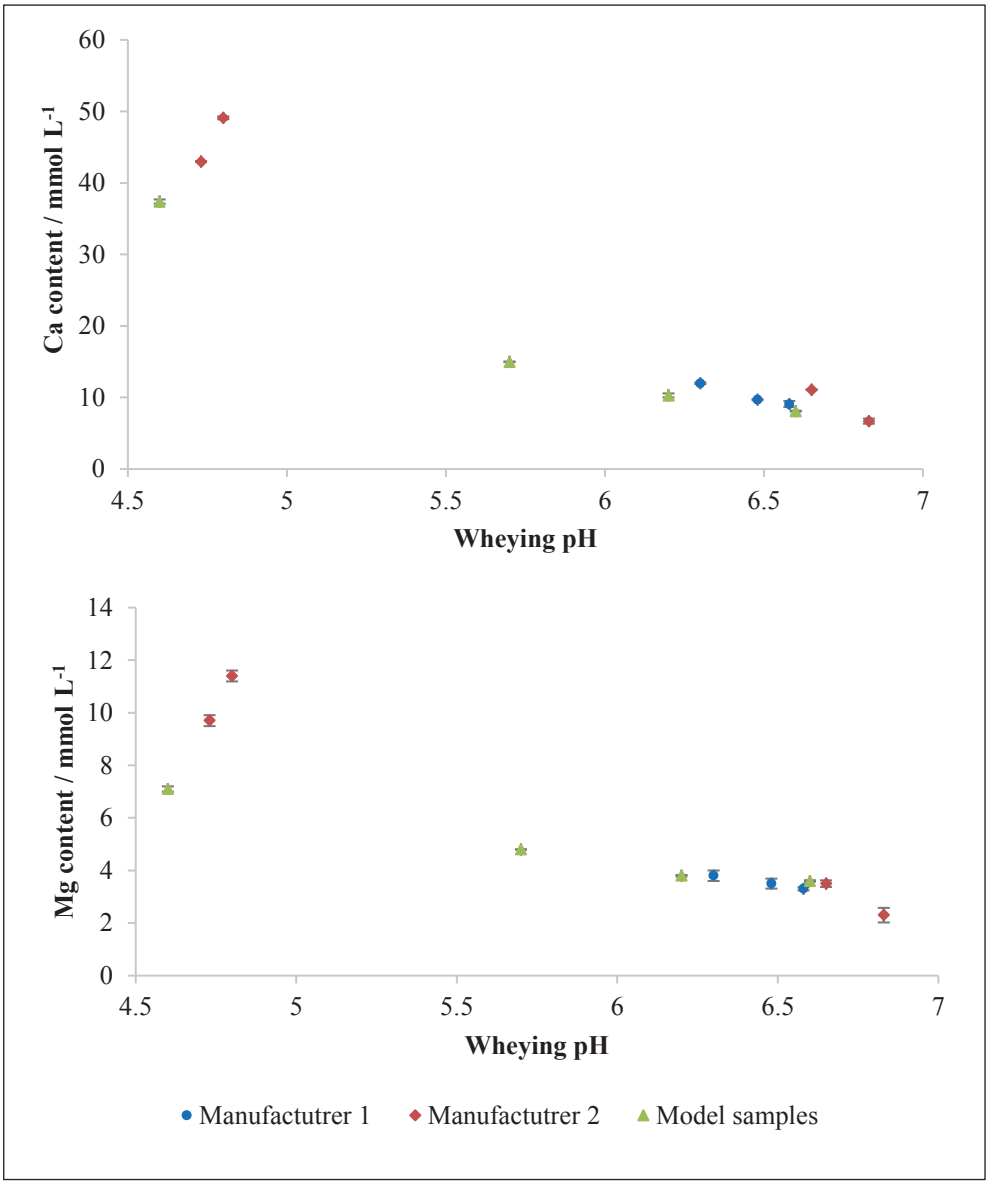


Figure 4.3: Effect of wheying pH on Ca and Mg contents of whey. Error bars represent standard deviation of triplicate.

Aside from characterising components likely to have an impact on the processing of a beverage, a preliminary NMR spectroscopy study was made of the minor components of the sheep whey. Information on minor components may provide important nutritional data that could assist with marketing a beverage. The small molecular metabolite composition of a pooled sheep whey sample as determined by NMR spectroscopy is shown in Figure 4.4 and Table 4.5. Sugars (lactose, galactose) and amino acids (leucine, tyrosine, valine, phenylalanine, taurine) are some of the minor constituents in the sheep whey which are known for nutritional and/or bioactive functionalities. Nevertheless, further studies on such compounds were not carried-out due to time limitations of this project.

The main difference in acid whey and rennet whey was evident in lactate and citrate concentrations (Figure 4.5). Lactate content increased with increasing pH at whey production, with respect to the added lactic acid, and thus reported the highest in acid whey sample. However, citrate content decreased with the increasing pH at whey separation. Citrate in casein micelles has reported increased solubility with acidification (Graet and Gaucheron, 1999 and Attia *et al.*, 2000) and was contradictory with the findings.

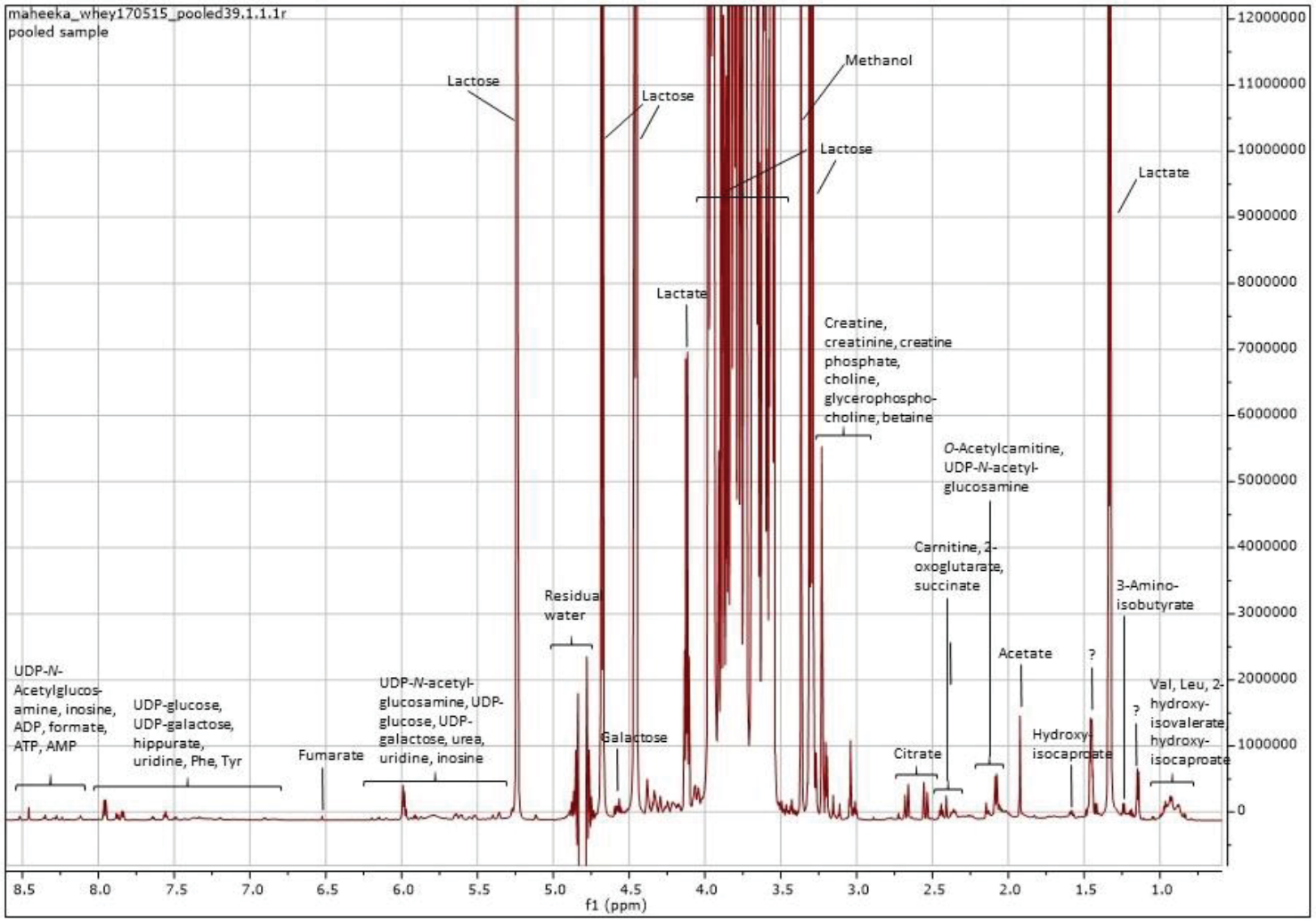


Figure 4.4: ^{1}H NMR spectrum of pooled whey (acid and rennet whey including 4 model whey and 7 commercial whey steams)

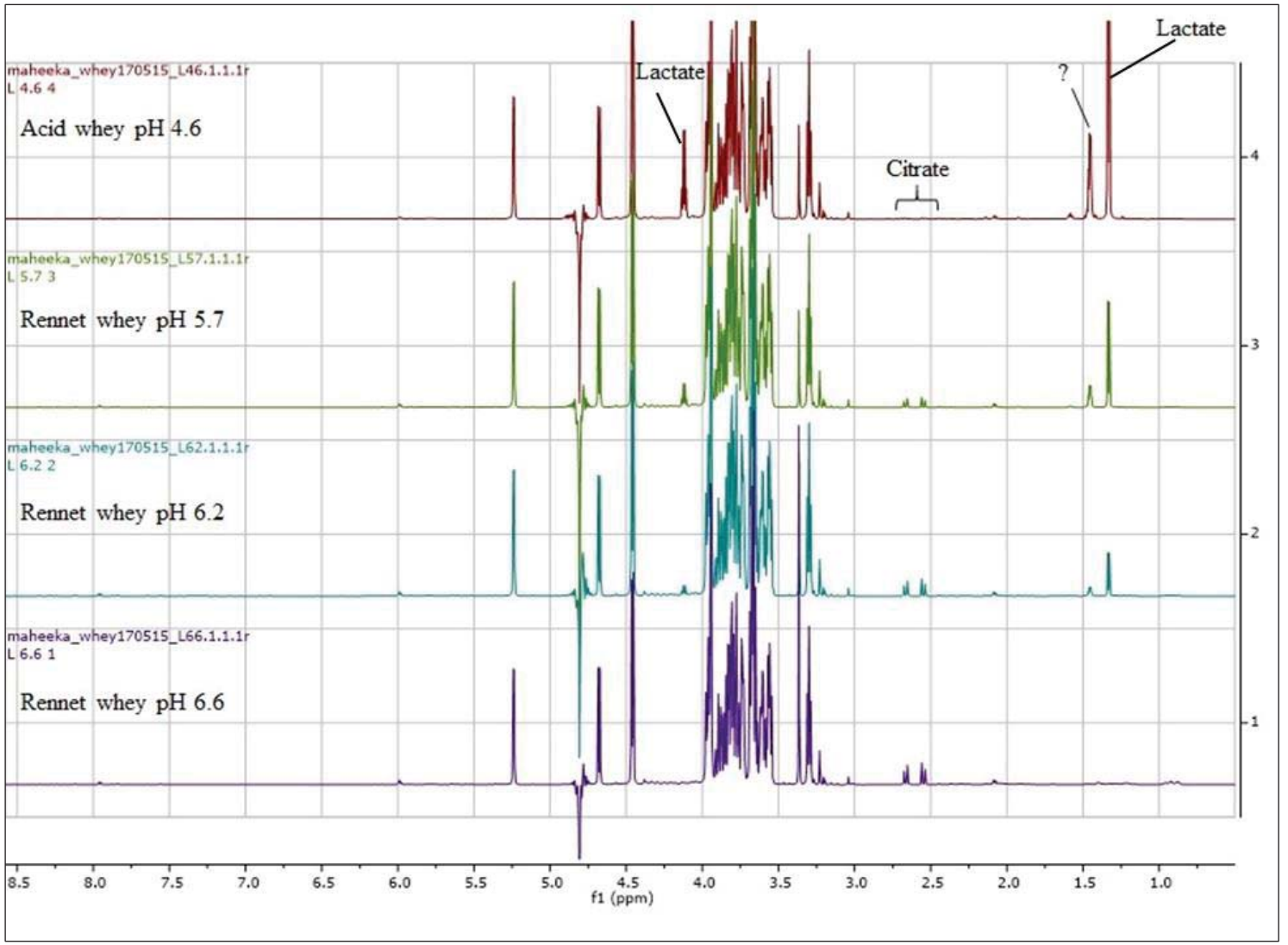


Figure 4.5: ^{1}H NMR spectrums of acid and rennet sheep whey

Table 4.5: Small molecular metabolites in pooled sheep whey

Metabolite	**Concentration (mM)**
Lactose	25.7
Lactate	7.5852
Methanol	5.1118
Urea	1.3213
Betaine	0.0255
sn-Glycero-3-phosphocholine	0.285
Galactose	0.2665
Acetate	0.2348
O-Phosphocholine	0.0399
Creatine phosphate	0.0376
Creatine	0.1114
UDP-glucose	0.1039
Taurine	0.0905
Formate	0.1269
UDP-galactose	0.1098
Carnitine	0.0700
Creatinine	0.0317
2-Oxoglutarate	0.1078
UDP-*N*-Acetylglucosamine	0.082
Choline	0.0188
Leucine	0.0352
2-Hydroxyisocaproate	0.028
3-Aminoisobutyrate	0.0271
Uridine	0.0326
O-Acetylcarnitine	0.0306
Succinate	0.029
ATP	0.0047
Dimethyl sulfone	0.0225
ADP	0.0048
Benzoate	0.0259
Hippurate	0.0669
2-Hydroxyisovalerate	0.0214
Inosine	0.0204
Citrate	0.2089
Tyrosine	0.0184
Fumarate	0.0139
Valine	0.0131
AMP	0.0049
Phenylalanine	0.0106
Dimethylamine	0.0079
trans-Aconitate	0.008

4.3. Effect of storage pH on Ca and Mg migration from contaminating curd in whey

Curd contamination was a key issue identified in the preliminary study of commercial whey (section 4.1). Colloidal minerals in casein micelles dissolve in acidic pH and produce whey with high mineral contents (section 2.3.2). Therefore, solubilisation of Ca and Mg in the contaminating curd (depending on storage pH) and their subsequent migration to whey during the storage were studied. The effect of storage pH (pH 4.5, 4.0 and 3.5) on mineral migration from contaminating curd in whey during overnight storage at 5°C were investigated in a model system. 5% (w/v) curd contamination was used based on the findings from the preliminary experiments (section 4.1). Model sheep whey prepared by rennet coagulation at pH 6.6 (i.e. pH of the sheep milk) (Figure 3.2) and its resulting curd were used for the study. pH 6.6 was chosen for the curd formation as higher levels of mineral retention occurs in casein curd prepared at higher pH (O'Mahony and Fox, 2013) and thus the maximum mineral concentration in contaminating curd was used to study its significance. Results of the experiment are summarised in Figure 4.6.

