A Novel Strategy for Minimizing Acid Whey Generation during Greek Yoghurt Production

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Abstract

Greek yoghurt is thicker, creamier and surpasses regular yoghurt in terms of protein richness, flavour, texture and taste. Greek yoghurt attains this unique combination by incorporating straining at the end of the production process. However, such straining also generates whey with high lactic acid content, which can cause serious environmental problems unless properly disposed. Difficulties in post-process treatment of this whey stream still presents a main challenge for the industry although various approaches have been attempted. The necessity of developing techniques to reduce the acid whey production are thus importantly emphasized by the dairy industry.

This present study aimed to explore an alternative strategy for Greek yoghurt production, which would reduce the amount of acid whey released. The main purpose of whey removal is to obtain desired concentration of total solids in the final Greek yoghurt. The proposed strategy thus aimed to increase the total solid level in initial milk base prior fermentation. This would potentially lead to lower levels of acid whey removal after fermentation. Therefore, the proposed technique would potentially provide a solution to the current acid whey issue. The study applied milk fortification and ultrafiltration techniques as two different approaches to obtain higher dry matter content of the initial milk base. Milk fortification was performed using milk protein concentrate (MPC) and skim milk powder (SMP) to obtain 15%, 20% and 23% w/w dry matter content in the initial milk base. Cloth bag filtering was performed where necessary to obtain a 23% w/w total solid level in yoghurt base. At the second trial, as alternative to milk fortification, ultrafiltration was performed prior fermentation to enhance initial milk total solid levels up to 13% and 17% w/w. Such adjustments were

investigated from the technological point including starter culture performance, chemical and physical properties of manufactured Greek yoghurt and generated acid whey. A comparison was also made to commercially available products.

Addition of MPC and SMP greatly influenced the starter culture activity during fermentation. Protein fortification significantly enhanced the *Lb. bulgaricus* growth rate and proteolytic activity. Higher solid levels significantly increased the lag phase of *St. thermophilus* growth due to the effect of osmotic pressure. Greek yoghurt prepared with 23% w/w total solid level, obviously resulted in zero amount of acid whey generation due to total avoidance of filtration after fermentation. However, poor gel structural properties were observed. Best structural properties including higher gel strength and lower syneresis were observed in the Greek yoghurt produced with 20% w/w initial milk total solid compared to manufactured or commercially available products, while acid whey generation was lowered by 47% w/w from typical values of acid whey generated in commonly used commercial processes.

Use of the ultrafiltration impacted more on properties of Greek yoghurt compared to milk fortification. Greek yoghurt prepared with 23% w/w total solid level resulted in zero acid whey discharge. However, this yoghurt exhibited inferior structural attributes negatively impacting quality. The Greek yoghurt produced with 20% w/w initial milk total solid level resulted in a 78% w/w reduction in released acid whey compared to the Greek yoghurt made with 15% w/w initial milk total solid. Increased gel hardness, lower syneresis, greater storage modulus for given forces, higher protein and higher fat contents depicted the properties of the Greek yoghurt produced with 20% w/w initial milk total solid. The resulting acid whey in this yoghurt also contained low amounts of lactic acid and Ca, measured to be ~ 0.55% and ~ 0.288% w/w, respectively. The

present study highlighted that combined use of pre-treating yoghurt base with ultrafiltration prior to fermentation and whey draining after fermentation would result in a yoghurt gel of acceptable physical properties with a concomitant release of very small volume of acid whey.

Declaration

‘‘I, Gangani Uduwerella, declare that the Master by Research thesis entitled “A Novel Strategy for Minimizing Acid Whey Generation during Greek Yoghurt Production” is no more than 60,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work”.

Signature: [redacted] **Date:** 03 – July – 2017

(Gangani Uduwerella)

Dedicated to

my Parents Sanath, Seetha and my husband Uditha

.....

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Refereed publications:

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Uduwerella, G., Chandrapala, J., & Vasiljevic, T. (2017). Pre-concentration of yoghurt base by ultrafiltration for reduction in acid whey generation during Greek yoghurt manufacturing. *International Journal of Dairy Technology*. doi: 10.1111/1471-0307.12393.