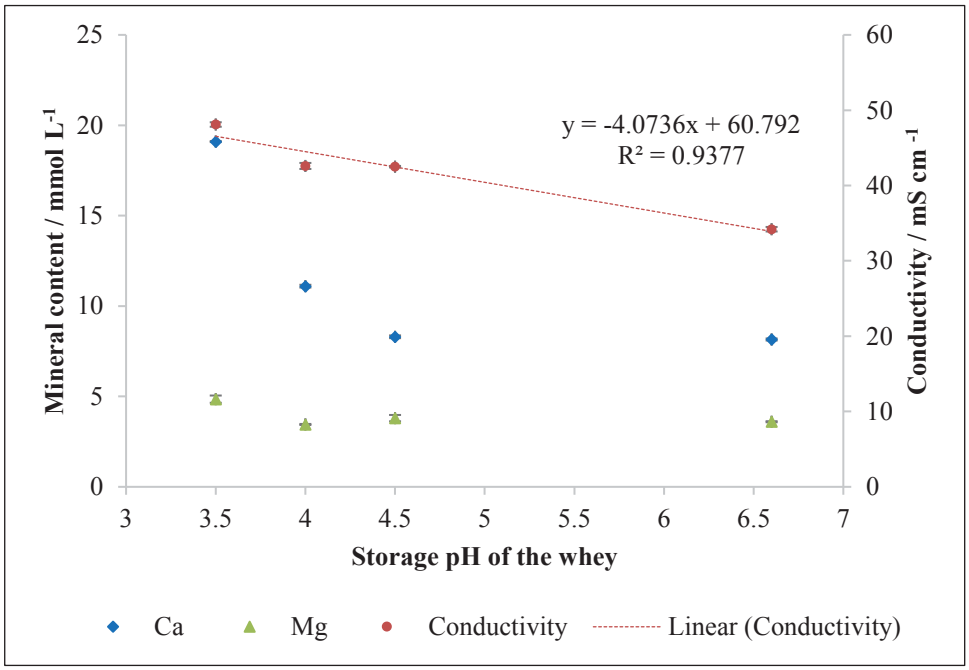


Figure 4.6: Effect of storage pH on mineral content of whey with contaminating curd. Error bars represent standard deviation of triplicate.

Storage at pH 4.5 had no effect on the Ca content in whey and presumably the solubility of Ca in contaminating curd. However, the Ca level in whey increased during storage at pH 4.0 and the Ca concentration in the whey was more than twice as high at the lowest pH measured i.e.

pH 3.5. In contrast, there was no significant change in the Mg level over the entire pH range studied. Therefore, contaminating curd only affects the mineral content of whey when stored at pHs lower than pH 4.5.

All of the samples heat treated at pH 3.5 were heat stable. Interestingly even the lactic acid whey containing 37 mmol L^{-1} Ca (Table 4.3) was stable during heat treatment at pH 3.5 (detailed in section 4.4). As the curd contamination model study was based on a 5% level of contamination and this only resulted in a highest Ca content of 19.1 mmol L^{-1} it is likely, based on a linear extrapolation that a whey containing 10% (w/v) curd would also be heat stable. An additional benefit of this finding is that drinks with increased mineral contents are possible which may increase the nutritional appeal of sheep whey drinks particularly as a base ingredient for electrolyte whey drinks.

The conductivity of the whey increased in a linear fashion (R^2=0.93) with increasing acidic pH. The trendline shown in Figure 4.6 under predicts slightly the data point at pH 3.5 which is probably due to the increased solubility of calcium, however the likely error in this region is in the order of 2-3 mS and, in light of the work reported in section 4.5, not of significance from a processing point of view.

4.4. Effect of pH at heat treatment on stability of whey

It was evident from the literature (section 2.5.3) that the pH at the point of heat treatment influences cow whey protein aggregation and thus it was assumed to also affect the stability of sheep whey. The heat stability of sheep whey was determined based on particle size, turbidity, colour, sedimentation and loss of SDS – monomeric whey proteins before and after the heat treatment of 90^{o}C for 5 min at pH 3.5 and pH 4.5 using the method described in section 3.7.

4.4.1.Particle size

Whey heated at pH 3.5 had multimodal particle size distributions while those heated at pH 4.5 had bimodal distributions (Appendix 1). However, the samples heated at pH 4.5 had large amounts of visible sediment and these visibly large particles were unable to be measured using the Mastersizer light scattering technique. Thus a discussion of the particle size data for these samples is meaningless.

4.4.2.Turbidity

Although heat treatment increased the turbidity of the whey samples with respect to the turbidity of unheated samples (Appendix 2), the initial turbidity of the samples was so high that the turbidity method was unable to provide useful information on characterizing changes in stability.

4.4.3.Colour

The colour of whey (Figure 4.7, Appendix 3, Appendix 4) was significantly affected by heat treatment depending on the pH with prominent effects observed on L value. Heating at pH 4.5 increased the L value of both the model and commercial whey steams (Figure 4.7). Changes in colour were likely dominated by whey protein aggregation during the heat treatment (section 2.5) and subsequent changes in particle size distribution (section 2.1.3). In contrast, heating at pH 3.5 had no significant effect on the L value with respect to the unheated whey in all cases except for commercial sample A. The general observation is in keeping with the literature of bovine whey where Jelen (2011) reported that the whey proteins develop resistance to heat below pH 3.6 (section 2.5.3). Therefore, heat treatment (90^{o}C for 5 min) of whey at pH 3.5 would yield more stable whey.

The exception, the commercial whey-A, in contrast experienced a significant increase in the L value when heated at pH 3.5. The only compositional factor that differed for this whey stream was ionic strength which was comparatively very high (i.e. conductivity 193.6 mS cm^{-1} compared to 30 to 56 mS cm^{-1} ; Table 4.4). Ionic strength is known (section 2.5.4 and O'Brien and Guinee, 2010) to alter the thermal aggregation behaviour of whey proteins. It was therefore hypothesised that ionic strength was responsible for the colour changes and aggregation observed on heating commercial sample A at pH 3.5.

From a beverage manufacturers point of view, it would be important to know whether any given whey stream or whey beverage formulation would be stable on processing. Therefore a systematic study of the effects of added salts on the stability of whey during heat treatment at pH 3.5 was undertaken (section 4.5).

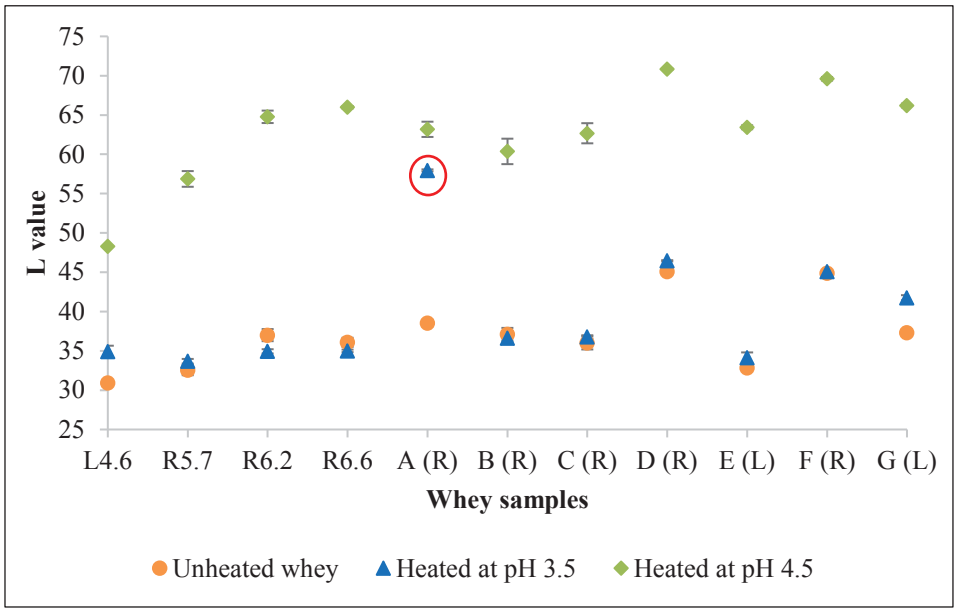


Figure 4.7: The change of L value (L = 0 – black, L = 100 – white) of whey as affected by the pH at heat treatment (90°C for 5min). L – acid whey, R – rennet whey, A – G - commercial whey. Error bars represent standard deviation of triplicate. Exception of A, heated at pH 3.5 is marked in the red circle.

4.4.4.Sedimentation

Stability of whey heat treated at pH 3.5 and 4.5 was also determined based on sedimentation after standing overnight in cold storage (5°C). This test was designed to mimic the sedimentation that might occur on normal shelf life. Sediment curd % was calculated according to the Equation 1.

Equation 1: $$\boldsymbol{Sediment\ curd\%} = \frac{\boldsymbol{curd\ height\ (cm)}}{\boldsymbol{total\ liquid\ height\ (cm)}} \times \mathbf{100}$$

Whey heated at pH 3.5 remained generally stable during the overnight storage, although a colour gradient was observed along the sample tube from being clear and transparent (at the top) to cloudy yellow (at the bottom) (Figure 4.8: a) indicating that some sedimentation was occurring. Whey sample A, on the other hand produced large volumes of sediments even when heated at pH 3.5 and thus was unstable (Figure 4.8, b). The results probably explain the deviation of L value on the same sample in Figure 4.7.

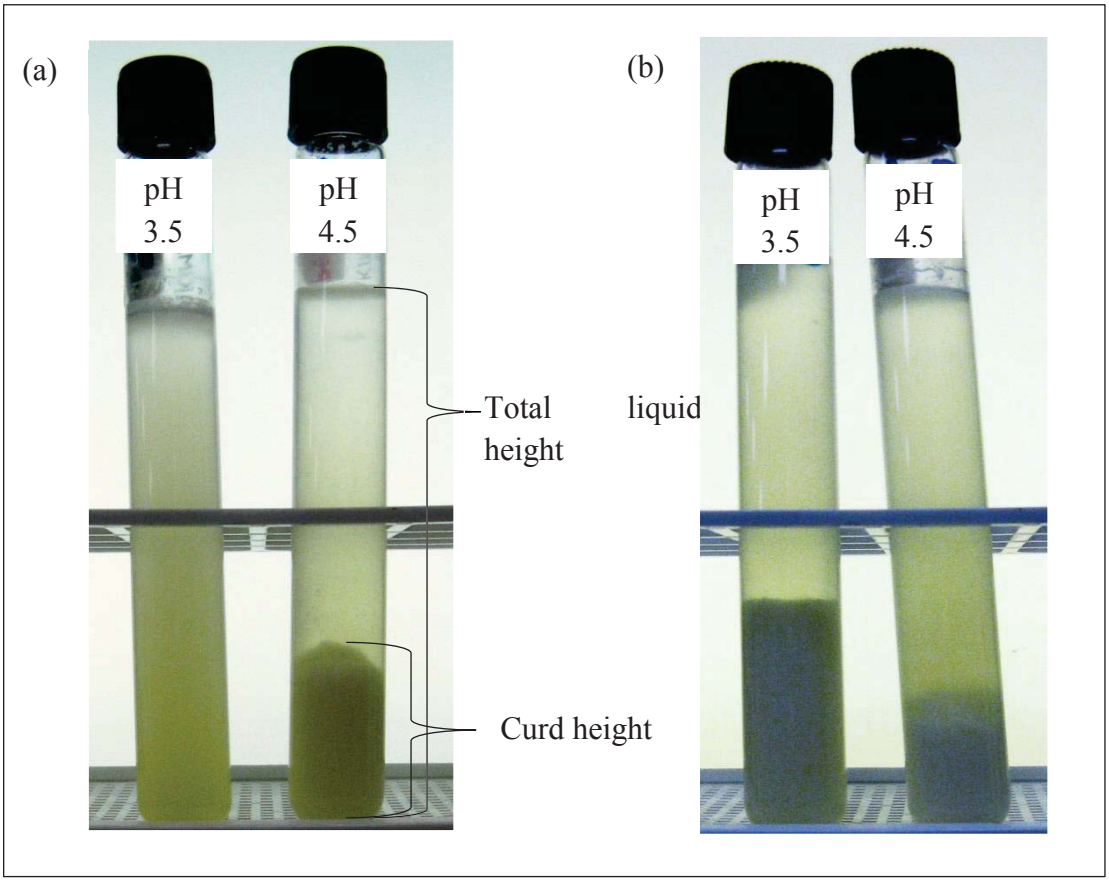


Figure 4.8: Sedimentation of heated (90ºC for 5min) whey after overnight storage at 5ºC; (a) commercial whey B and (b) commercial whey A.

Whey stream-A produced powdery aggregates after the heat treatment comparative to the lumpy cohesive aggregates observed in the other heated whey. Powdery aggregates produced a clear demarcation in between the serum phase and sediments and probably a result of compact settling. This observation added to the impetus to elucidate the effect of added salt on the heat stability of whey (section 4.5).

To gather an insight into the nature of the sedimentable material, the model heated whey samples were centrifuged (after the overnight storage) at increasing speeds from 0×g to 1000×g for 5 min at each speed and the results are presented in Figure 4.9.

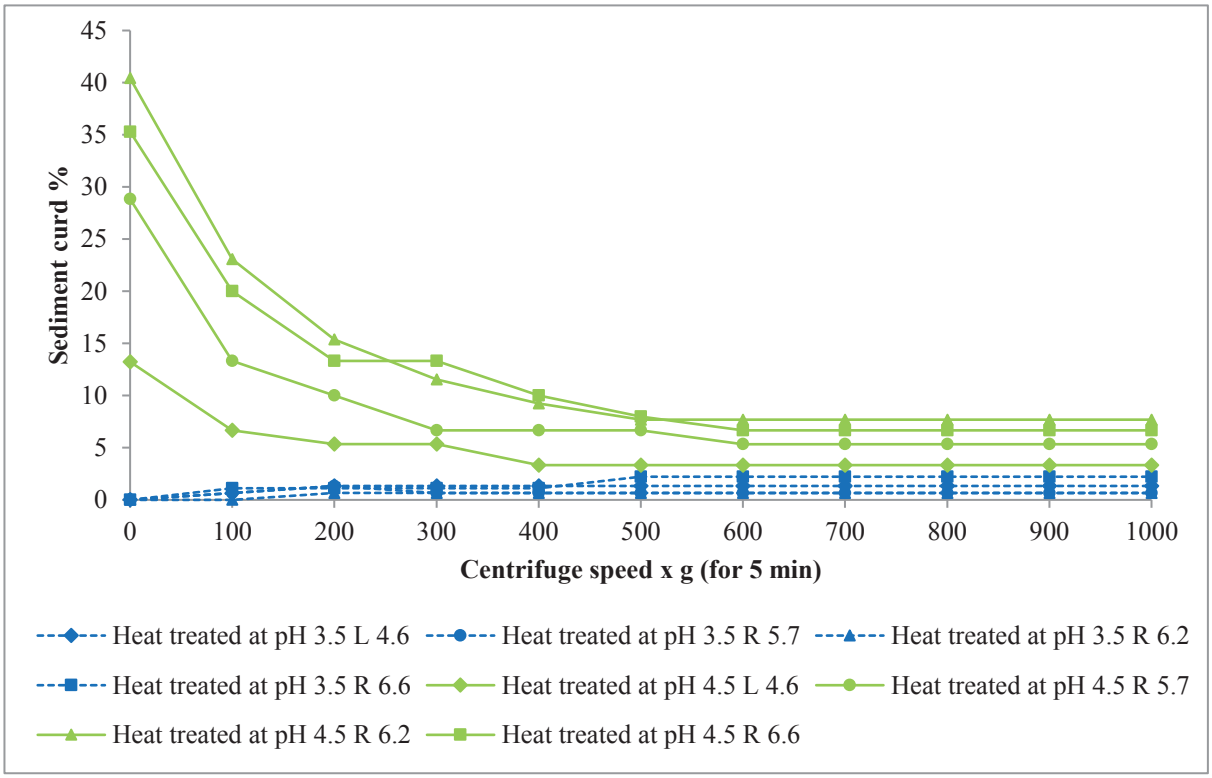


Figure 4.9: Effect of centrifugation on sedimentation of heated model whey streams (L 4.6 - acid whey and R 5.7, 6.2 and 6.6 – rennet whey, produced at pH 4.6, 5.7, 6.2 and 6.6, respectively).

The model acid and rennet whey demonstrated similar behaviours upon heat treatment at pH 3.5 with sedimentation less than 1% (Figure 4.9). However, centrifugation of the pH 3.5 treatment at 500×g increased sediments up to 3.3% which was likely due to settling of small suspended aggregates or casein residues. In comparison, heat treatment at pH 4.5 produced 13.2 – 40.4% sediments which compressed to 3.3 – 7.7% with centrifugation at 600×g indicating that the sedimented material has a relatively low density. Inspection of the sediment itself indicated that the heat treatment at pH 3.5 and pH 4.5 produced two types of particles. For instance, heating at pH 3.5 probably produced aggregates smaller than 1µm range and thus remained in colloidal suspension (section 2.1.3, Damodaran *et al.*, 2008).

The commercial whey streams showed similar behaviours during the heat treatments (Figure 4.10 and Figure 4.11) with the exception in sample A.

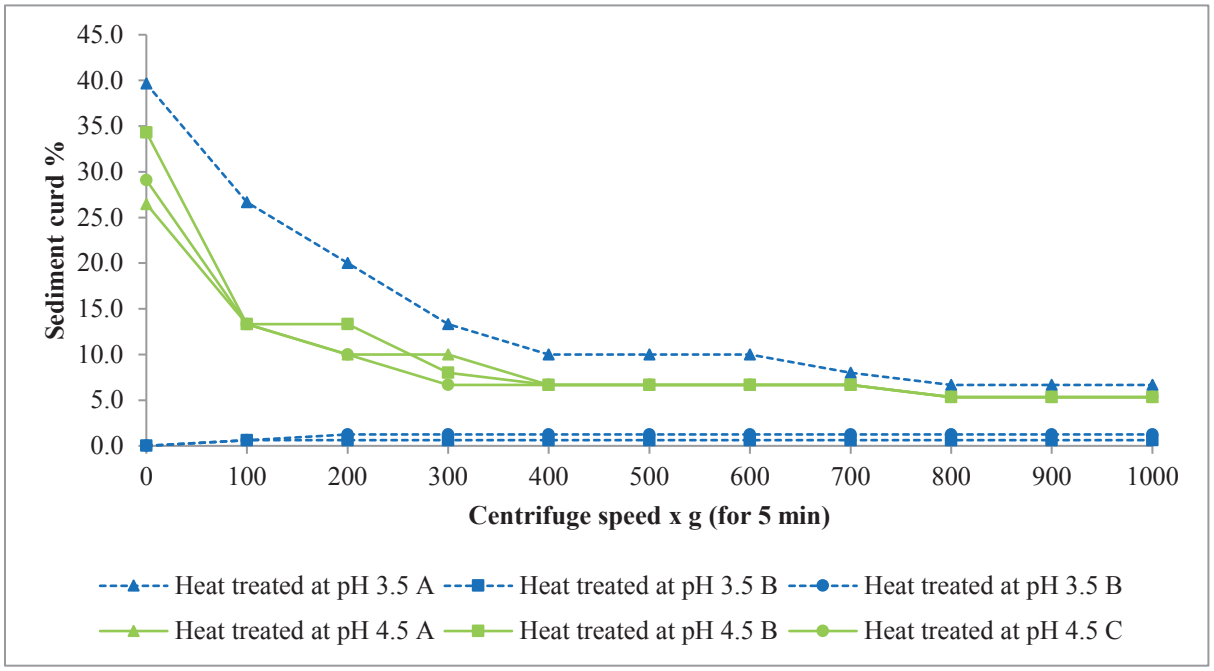


Figure 4.10: Effect of centrifugation on sedimentation of heated whey (A, B, and C) obtained from manufacturer 1.

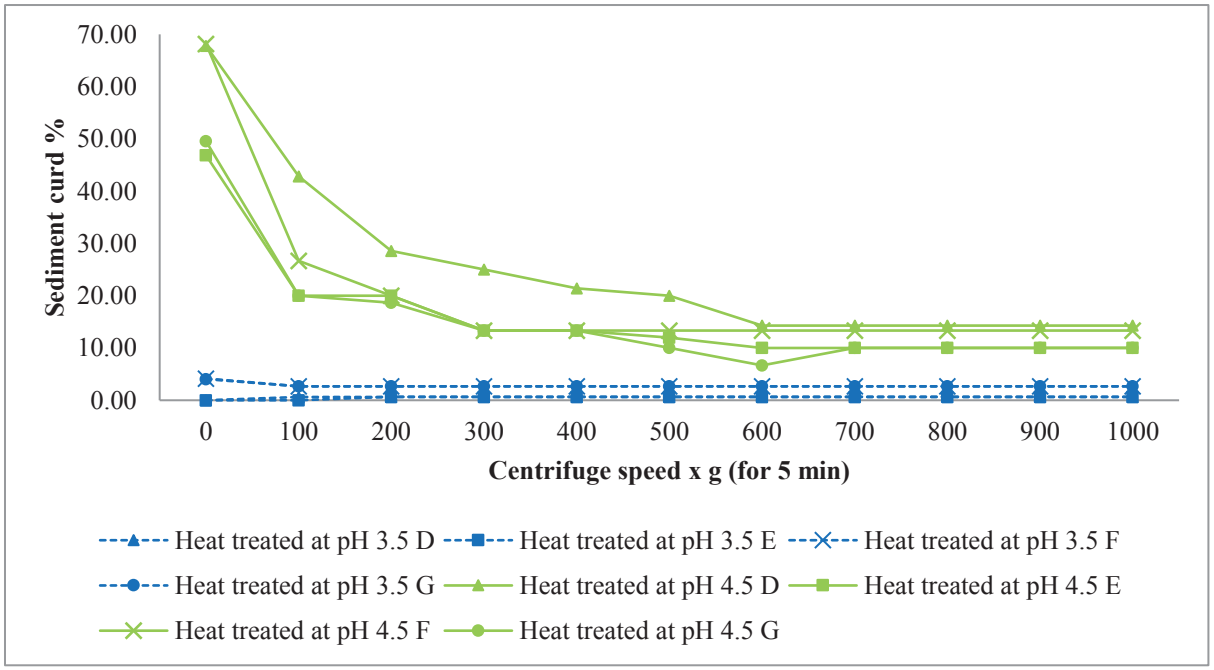


Figure 4.11: Effect of centrifugation on sedimentation of heated whey (D, E, F and G) obtained from manufacturer 2

4.4.5. Loss of monomeric whey proteins

Heated whey was further analysed using SDS – PAGE (section 3.2.8) to determine effects of pH (pH 3.5 and 4.5) and heat treatment (90ºC for 5 min) on the loss of monomeric whey proteins, protein aggregation, sedimentation and subsequent stability of the sheep whey.

According to the SDS – PAGE, heated whey showed less intensities in protein bands than those in the unheated whey (Figure 4.12 and Figure 4.13). Whey heated at pH 4.5 showed the least intensities for whey protein bands and perhaps explains the heat induced aggregations and subsequent loss of individual whey proteins from the serum phase. Further, the results support the findings discussed under sections 4.4.3 and 4.4.4. Additionally, commercial whey streams heat treated at pH 4.5 also showed a comparatively high loss of monomeric whey proteins from the serum phase (Figure 4.14) nevertheless with a wide variation among the samples. The variation perhaps caused by the diverse compositions (Table 4.4) of the commercial whey samples and their respective effects on heat induced whey protein aggregation (section 2.5). For Sample-A, for instance, the difference in residual whey proteins among the two pH levels were less than 10% compared to 10 to 60% for other samples (Figure 4.14). This supports the deviated results revealed in colour (section 4.4.3) and sedimentation (section 4.4.4) analyses.

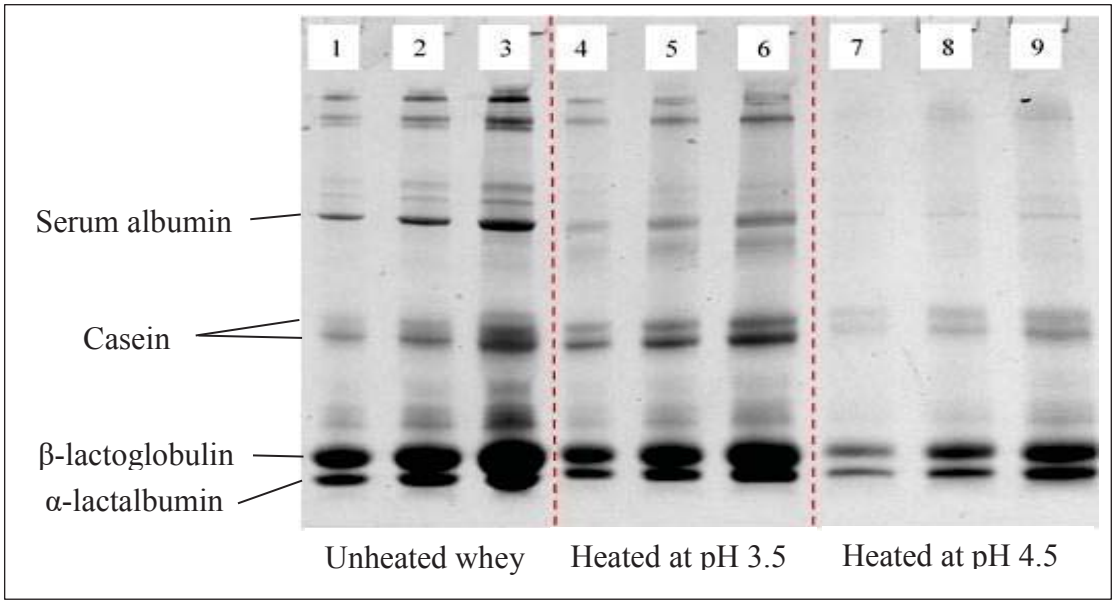


Figure 4.12: The effect of pH at heat treatment (90ºC for 5 min) on loss of SDS – monomeric whey proteins in model rennet whey (R6.6). Whey serum % (v/v) in loading samples were 0.05 % - lane 1, 4 and 7, 0.1% - lane 2, 5 and 8, and 0.2% - lane 3, 6 and 9.

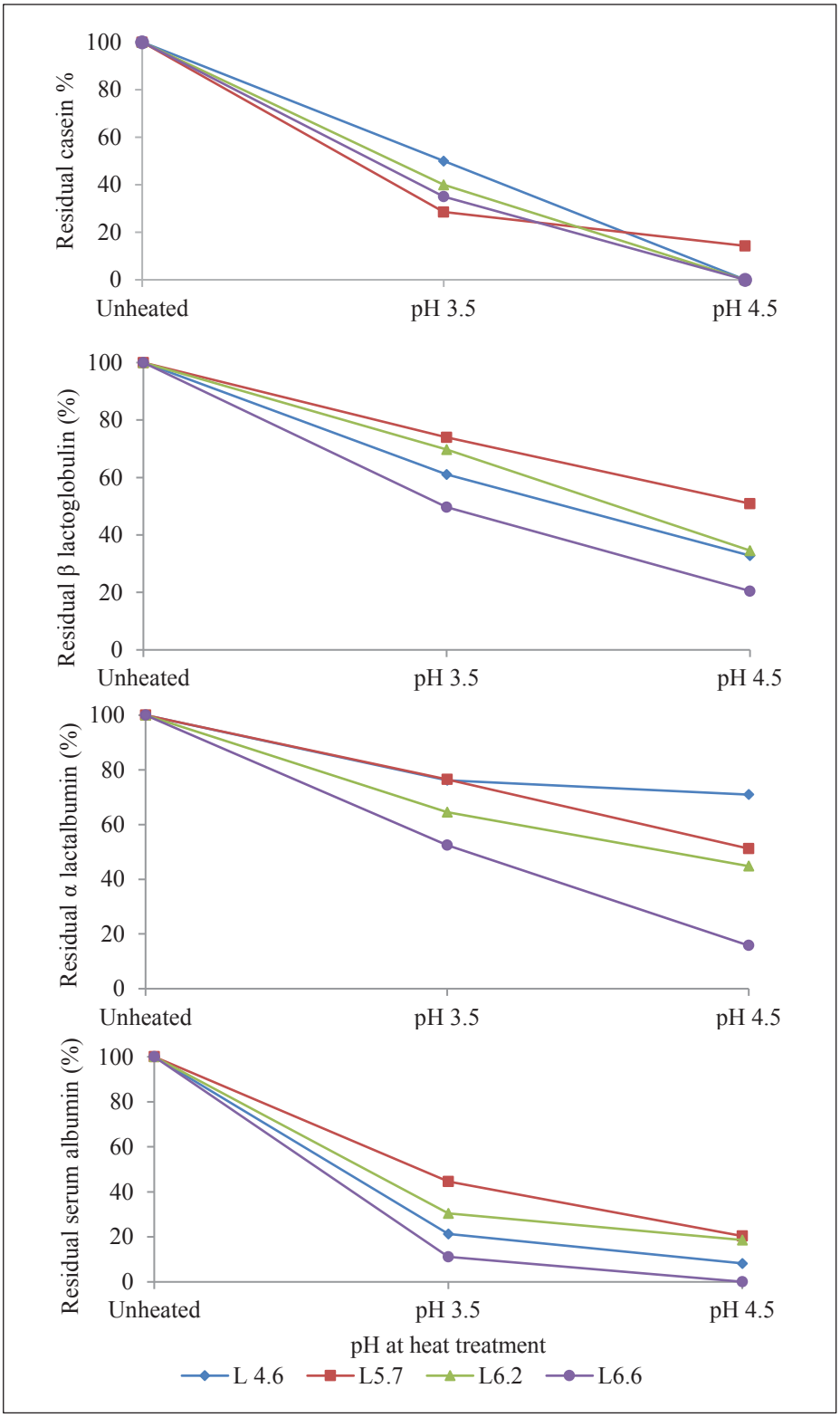


Figure 4.13: Loss of SDS – monomeric whey proteins during heating of model whey at 90°C for 5 min. Peak area of each protein band is presented as a percentage of the respective band in the unheated control whey sample.

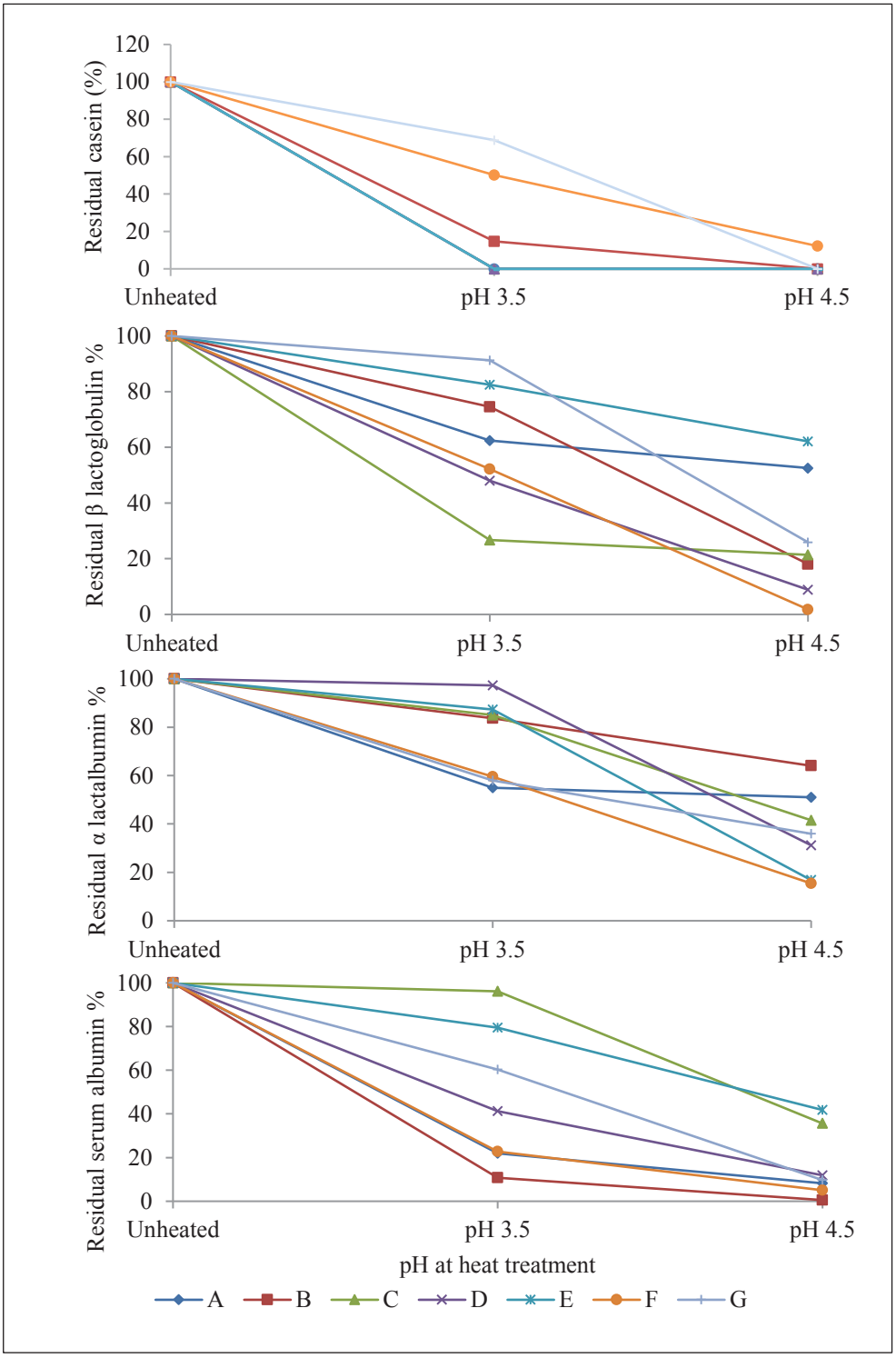


Figure 4.14: Loss of SDS – monomeric whey proteins during heating of commercial whey at 90^{o}C for 5 min. Peak area of each protein band is presented as a percentage of the respective band in the unheated control whey sample.

4.5. Effect of added NaCl on stability of whey during heat treatment

Sheep whey steams were generally stable during heat treatment at pH 3.5 (section 4.4). However, the colour (section 4.4.3), sedimentation (section 4.4.4) and loss of monomeric whey proteins (section 3.2.8) of commercial sample-A deviated from the other whey streams during the heat treatment at pH 3.5. Moreover, the sample-A has been released from a cheese production with salting in cheese milk. The excess salt would have drained off with whey and consequently produced a whey with high ionic strength (i.e. conductivity 193.6 mS cm^{-1}; Table 4.4). NaCl is often used in such salting processes (Fox *et al.*, 2000). Therefore, an experiment was designed to study the effect of added NaCl in the range of 0.00 – 0.35 mol L^{-1} (including NaCl concentration respective to the conductivity of the sample-A) on stability of whey during heat treatment of 90^{o}C for 5 min at pH 3.5. Model whey prepared by rennet coagulation at pH 6.6 (section 3.4) was used in this study.

The stability of heat treated whey was determined based on particle size (section 3.2.9), turbidity (section 3.2.10), sedimentation (after overnight storage at 5^{o}C) and loss of monomeric whey proteins (section 3.2.8). However, particle size and turbidity measurements were not satisfactory in explaining the observations of the experiment (Appendix 6) for the same reasons as discussed in sections 4.4.1 and 4.4.2.

Sediment curd% increased slightly up to 0.1 mol L^{-1} NaCl (Figure 4.16; A), but the levels were still low and might be acceptable by consumers. With further increases in salt concentration from 0.1 mol L^{-1} to 0.15 mol L^{-1} there was a dramatic decrease in stability from 6% sediment to 69 % sediment. This indicates that there is a critical salt concentration/ionic strength at which the whey system becomes unstable and this point has been used to separate the stability behaviours in Figure 4.16. As expected the SDS – PAGE analysis showed reducing intensities of protein bands (Figure 4.17) with increasing NaCl concentration. This indicates loss of whey proteins from the serum phase due to aggregate formation during the heat treatment. Thus, the results of SDS – PAGE support the above findings.

Clearly the salt composition of sheep whey is likely a significant factor which could determine its behaviour during thermal processing and thus its applicability in whey beverage prodution. However, quantification of NaCl in whey for such on-site decision making is complex and costly. In comparison, a conductivity measurement would be a cost effective rapid method to determine (indirectly) the NaCl concentration in whey. A linear regression of the data on conductivity versus added NaCl is quantified in the relationship shown in Equation 2 with a

R^2 coefficient of 0.98 at a 95% level of confidence (Figure 4.15). Therefore, conductivity would predict the suitability of a sheep whey stream or formulation for further processing and likely applicable in small scale dairy sheep operations.

Equation 2: $Y = 576.4\ X + 44.98$

Where; Y = conductivity (mS cm^{-1}) of whey and

X = added NaCl concentration (mol L^{-1})

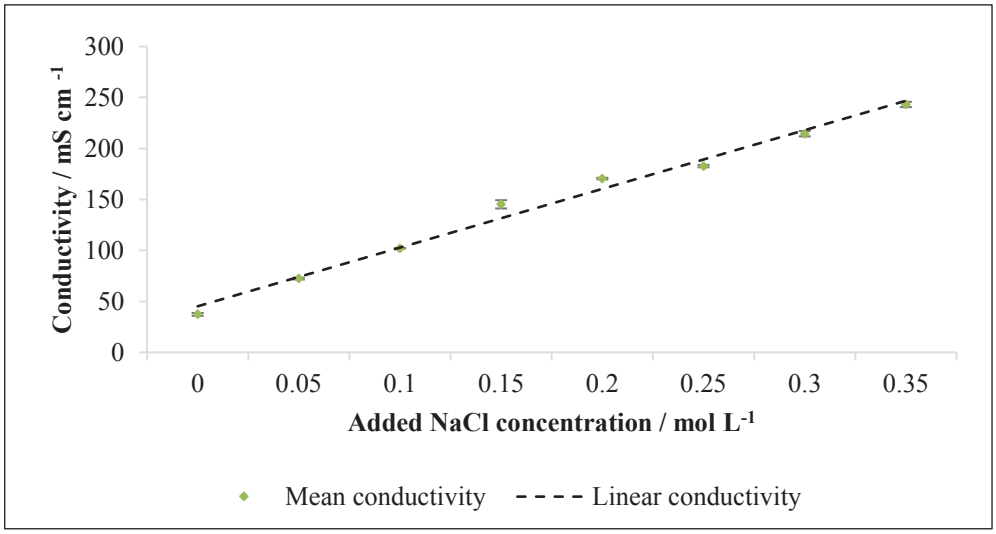


Figure 4.15: The change of conductivity (at 20°C) with increase of added NaCl. Error bars represent standard deviation of triplicate.

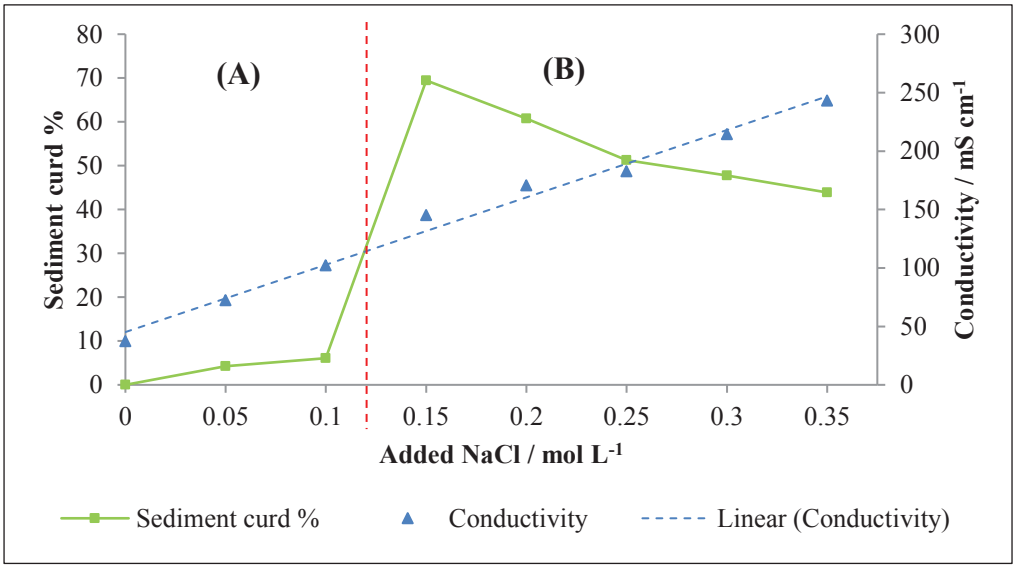


Figure 4.16: Effect of added NaCl on sedimentation (after overnight storage at 5°C) of heated whey (90°C for 5 min at pH 3.5)

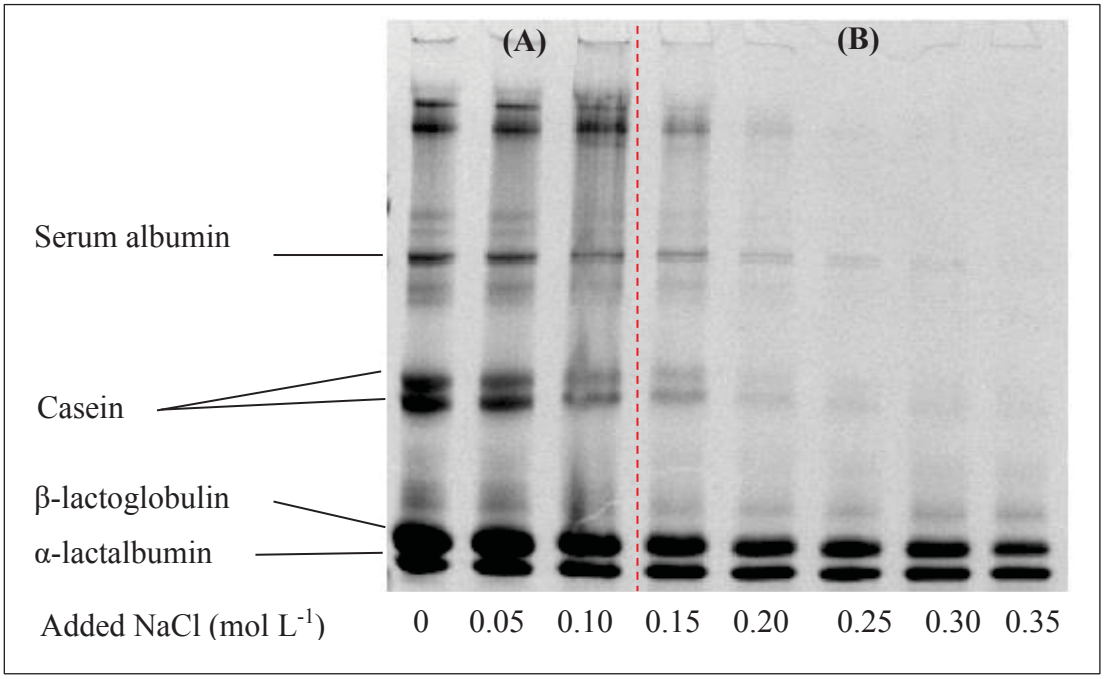


Figure 4.17: SDS-PAGE patterns of rennet whey serum (heated at pH 3.5, 90ºC for 5 min)

An additional observation was that increasing the NaCl concentration beyond the critical salt concentration of 0.1 mol L^{-1} showed a clear separation of sediment phase and serum phase (Figure 4.18) and produced powdery like aggregates upon the overnight storage of heated whey (visual observations). These sediments were further investigated using TEM images. Changes of sediment structure with increasing NaCl concentration were evident in the TEM micrographs (Figure 4.19). Sediments showed hairy like structure at 0.15 mol L^{-1} NaCl and respectively dense localised aggregates at 0.20 mol L^{-1} NaCl and above. The dense localised aggregates perhaps settled compactly and thus produced comparatively clear serum phase and demarcation, observed in Figure 4.18. These results help to explain a key feature associated with the higher salt samples shown in region B of Figure 4.16 which shows that the sediment volume in this region decreases with added NaCl i.e. as the salt concentration increases and the aggregates become more compact this manifests itself on the macro-scale as an increase in sediment bulk density and thus a reduction in total sediment volume. These findings are in keeping with published data for the effects of Na on the thermal aggregation behaviour of bovine whey systems (Varunsatian *et al.*, 1983; Mulvihill and Donovan, 1987; Xiong, 1992; Bryant and McClements, 2000 and Havea *et al.*, 2002). High NaCl concentration decreases the heat denaturation rate of proteins through stabilising its native configuration. However, low solubility of proteins at high NaCl concentration assists their physical aggregation and thus

produces large sediment particles (Verheul *et al.*, 1998). Therefore, the observations in this experiment would be a simultaneous effect of the two phenomena and a concern in sheep whey procesing.

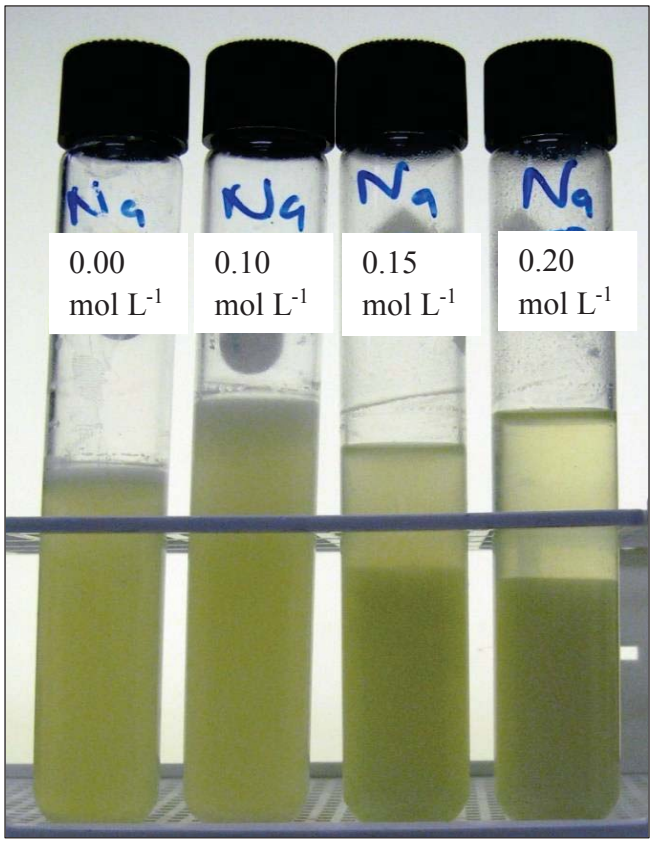


Figure 4.18: Effect of added NaCl on sedimentation upon heating of whey at 90ºC for 5min at pH 3.5. (Photographed after overnight storage at 5ºC).

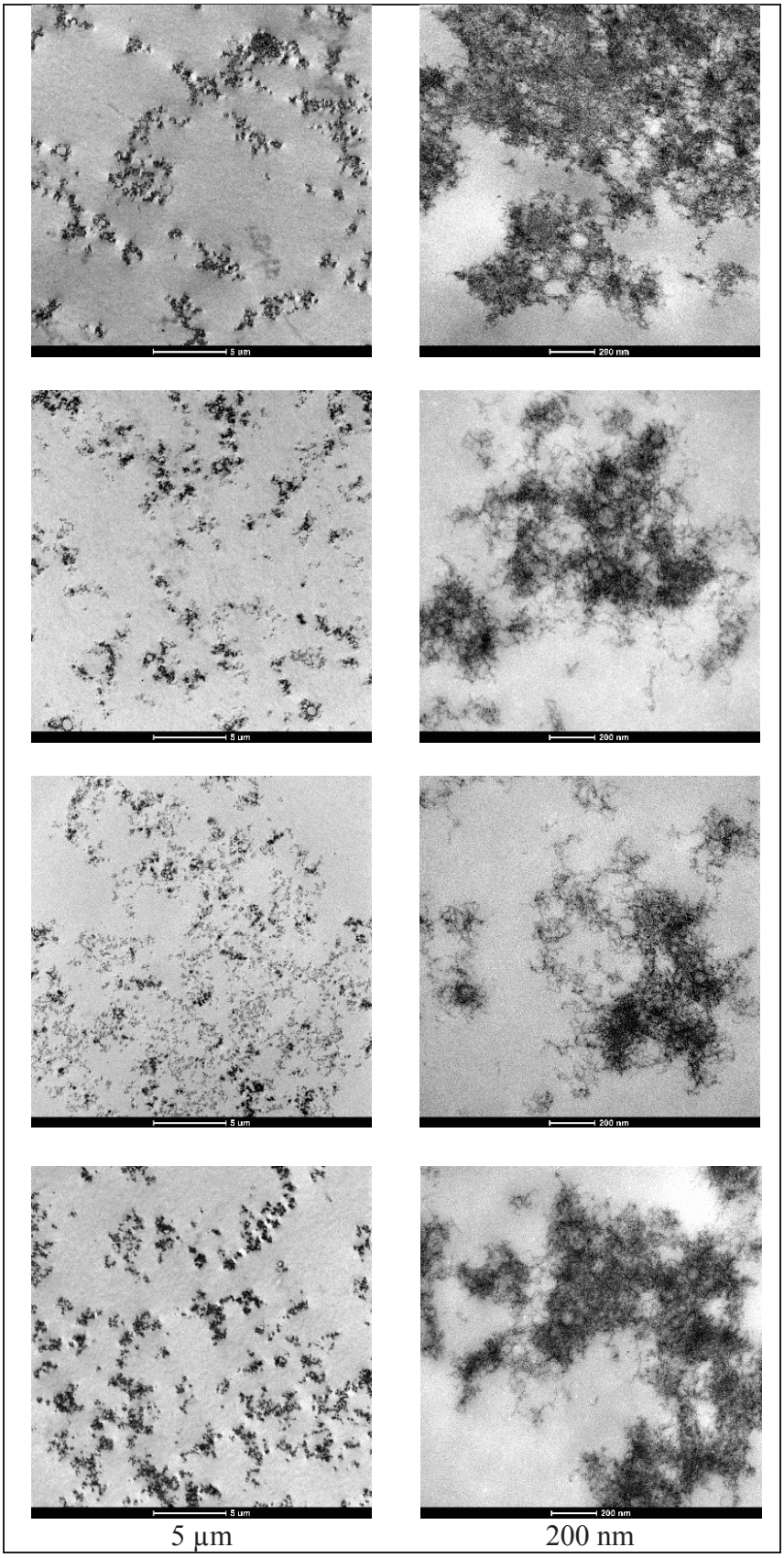


Figure 4.19: TEM micrographs of rennet whey aggregates produced after heating at pH 3.5, 90ºC for 5 min with added NaCl

4.6. Prototype sheep whey beverage development

A prototype lemonade was developed using sheep whey and lemon juice concentrate as described in Figure 3.3 and the physicochemical composition of the final product was analysed (Table 4.6). Conductivity of the whey beverage was below the critical limit (i.e. 102.1 mS cm^{-1}) for significant sedimentation, which was the value identified in section 4.5 (Figure 4.16). Further, the formulation contained sugar which is a constituent in the lemon juice concentrate. The addition of sugar, besides increasing palatability may also have beneficial processing effects. Kulmyrzaev *et al.* (2000) showed that a 6 – 8ºC increase of denaturation temperature of a 0.2% (w/w) whey protein isolate solution could be obtained in the presence of 40% (w/w) sucrose at pH 7. Moreover, Jelan and Bernal (1985) reported increased resistance to thermal denaturation of β-lactoglobulin in the presence of sugars. Although it is possible that sugars in the current sheep whey beverage formulation probably improved the denaturation of whey proteins during thermal processing it was not possible to ascertain this within the project scope. It should be noted that the level of added sugar was significantly less than that reported by Kulmyrzaev *et al.* (2000) and that the pH was acid. Thus the effects would vary depending on protein and sugar concentrations in the beverage.

Table 4.6: Physicochemical composition of the prototype sheep whey beverage

Physicochemical parameters	**Composition**
Moisture (%)	87.3
Protein (%)	0.72
Fat (%)	0.09
Ash (%)	0.34
pH	3.5
Total soluble solids (Brix %)	16.7
Conductivity (mS cm^{-1})	28.0

4.6.1.Stability of the whey beverage during cold storage

4.6.1.1. Laboratory scale study

The stability of the beverage during cold storage (5^{o}C) was determined using a model study. Aliquots (15 ml) of the beverage were filled into 20 ml glass tubes (with screw caps) and heated in a water bath set at 90^{o}C (section 3.10.1). The time taken for the samples to attain 90^{o}C was 2.5 min (Figure 4.23). The prototype beverage formulation was stable and produced no sediments during overnight cold storage after the heat treatment (Figure 4.20), and thus confirms the findings of the previous studies (section 4.4 and 4.5). However, a white floating layer of aggregates and reduced opaqueness of the liquid phase were observed (visual observations) after the overnight storage.

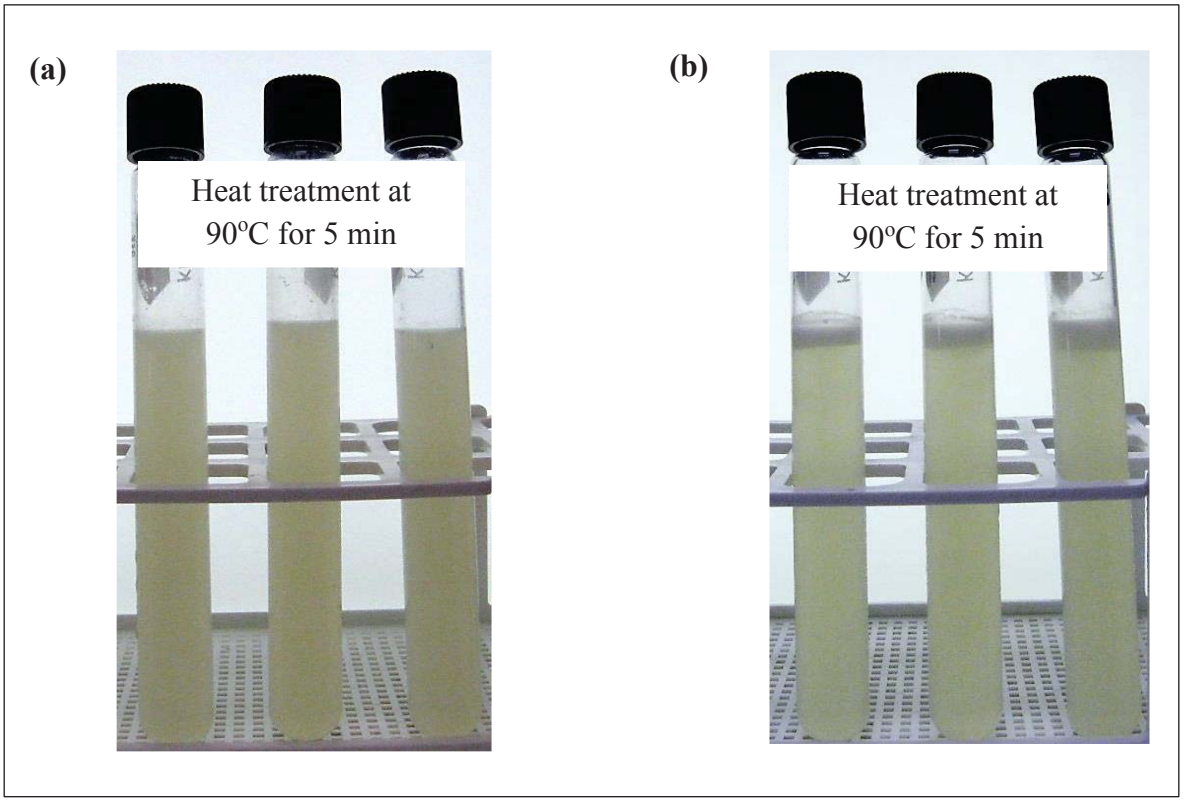


Figure 4.20: Heat treated prototype whey beverage (a) cooling over ice for 1 h and (b) after overnight storage at 5^{o}C

4.6.1.2. Pilot scale study (in-bottle heat treatment)

Subsequently, the model experiment was extended to a pilot scale study and thus to investigate the stability of the whey lemonade. The beverage was manufactured according to Figure 3.3 and the contents attained 90ºC (from 20ºC) within 36 min during in-bottle heat treatment (400 ml beverage in 450 ml glass bottle with lug caps). The resulting beverage bottles were stored overnight at 5ºC and the stability was determined based on sedimentation. These beverages produced significant sedimentation upon overnight storage (Figure 4.21) and were deemed unstable. The sediment height increased from day 1 to day 5, and the liquid over the sediments became more transparent over the time. A thin white floating layer of aggregates was observed on the 5th day. Moreover, no visible changes in sediment layer were observed from day 5 to day 10 during the storage. However, the sediments were rendered colloidally stable upon two inversions of the bottle.

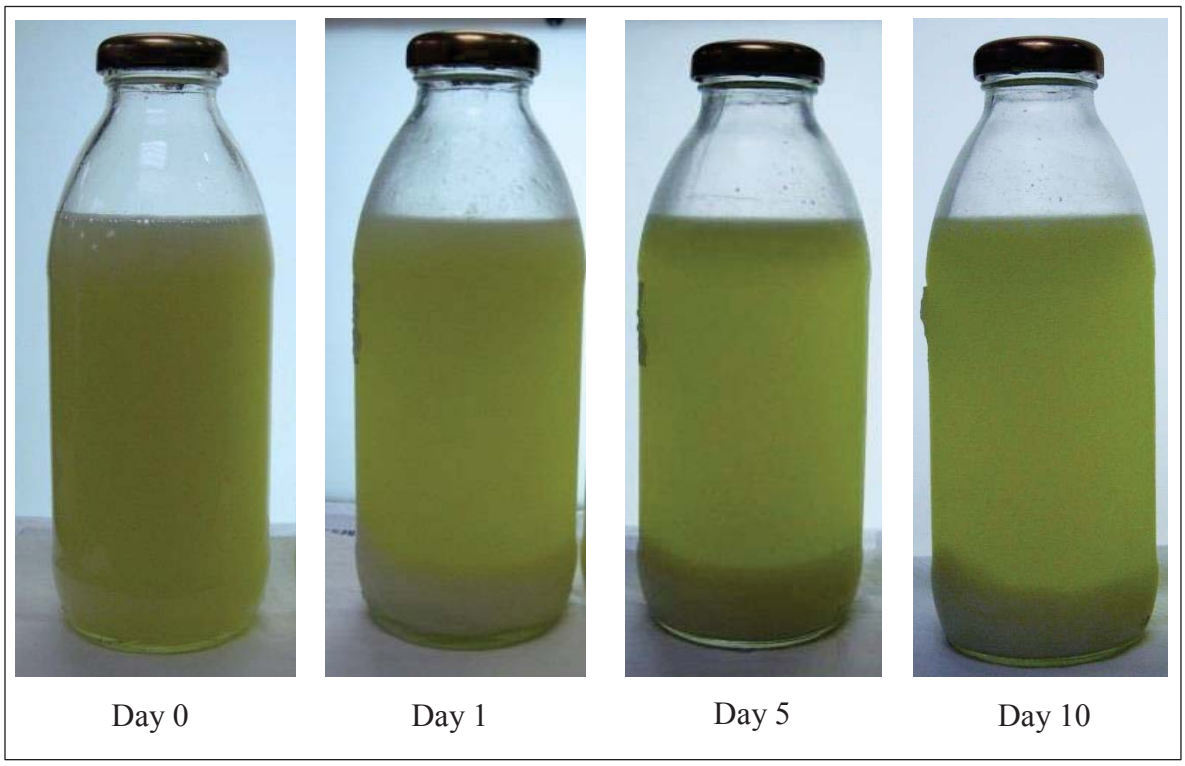


Figure 4.21: Sedimentation of the whey beverage upon cold storage (in-bottle heat treatment)

Based on the conductivity (i.e. 28.0 mS cm^{-1};Table 4.6) the beverage is likely stable during heat treatment of 90°C for 5 min at pH 3.5. Additionally, the model study (Figure 4.20) confirmed its stability under above treatment conditions. The reason for the observed instability on scale-up from the model study to the pilot plant study was thought to be related to the time/temperature profile during thermal processing between the two systems. In the model system the beverage attained 90°C within 2.5 min whereas it took 36 min in the pilot scale study. It is important when analysing this data to note that the heat induced denaturation of whey proteins occurs above 62°C (Table 2.3) which results in aggregation and subsequent sedimentation (section 2.5). Therefore, the slow heating and cooling rates during the pilot scale beverage production probably extended the time held above the whey protein denaturation temperature (above 62°C) (Figure 4.23) and thus resulted in the extensive denaturation of whey proteins followed by aggregation and sedimentation observed during the storage. Therefore, an alternative rapid heating method was investigated to overcome the above product defect.

4.6.1.3. Pilot scale study (rapid heat treatment)

In this study the beverage formulation was heated rapidly (8.5 min from 62 to 90°C) in a stainless steel bowl prior to bottling. Thereafter, the beverage was bottled hot and cooled by immersion in a water bath at room temperature (15°C) for 1 h (section 3.10.1) and held overnight at 5°C. Sedimentation was not observed after the overnight storage but a white floating layer of aggregates was observed (Figure 4.22) (similar results were evident in the section 4.6.1.1; Figure 4.20). However, the aggregates were soluble upon two inversions of the bottle. Moreover, there was no increase of the floating aggregate layer or sedimentation over observation for 20 days from the manufacture.

The effects of heat treatment, pH and ionic strength on heat induced aggregation of whey proteins are well established (section 2.5). The current study demonstrated that heating and cooling rates are likely influence the properties of whey protein aggregates. It is hypothesised that sediment formed during the first in-bottle treatment (Figure 4.21) was likely caused by high density aggregates whereas the floating layer in the second treatment (Figure 4.22) were probably produced by low density aggregates. This finding led to a further study that investigated the behaviour of aggregates during extended holding time during heat treatment.

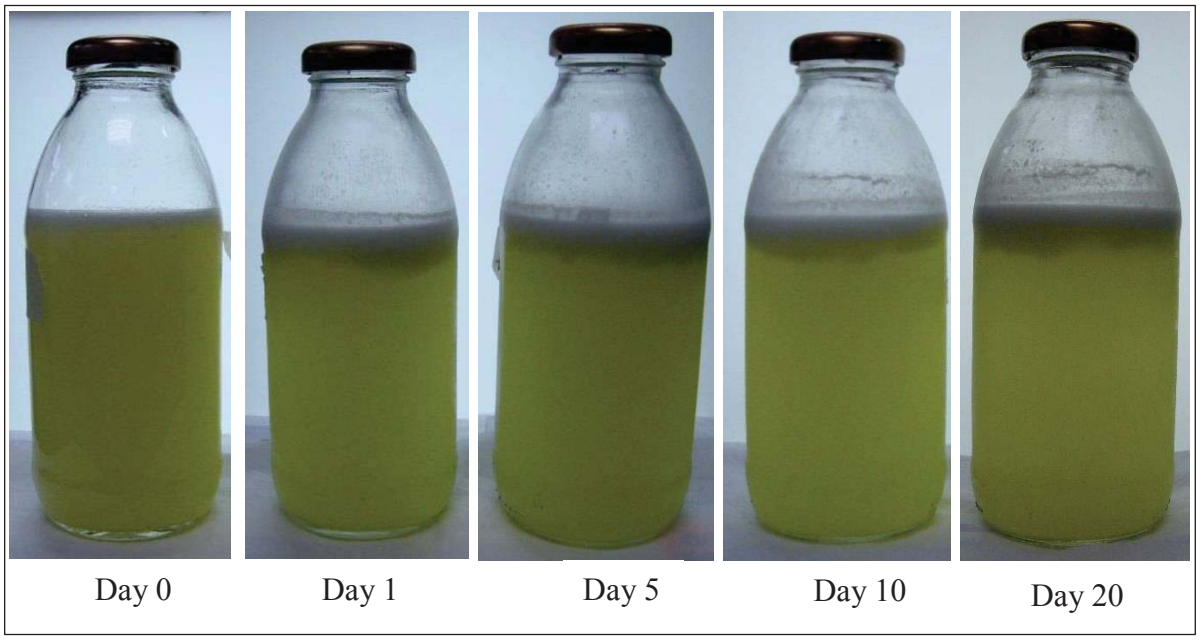


Figure 4.22: Beverage produced by heat treatment in stainless steel bowl

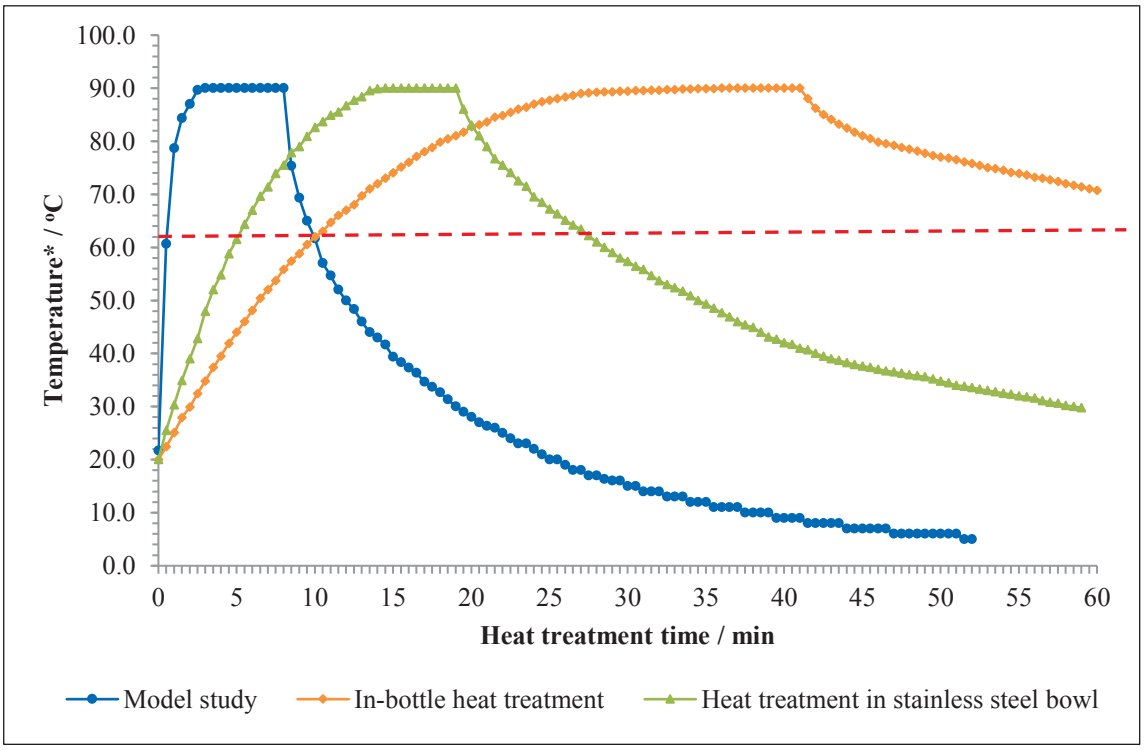


Figure 4.23: Heating and cooling curves of the whey beverage used in the three studies.

* Mean temperature of triplicate.

4.7. Effect of holding time at heat treatment on properties of whey protein aggregates

Aliquots (15 ml) of the whey beverage formulation were filled into 20 ml glass tubes (with screw caps) and dipped in a water bath set at 90ºC (section 3.11). The contents reached 90ºC within 2.5 min and produced no sedimentation on holding for 6 min (Figure 4.24). However, heating above 6 min produced visible aggregates and were thus unstable. Moreover, the aggregates produced at 8 min were floating on the top and aggregates produced at 10 min and above settled at the bottom of the tubes. The results show the significance of holding time at high temperature on the properties of whey protein aggregates. The phenomenon supports the difference in aggregate behaviour observed in Figure 4.21 and Figure 4.22. Therefore, rapid heating and cooling by manufacturers is necessary to ensure that the minimum holding time within the critical temperature limits (i.e. above 62ºC) so that a whey beverage can have optimal product stability.

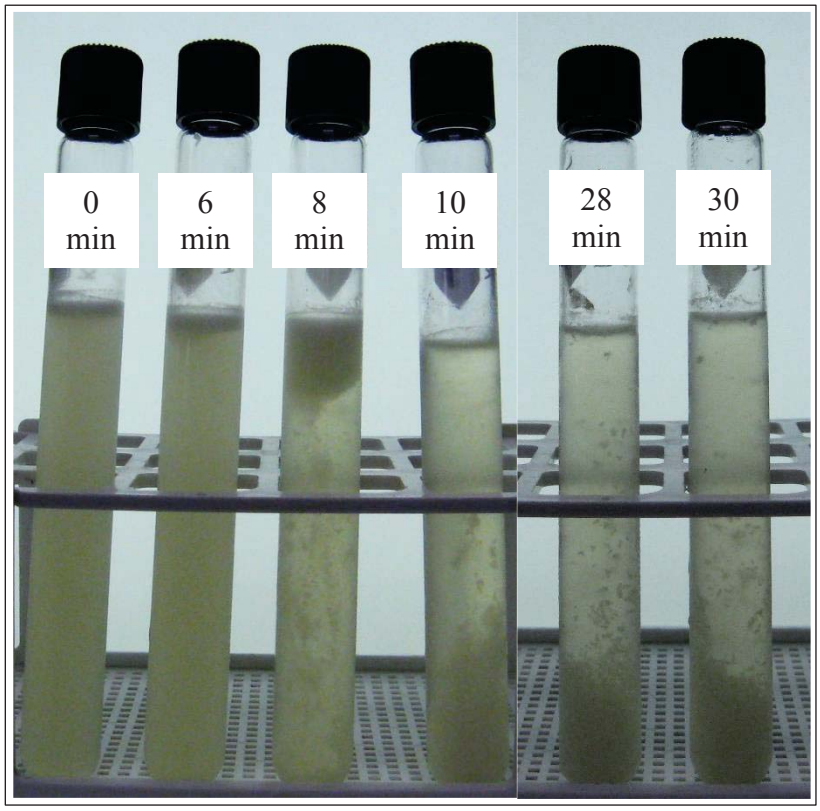


Figure 4.24: Whey beverage heated at 90ºC for 30 min (photographed after 1 h cooling in ice)

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Composition analysis of model whey and commercial whey streams

The compositions of model sheep whey showed characteristic physicochemical properties such as high mineral content and low total proteins in acid whey with respect to low minerals and high total proteins in rennet whey. The commercial whey streams showed composition variations based on the cheese type and respective cheese processing conditions. Therefore, processing of sheep whey into beverages and thus the beverage composition would vary depending on physicochemical composition of the base whey stream.

5.2. Effect of storage pH on Ca and Mg migration from contaminating curd in whey

Commercial whey from artisan sheep cheese manufacturers is often contaminated with casein curd fines. The impact of curd contamination on processability of the whey into a beverage has been characterised. Storage pH of sheep whey with 5% (w/v) contaminating curd (produced by rennet coagulation at pH 6.6) was shown to significantly affect the solubilisation of minerals in the curd and subsequently produces whey with high Ca while having a minimal effect on the Mg content. The phenomenon could be beneficial in an electrolyte whey beverage product.

5.3. Effect of pH at heat treatment on stability of whey

Heat treatment (90^{o}C for 5 min) of sheep whey at pH 4.5 and subsequent overnight storage at 5^{o}C resulted in large amounts of sedimentation (about 13 - 40%, v/v) that would not be accepted by consumers. Additionally, notable increases in the L value (white colour) was evident in the heated whey. Therefore, heat treatment at pH 4.5 is likely to produce unstable whey. Comparatively, whey remains generally stable during the heat treatment at pH 3.5. This heat treatment only produced less than 1% (v/v) sedimentation and no significant colour changes. It is recommended that commercial manufacturers of a whey beverage have tight quality control over the pH of the beverage pre-thermal treatment.

An additional key finding was that whey streams with high ionic strength (conductivity 193.6 mS cm^{-1}) are unstable even when heated at pH 3.5. Therefore, salty whey streams (particularly where salting is performed in cheese milk) probably require additional processing steps such as combination with other whey streams to reduce the salt content to within the safe range and thus to make stable product upon heat treatment.

5.4. Effect of added NaCl on stability of whey during heat treatment

Heat treatment of sheep whey at pH 3.5 with 0.1 mol L^{-1} added NaCl only produced about 6% (v/v) sediments and thus are likely to be stable. Above a critical concentration of 0.15 mol L^{-1} NaCl caused a dramatic increment in sedimentation (i.e. about 60%, v/v) with a gradual reduction with subsequent addition of NaCl.

The actual mechanism of NaCl instability is likely to be based on ionic strength. Therefore, a useful tool for manufacturers to predict the stability of a given whey stream or beverage formulation would be conductivity. A calibration curve for added NaCl concentration (0.1 – 0.35 mol L^{-1}) against conductivity showed a linear relationship with a R^2 coefficient of 0.98 at a 95% level of confidence. Therefore, conductivity measurement would provide an indication about the ionic strength of respective whey streams and thus assist with on-site decision making on the potential use of whey as a base ingredient in beverage processing. Conductivity measurement is comparatively cost effective and convenient method which could be applied in small scale sheep dairy operations.

5.5. Prototype sheep whey beverage development

Sheep whey can be developed into a stable beverage (lemonade) with heat treatment of 90°C for 5 min at pH 3.5. However, heating and cooling rates significantly affect the whey protein aggregation. Therefore, it is essential to minimise the holding time above the whey protein denaturation temperature (i.e. above 62°C). Results from a pilot plant study showed that in-bottle heating was not effective and resulted in unstable product due to the long heating time. An improved method was shown to be effective. This method involved heating the beverage formulation in a stainless steel bowl up to 90°C (8.5 min from 62 to 90°C) and holding for 5 min followed by rapid cooling in a cold water bath to limit heat induced aggregation of whey proteins and thus the sedimentation over product shelf life.

5.6. Effect of holding time at heat treatment on properties of aggregates

The holding time of whey in heat treatment at 90°C (at pH 3.5) has been shown to be critical and to alter the properties of whey protein aggregates. Sheep whey was stable in heating at 90°C up to 6 min. However, floating aggregates (at 8 min) and sediments (at 10 min or above holding time) were observed during the heat treatment, respectively. These results further emphasise the significance of heating and cooling rates on whey protein aggregation and

sedimentation. Moreover, heating and cooling rates could be selected based on the intended use of whey.

5.7. Application of research outcomes to industry

New Zealand sheep whey is primarily utilised for animal feed. However, sheep whey contains valuable functional constituents as such could be further processed into beverages. Sheep whey composition varies depending on the type of cheese processing. The results in this study assist knowledge on the composition variables, their respective effects on processing and subsequent on-site decision making for individual cheese manufacturers on the suitability of specific whey streams as a stable base ingredient in beverage processing.

5.8. Recommendations for future studies

Future studies could be focused on;

Mathematical modelling quantifying relationships between:

a) physicochemical composition of whey (specifically, protein, minerals, ionic strength and pH)
b) heat treatment conditions (time, temperature, heating and cooling rates, pH and ionic strength at heat treatment) and,
c) chemical properties of resulting whey protein aggregates (such as; non-reducible covalently cross-linked, reducible disulphide cross-linked, hydrophobic linked aggregates and native-like proteins).

Further, the chemical characteristics could be related with the behaviour (colloidal suspension, flocculation or sedimentation) of heat induced protein aggregates and thus to predict the stability of sheep whey as a base ingredient in beverage manufacturing.

6. REFERENCES

AOAC. (2005). *Official Methods of Analysis.* Virginia: Association of Official Analytical Chemists, Arlington, Virginia.

Attia, H., Kherouatou, N., and Nasri, M. (2000). Characterization of the dromedary milk casein micelle. *EDP Sciences, 80*, 503 - 515.

Baccouche, A., Ennouri, M., Felfoul, I., and Attia, H. (2013). A Physical Stability Study of Whey-based Prickly Pear Beverages. *Food Hydrocolloids, 33*, 234 - 244.

Banavara, D. S., Anupama, D., and Rankin, S. A. (2003). Studies on Physicochemical and Functinoal Properties of Commercial Sweet Whey Poweders. *Journal of Dairy Science, 86*(12), 3866 - 3875. Retrieved October 03, 2016, from http://www.sciencedirect.com/science/article/pii/S0022030203739940

Bio-Rad. (2011). *Criterion Precast Gels Instruction Manual and Application Guide.* USA: Bio-Rad Laboratories.

Bonzanic, R., Barukcic, I., Lisak, K., and Tratnik, L. (2014). Possibilities of whey utilization. *Austin Journal of Nutrition and Food Sciences, 2*(7), 1036.

Britz, T. J., and Robinson, R. (2008). *Advanced Dairy Science and Technology.* Wiley-Blackwell Publications.

Bryant, C. M., and McClements, D. J. (2000). Influence of NaCl and CaCl2 on Cold-Set Gelation of Heat Denatured Whey Proteins. *Journal of Food Science, 65*(5), 801 - 804.

Casper, J. L., Wendorff, W. L., and Thomas, D. L. (1998). Seasonal changes in Protein Composition of Whey from Commercial Manufacture of Caprine and Ovine Specialty Cheeses. *Journal of Dairy Science, 81*, 1317 - 1322.

Chavan, R. A., Shraddha, R. C., Kumar, A., and Nalawade, T. (2015). Whey Based Beverage: Its Functionality, Formulations, Health Benefits and Application. *Journal of Food Processing and Technology, 6*(10), 1 - 8. doi:doi:10.4172/2157-7110.1000495

Childs, J. L., Yates, M. D., and Drake, M. A. (2007). Sensory Properties of Meal Replacement Bars and Beverages Made from Whey and Soy Proteins. *Journal of Food Science, 72*(6), 425 - 434.

Chung, C., Rojanasasithara, T., Mutilangi, W., and McClements, D. J. (2015). Enhanced Stability of Anthocyanin - Based Colour in Model Beverage Systems through Whey Protein Isolate Complexation. *Food Research International, 76*, 761 - 768.

Correa, A. P., Daroti, D. J., Fontoura, R., Meira, S. M., Segalin, J., and Brandelli, A. (2014). Hydrolysates of sheep cheese whey as a source of bioactive peptideswith antioxidant and angiotensin-converting enzyme inhibitory activities. *Peptides, 61*, 48 - 55.

Damodaran, S. (2008). Amino Acids, Peptides, and Proteins. In S. Damodaran, K. L. Parkin, and O. R. Fennema (Eds.), *Fennema's Food Chemistry* (4th ed., pp. 217 - 330). New york: CRC Press.

Damodaran, S., Parkin, K. L., and Fennema, O. R. (Eds.). (2008). *Fennema's Food Chemistry* (4th ed.). New York: CRC Press.

Day, L., Broadhurst, M., and Samuelsson, L. (2016). New Zealand Sheep Milk - Nutritional Composition. *Food New Zealand, 16*(4), 20 - 21.

Diaz, O., Pereira, C. D., and Cobos, A. (2004). Functinoal Properties of Ovine Whey Protein Concentrates Produced by Membrane Technology after Clarification of Cheese MAnufacture By-products. *Food Hydrocholloids, 18*, 601 - 610.

Divya, and Kumaria, A. (2009). Effect of Different Temperatures, Timing and Storage Periods on the Physico-Chemical and Nutritional Characteristics of Whey - Guava Beverage. *World Journal of Dairy and Food Science, 4*(2), 118 - 122.

Djuric, M., Caric, M., Milanovic, S., Tekic, M., and Panic, M. (2004). Development of whey based beverages. *Journl of Food Research and Technology, 219*, 321 - 328. doi:10.1007/s00217-004-0950-1

Donato, L., and Guyomarc, F. (2009). Formation and properties of whey proteins/k-caseins complexes in heated skim milk; A Review. *Journal of Dairy Science and Technology, 89*, 3 - 29.

Dragone, G., Mussatto, S. I., Oliveira, J. M., and Teixeira, J. A. (2009). Characterisation of volatile compounds in an alcoholic beverage. *Food Chemistry, 112*, 929 - 935.

Durham, R. J., and Hourigan, J. A. (2007). Wast Management and Co-product Recovery in Dairy Processing. In K. Waldron (Ed.), *Handbook of Wast Managemetn and Co-product Recovery in Food Processing* (Vol. 1, pp. 332 - 387). New York: CRC Press.

Fox, P. F., and McSweeney, P. L. (2003). *Advanced Dairy Chemistry* (3rd ed., Vol. 1). New York: Kluwer Academic/ Plenum Publishers.

Fox, P. F., Guinee, T. P., Cogan, T. M., and McSweeney, P. L. (2000). *Fundamentals of Cheese Science.* US: Aspen Publishers, Inc.

Fox, P. F., Guinee, T. P., Cogan, T. M., and McSweeney, P. L. (2000). *Fundamentals of Cheese Science.* US: Aspen Publishers, Inc.

Gezimati, J., Creamer, L. K., and Singh, H. (1997). Heat Induced Interactions and Geleation of Mixtures of B-Lactoglobulin and a-Lactalbumin. *Journal of Agriculture and Food Chemistry, 45*, 1130 - 1136.

Gezimati, J., Singh, H., and Creamer, L. K. (1996). Heat-Induced Interatins and Geleation of Mixtures of Bovine B-lactoglobulin and Serum Albumin. *Journal of Agriculture and Food Chemistry, 44*, 804 - 810.

Goyal, N., and Gandhi, D. N. (2009). Comparative analysis of Indian paneer and cheese whey for electrolyte whey drink. *World Journal of Dairy and Food Sciences, 4*(1), 70 - 72.

Goyal, N., and Gandhi, D. N. (2009). Comparative analysis of Indian paneer and cheese whey for electrolyte whey drink. *World journal of Dairy and Food Sciences, 4*(1), 70 - 72.

Graet, Y., and Gaucheron, F. (1999). pH-induced solubilization of minerals from casein micelles: influence of casein concentration and ionic strength. *Journal of Dairy Reseaarch, 66*(6), 1999.

Griffiths, L. (2015). *Business Plan for the New Zealand Sheep Dairy Industry.* Masterton, NZ: Nuffield NZ.

Guinee, T. P., and O'Brien, B. (2010). The Quality of Milk for Cheese Manufacture. In B. A. Law, and A. Y. Tamime (Eds.), *Technology of Cheese Making* (2nd ed., pp. 1 - 67). USA: Blackwell Publishing Ltd.

Hamada, D., Tanaka, T., Tartaglia, G. G., Pawar, A., Vendruscolo, M., Kawamura, M., . . . Dobson, C. M. (2009). Competion between Folding, Native-State Dimerisation and Amyloid Aggrgation in B-lactoglobulin. *Journal of Molecular Biology, 386*, 878 - 890.

Havea, P., Singh, H., and Creamer, L. K. (2002). Heat - Induced Aggregation of Whey Proteins: Comparison of Cheese WPC with Acid WPC and Relevance of Mineral Composition. *Journal of Agriculture and Food Chemistry, 50*, 4674 - 4681. doi:10.1021/jf011583e

Havea, P., Singh, H., and Creamer, L. K. (2002). Heat-Induced Aggregation of Whey Proteins: Comparison of Cheese WPC with Acid WPC and Relevance of Mineral Composition. *Journal of Agriculture and Food Chemistry, 50*, 4674 - 4681.

Havea, P., Singh, H., Creamer, L. K., and Campanella, O. H. (1998). Electrophoretic Characterization of the Protein Products formed During Heat Treatment of Whey Protein Concentrate Solutions. *Journal of Dairy Research, 65*, 79 - 91.

Hejtmankova, A., Pivec, V., Trnkova, E., and Dragounova, H. (2012). Differences in the composition of total and whey proteins in goat and ewe milk and their changes throughout the lactation period. *Czech Journal of Animal Science, 57*(7), 323 - 331.

Hollar, C. M., Parris, N., Hsieh, A., and Cockley, K. D. (1995). Factors Affecting the Denaturation and Aggregation of Whey Proteins in Heated Whey Prtoein Concentrate Mixtures. *Journal of Dairy Science, 78*, 260 - 267.

Holsinger, V. H., Posati, L. P., and Vilbiss, E. D. (1974). Whey Beverages: A Review. *Journal of Dairy Science, 57*(8), 849 - 859.

Jain, S., Gupta, R., and Jain, S. (2013). Development of Low Cost Nutritional Beverage from Whey. *IOSR Journal Of Environmental Science, Toxicology And Food Technology, 5*(1), 73 - 88.

Jay, J. M., Loessner, M. J., and Golden, D. A. (2005). *Modern Food Microbiology* (7th ed.). US: Springer.

Jelan, P. (2009). Whey Based Functional Beverages. In P. Paquine, *Functional and Specialty Beverage Technology* (pp. 259 - 280). New York: CRC Press.

Jelan, P., and Bernal, V. (1985). Thermal Stability of Whey Proteins - A Calorimetric Study. *Journal of Dairy Science, 68*(11), 2847 - 2852.

Jelen, P. (2011). Whey Processing. *Encyclopedia of Dairy Sciences, 2nd*, 731 - 737. (J. F. Fuquay, Ed.)

Kannan, K. P., Vijayakumar, L. N., Manogaran, S., Karthikeyan, A., and Vignesh, M. (2015). Development of an Energy Drink from Sweet Liquid Whey. *International Journal of Research in Engineering and Advanced Technology, 3*(4), 117 - 126.

Kingsmeade. (2016). *Kingsmeade Artisan Cheese*. Retrieved from http://www.kingsmeadecheese.co.nz/products

Koffi, E., Shewfelt, R., and Wicker, L. (2005). Storage Stability and Sensory Analysis of UHT Processed Whey - Banana Beverages. *Journal of Food Quality, 28*, 386 - 401.

Kulmyrzaev, A., Bryant, C., and McClements, D. J. (2000). Influence of Sucrose on the Thermal Denaturation, Gelation, and Emulsion Stabilization of Whey Proteins. *Journal of Agriculture and Food Chemistry, 48*, 1593 - 1597.

Kumar, S. (2015). Development, Quality Evaluation and Shelf Life Studies of Probiotic Beverages using Whey and Aloe vera Juice. *Journal of Food Processing and Technology, 6*(9), 1 - 5.

LaClarie, E. C., and Etzel, M. R. (2009). Turbidity and Protein Aggregation in Whey Protein Beverages. *Journal of Food Science, 74*(7), C526 - 535.

Lawless, H. T., and Heymann, H. (2010). *Sensory Evaluation of Food; Principles and Practices* (Vol. 2nd). New York: Springer.

Ledesma, B. H., Ramos, M., and Ruiz, J. A. (2011). Review: Bioactive components of ovine and caprine cheese whey. *Small Ruminent Research, 101*, 196 - 204.

Ljutovac, K. R., Lagriffoul, G., Paccard, P., Guillet, I., and Chilliard, Y. (2008). Composition of Goat and Sheep Milk Products: An Update. *Journal of Small ruminent Research, 79*, 52 - 57.

Magalhaes, K. T., Pereira, M. A., Nicolau, A., Dragone, G., Domingues, L., Teixeira, J. A., . . . Schwan, R. F. (2010). Production of fermented cheese whey-based beverage using

kefir grains as starter culture: Evaluation of morphological and microbial variations. *Bioresource Technology, 101*, 8843 - 8850.

Mazedo, A., Duarte, E., and Carvalho, F. (2012). Application of the Sequence Ultra/nanofiltration for Valorization and Reduction of the Environmental Impact of Ovine Cheese Whey. *20th APDR Congress*, (pp. 587 - 591).

McMahon, D. J., and Oommen, B. S. (2013). Casein Micelles Structure, Fucntions and Interactions. In P. H. Fox, and P. L. McSweeney (Eds.), *Advanced Dairy Chemistry* (4th ed., Vol. 1A, pp. 185 - 210). New York: Springer.

Monahan, F. J., German, J. B., and Kinsella, J. E. (1995). Effect of pH and Temperature on Protein Unfolding and Thiol/Disulfide Interchange Reactions duringHeat-Induced Gelatino of Whey Proteins. *Journal of Agriculture and Food Chemistry, 43*, 46 -52.

Mulvihill, D. M., and Donovan, M. (1987). Whey Proteins and their Thermal Denaturation. *Irish Journal of Food Scienc and Technology, 11*, 43 - 75.

Mulvihill, D. M., and Donovan, M. (1987). Whey Proteins and their Thermal Denaturation - A Review. *Irish Journal of Food Scienc and Technology, 11*(1), 43 - 75.

Neelima, Sharma, R., Rajput, Y. S., and Mann, B. (2013). Chemical and functional properties of glycomacropeptide (GMP) and its role in the detection of cheese whey adulteration in milk: a review. *Journal of Dairy Science and Technology, 93*, 21 - 43.

Nudda, A., Battacone, G., Neto, O. B., Cannas, A., Francesconi, A. H., Atzori, A. S., and Pulina, G. (2014). Feeding Stratergies to Design the Fatty Acid Profile of Sheep Milk and Cheese. *Revista Brasileria de Zootecnia, 43*(8), 445 - 456.

O'Brien, B., and Guinee, T. P. (2010). The Quality of Milk for Cheese Manufacture. In B. A. Law, and A. Y. Tamime (Eds.), *Technology of cheesemaking* (pp. 1 - 67).

O'Mahony, J. A., and Fox, P. H. (2013). Milk Proteins: Introductino and Historical Aspects. In P. L. McSweeney, and P. F. Fox (Eds.), *Advanced Dairy Chemistry* (4th ed., Vol. 1A, pp. 43 - 86). New York: Springer.

O'Mahony, J. A., and Fox, P. H. (2013). Milk Proteins: Introduction and Historical Aspects. In P. L. McSweeney, and P. F. Fox (Eds.), *Advanced Dairy Chemistry* (4th ed., Vol. 1A, pp. 43 - 86). New York: Springer.

Panesar, P. S., Kennedy, J. F., Gandhi, D. N., and Bunko, K. (2007). Bioutilisation of whey for lactic acid production. *Journal of Food Chemistry, 105*, 1 - 14.

Park, Y. W., and Haenlein, G. F. (2008). *Handbook of Milk of Non-Bovine Mammals.* Blackwell Publishers.

Park, Y. W., Juarez, M., Ramos, M., and Haenlein, G. F. (2007). Physico-chemical Characteristics of Goat and Sheep Milk. *Journal of Small Ruminent Research, 68*, 88 - 113.

Parris, N., Anema, S. G., Singh, H., and Creamer, L. K. (1993). Aggregation of Whey Proteins in Heated Sweet Whey. *Journal of Agriculture Food Chemistry, 41*, 460 - 464.

Pescuma, M., Hebert, E. M., Mozzi, F., and Valdez, G. F. (2010). Functional fermented whey-based beverage using lactic acid bacteria. *Internatinoal Journal of Food Microbiology, 141*, 73 - 81.

Pesta, G., Pitroff, R. M., and Russ, W. (2007). Utilization of Whey. In V. Oreopoulou, and W. Russ (Eds.), *Utilisation of By-Products and Treatment of Waste in the Food Industry.*

Peterson, S., and Prichard, C. (2015). The sheep dairy industry in New Zealand: a review. *Proceedings of the New Zealand Society of Animal Production 2015*, *75*, pp. 119 - 126.

Pogon, K., Sady, M., Jaworska, G., and Grega, T. (2015). Characterstics of Orange - Wehy Fermented Beverages. *Acta Alimentaria, 44*(2), 170 - 177.

Sady, M., Jaworska, G., Grega, T., Bernas, E., and Domagala, J. (2013). Application of Aicd whey in Orange Drink Production. *Journal of Food Technology and Biotechnology, 51*(2), 266 - 277.

Sakurai, K., Konuma, T., Yagi, M., and Goto, Y. (2009). Review: Structural Dynamics and Folding of B-lactoglobulin Probed by Heteroneuclear NMR. *Biochimica et Biophysica, 1790*, 527 - 537.

Sanmartin, B., Diaz, O., Turienzo, L. R., and Cobos, A. (2012). Composition of Caprine Whey Protein Concentrates Produced bu Membrane Technology After Clarification of Cheese Whey. *Journal of Small Ruminent Research, 105*, 186 - 192.

Sawyer, L. (2013). B-Lactoglobulin. In P. L. McSweeney, and P. F. Fox (Eds.), *Advanced Dairy Chemistry* (4th ed., Vol. 1A, pp. 211 - 260). New York: Springer.

Schlabitz, C., Gennari, A., Araujo, A. L., Bald, J. A., De Souza, C. F., and Hoehne, L. (2015). Shelflife of a Synbiotic Fermented dairy Beverage using Ricotta Cheese Whey. *American Journal of Food Technology, 10*(6), 254 - 264.

Shi, Q., and Jackowski, G. (1998). One-dimentional Polyacrylamide Gel electrophoresis. In E. D. Hames (Ed.), *Gel Electrophoresis of Proteins, A Practical Approach* (3rd ed., pp. 1 - 52). New York: Oxford University Press Inc.,.

Sienkiewicz, T., and Riedel, C. L. (1990). *Whey and Whey Utilisation: Posibilities for Utilisation in Agriculture and Food Stuffs Production.* German: Gelsenkirchen-Buer.

Singh, H., and Havea, P. (2003). Thermal denaturation, aggregation and gelation of whey proteins. In P. F. Fox, and P. L. McSweeney (Eds.), *Advanced Dairy Chemistry* (3rd ed., Vol. 1, pp. 1261 - 1287). New York: Kluwer Academic/ Plenum Publisher.

Smithers, G. W. (2008). Whey and whey proteins—From 'gutter-to-gold'. *Internatinoal Dairy Journal, 18*, 695 - 704.

Smithers, G. W. (2015). Whey-ing up the options e Yesterday, today and tomorrow. *International Dairy Journal, 48*, 2 - 14.

Swaisgood, H. E. (2007). Characteristics of milk. In S. Damodaran, K. L. Parkin, and O. R. Fennema (Eds.), *Fennema's Food Chemistry* (4th ed., pp. 886 - 918). New York: CRC Press.

Varunsatian, S., Watanabe, K., Hayakawa, S., and Nakamura, R. (1983). Effects of Ca++, Ma++ and Na+ on Heat Aggregation of Wehy PRotein Concentrates. *Journal of Food Science, 48*, 42 - 46.

Verheul, M., Roefs, S. P., and de Kruif, K. G. (1998). Kinetics of Heat-Induced Aggregation of b-Lactoglobulin. *Journal of Agriculture and Food Chemistry, 46*, 896 - 903.

Walstra, P., and van Vliet, T. (2007). Dispersed Systems: Basic Consisderations. In O. R. Fennema, K. L. Parkin, and S. Damodaran (Eds.), *Fennema's Food Chemistry* (4th ed., pp. 383 - 847). CRC Press.

Wong, N. P., Lacroix, D. E., and McDonough, F. E. (1978). Minerals in whey and whey fractions. *Journal of Dairy Science, 61*, 1700 - 1703.

Xiong, Y. L. (1992). Influence of pH and Ionic Environment on Thermal Aggregation of Whey Proteins. *Journal of Agriculture and Food Chemistry, 40*, 380 - 384.

Zhu, H., and Damodaran, S. (1994). Effects of Calcium and Magnesium Ions on Aggregation of Whey Protein Isolate and Its Effect on Foaming Properties. *Journal of Agriculture and Food Chemistry, 42*, 856 - 862.

APPENDICES

Appendix 1: Effect of pH at heat treatment (90ºC/ 5min) on particle size of whey

Whey sample	Particle size diameter at pH 3.5 / µm				Particle size diameter at pH 4.5 / µm			
	D(4,3)	**d(0.1)**	**d(0.5)**	**d(0.9)**	**D(4,3)**	**d(0.1)**	**d(0.5)**	**d(0.9)**
L4.6	20.845	2.862	7.209	62.27	20.142	5.37	16.366	39.822
R5.7	21.824	0.152	4.536	58.334	37.709	12.227	33.186	69.635
R6.2	33.406	0.126	9.298	98.423	41.366	13.084	35.816	77.711
R6.6	34.43	0.373	7.587	77.651	39.464	12.151	33.7	75.047
$A_{(R)}$	9.609	3.645	7.768	16.505	17.831	6.375	15.529	32.316
$B_{(R)}$	27.404	2.767	9.355	82.069	44.258	12.136	37.228	86.25
$C_{(R)}$	28.244	2.874	13.493	75.252	46.925	13.269	39.326	91.089
$D_{(R)}$	28.926	2.681	7.297	46.582	32.678	9.644	28.684	61.069
$E_{(L)}$	86.086	2.887	9.726	141.107	33.956	11.314	29.769	62.627
$F_{(R)}$	31.175	4.05	14.07	66.282	43.471	11.863	37.002	84.159
$G_{(L)}$	34.852	5.199	20.784	67.362	33.161	9.711	28.907	62.412

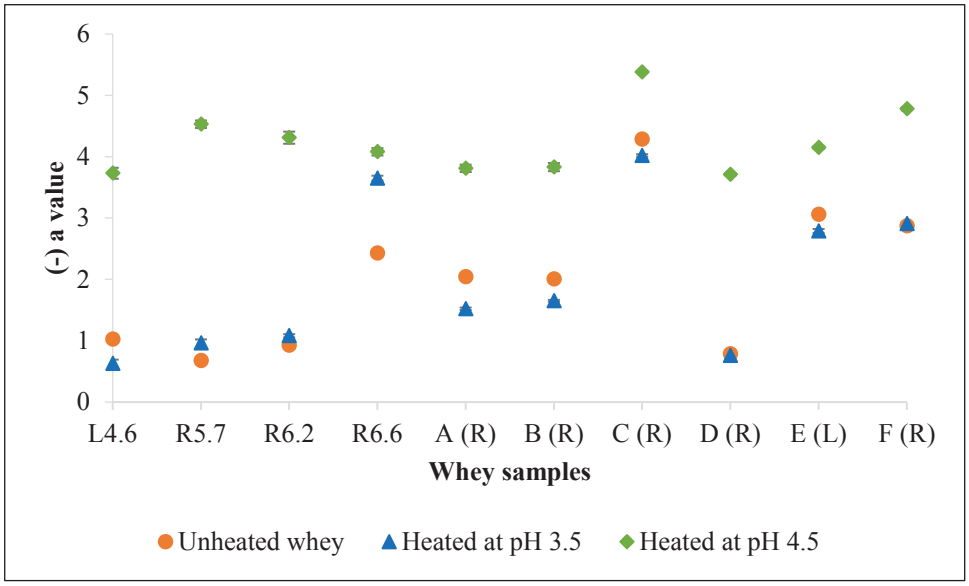
D (4,3) – volume mean diameter, d(0.1), d(0.5) and d(0.9) represents particle diameters covering 10%, 50% and 90% fractions respectively. L – acid whey, R – rennet whey and A – G – commercial whey.

Appendix 2: Effect of pH at heat treatment (90ºC/ 5min) on turbidity of whey

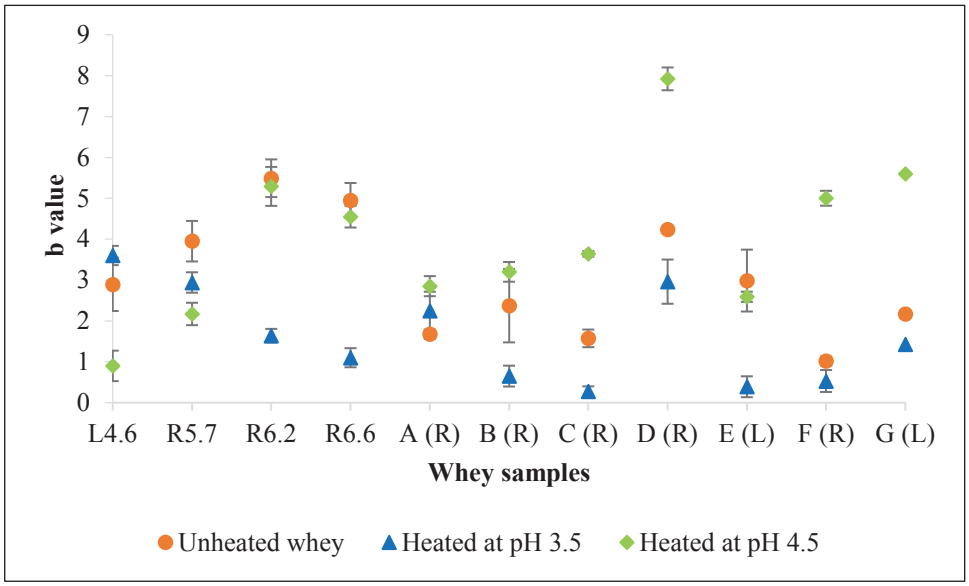
Whey sample	Initial turbidity %	Turbidity % at pH 3.5	Turbidity % at pH 4.5
L4.6	94.4±0.0	94.0±0.0	99.7±0.2
R5.7	61.4±0.0	88.7±0.0	99.8±0.0
R6.2	75.6±0.0	94.5±0.0	99.8±0.0
R6.6	92.5±0.0	98.5±0.1	99.8±0.3
$A_{(R)}$	99.4±0.0	100.0±0.0	100.0±0.0
$B_{(R)}$	99.1±0.0	99.3±1.0	100.0±0.0
$C_{(R)}$	99.0±0.0	99.3±0.7	100.0±0.0
$D_{(R)}$	99.9±0.0	99.9±1.0	100.0±0.0
$E_{(L)}$	97.6±0.0	98.2±0.3	99.8±0.0
$F_{(R)}$	99.9±0.0	99.9±0.8	100.0±0.0
$G_{(L)}$	99.4±0.0	99.8±0.5	100.0±0.0

L – acid whey, R – rennet whey and A – G – commercial whey.

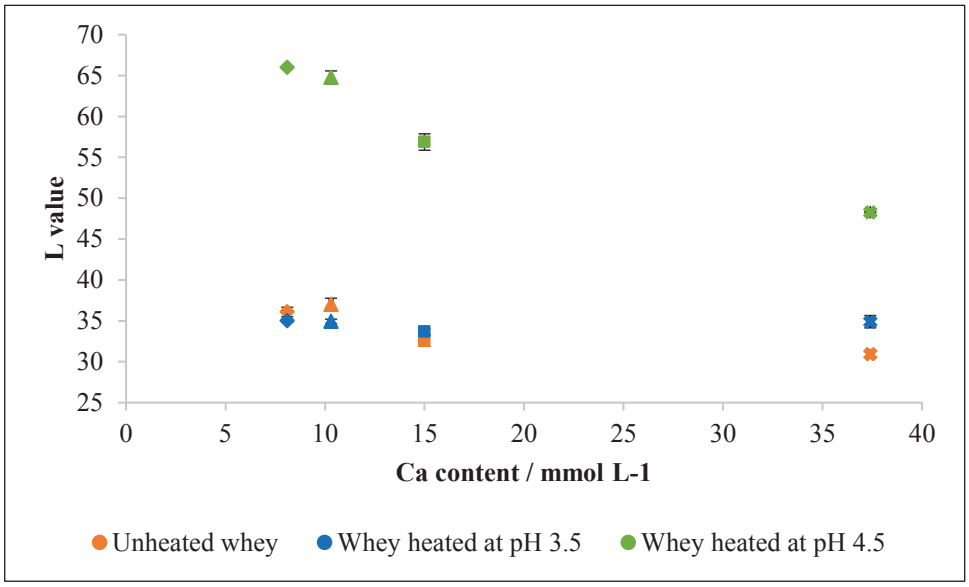
Appendix 3: The change of (-) a value as affected by the pH at heat treatment (90°C/ 5min). a < 0; green. L – acid whey, R – rennet whey, A – G - commercial whey. Error bars represent standard deviation of triplicate.



Appendix 4: The change of b value as affected by the pH at heat treatment (90°C/ 5min). b > 0; yellow. L –acid whey, R –rennet whey, A – G commercial whey. Error bars represent standard deviation of triplicate.



Appendix 5: The variation of L value (L = 0; black, L = 100; white) upon heating of model whey produced at; ◆ - pH 6.6, ▲ - pH 6.2, ■ - pH 5.7 and x - pH 4.6.

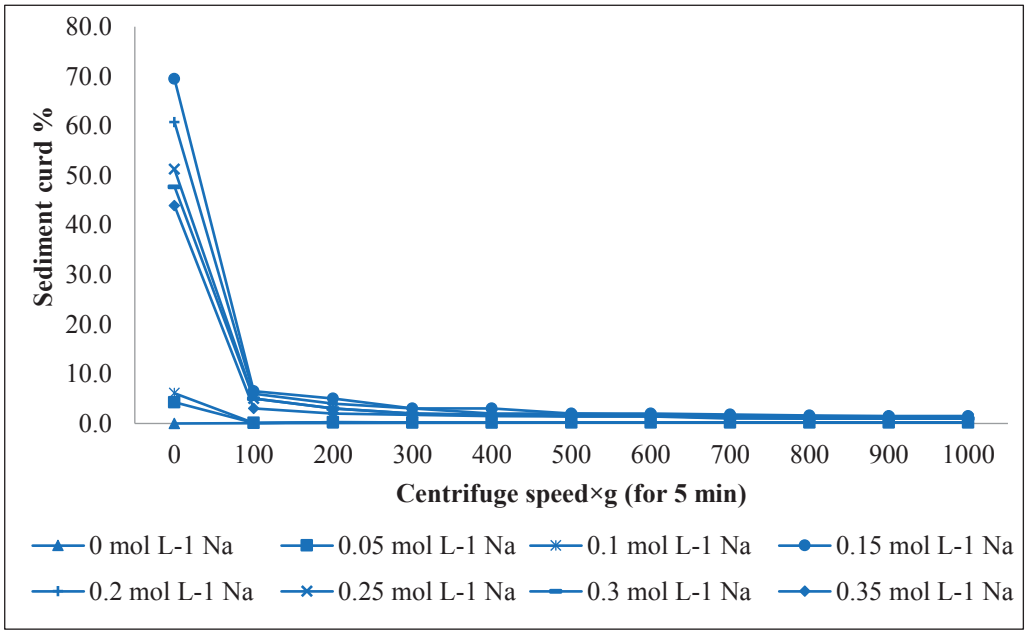


Appendix 6: Effect of added NaCl on whey protein aggregation upon heating (90 ^{o}C/ 5min) at pH 3.5

Added NaCl concentration in whey / mol L^{-1}	Sediment curd %	Turbidity%	Particle size diameter / µm			
			D (4,3)	d (0.1)	d (0.5)	d (0.9)
0	0.0	92.3	31.355	3.108	8.093	95.125
0.05	4.3	99.2	31.536	3.057	8.172	111.153
0.1	6.1	99.4	73.105	3.181	10.67	250.827
0.15	69.4	99.7	70.108	3.49	9.074	253.285
0.2	60.8	99.7	15.896	4.877	9.516	19.723
0.25	51.3	99.8	20.275	4.748	10.258	22.886
0.3	47.8	100	28.382	5.382	11.492	97.992
0.35	43.9	100	15.00	5.621	12.433	25.997

Note: D (4,3) – volume mean diameter, d(0.1), d(0.5) and d(0.9) represents particle diameters covering 10%, 50% and 90% fractions respectively

Appendix 7: The change of sediment curd% upon centrifugation of heated whey with added NaCl.



Appendix 8: Conductivity and Na content of some commercial samples, as a guideline for prototype product development

Product name	**pH**	**Conductivity / mS cm^{-1}**	**[1]mg of Na/ 100 ml**
Carbonated soft drink	2.38	13.16	10.0
Orange juice	3.42	15.05	12.9
Chicken stock	5.14	128.2	447.0
Chicken stock (low salt)	5.14	77.6	28.0
Vegetable stock	5.36	136.9	541.0

[1]Details from the product labels.