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Chapter 1

Introduction

1.1 Background

Yoghurt has become an essential part of daily dietary intake from infants to adults around the globe due to its high acidity level, presence of favourable aroma compounds, desirable sensory profile and availability of essential nutrients such as proteins, fats, carbohydrates and minerals (El-Salam, 2002). With the widespread introduction of commercially made Greek yoghurt, people have become more attracted to Greek yoghurt compared to regular yoghurt due to its unique favourable properties. These properties include milky white appearance with a soft, smooth and spreadable texture, a clean acidic flavour and an increased product quality with a high protein content and a minimum wheying-off (El-Salam, 2002; Xu et al., 2008). Composition of Greek yoghurt has been studied by different researchers and reported to contain between 23 – 25% w/w total solids (Arls Food Ingredients, 2014), 8 – 11% w/w fat, 1.4 – 2.8% acidity and pH value in the range from 3.6 – 4.0 (Abu-Jdayil et al., 2002) In comparison to regular yoghurt, this composition directly contributes to the remarkable increment in viscous body, while improving creamy flavour and moderating acidic flavour (Chandan, 2006; Tamime & Robinson, 1999).

Technically, Greek yoghurt is defined as a semi-solid product derived from regular yoghurt by draining away part of its water and water soluble components, mainly lactose and salts (Nsabimana et al., 2005). This draining process, which takes place after controlled fermentation of milk, concentrates insoluble macro nutrients in the yoghurt mixture and strengthen the protein gel structure. Additionally, higher lactic acid concentration and drainage of significant amounts of water from the fermented product increases many desirable qualities in the final yoghurt (Tamime & Robinson, 1999). Currently, several well-known processes are used by the industry for this whey draining

process. These techniques include the traditional method using a cloth bag and modern technical methods such as the use of ultrafiltration, centrifugation, direct reconstitution and reverse osmosis (Nsabimana et al., 2005; Tamime & Robinson, 1999). The selected concentration techniques affect the chemical composition and the structure of the final product (Ozer et al., 1999b).

Although whey draining enhances the properties of Greek yoghurt, it introduces a main drawback to the production process due to the release of acid whey as a by-product. The major problem caused by acid whey is the pollution it creates when released into the natural environment due to its high toxicity and pollutant properties. Acid whey comprises of mainly lactose (70 – 72% w/w of the total solids), whey proteins (8 – 10% w/w), minerals (8 – 10% w/w) and lactic acid (1-2% w/w) (Tamime & Robinson, 1999). The pH of acid whey varies within 3.57 – 4.34 (Alsaed et al., 2013) due to the presence of lactic acid. Release of acid whey into waterways creates danger to the aquatic life, while spreading it onto the fields affects the physical and chemical structure of soil causing a decrease in crop yield.

Currently, industry has found only a handful uses for acid whey as a by-product including: mixing acid whey with silage to feed cattle, combining with manure to make fertilizer and treating as a bio-digester to generate electricity (Elliott, 2013; Prazeres et al., 2012). However, these current solutions are limited in practice due to many reasons. When mixing with cattle feed, caution is required due to possibility of energy overload and the suppression of metabolic activity of cows. The use as a fertilizer is also limited as acid whey can disturb the pH balance of soil making it too acidic for plants to grow. Converting whey into biogas requires higher capital investment, mainly due to the higher cost of the anaerobic digesters and requirement for underground installation

which deems unfeasible for small scale farms. Due to the above limitations, industry is still struggling to find an economical and effective solution for the acid whey issue.

There are two main approaches that can be applied in controlling the problems associated with acid whey. These include either minimizing generation of acid whey at the point of its origin, or finding processing solutions to already generated acid whey and converting it into value added products, which could be used as food ingredients. However, due to presence of lactic acid further processing of this whey stream is extremely difficult (Anand et al., 2013). For example, lactic acid hinders the ability of lactose to be crystallized causing difficulty in processing through spray drying during manufacture of whey powders (Wijayasinghe et al., 2015; Ganzle et al., 2008). Therefore, regulating the Greek yoghurt production process to avoid acid whey generation at the point of origin appears as a more feasible approach for sustainability of Greek yoghurt industry.

Few studies have been reported to produce Greek yoghurt with reduced acid whey generation. For example, fortification of the milk base using micellar casein concentrates to enhance milk protein levels has been discussed (Bong & Moraru, 2014; Merrill, 2014). However, those approaches are more related to Greek style yoghurt which obtains similar chemical and physical quality of Greek yoghurt through additions of different ingredients, instead of whey drainage. Although these studies show an attractive strategy for Greek style yoghurt production without generating acid whey, they do not meet the technical definition of real Greek yoghurt production, which incorporate draining of whey after fermentation. Furthermore, the higher cost of powder ingredients may not be economically attractive for the large scale production (Robinson & Tamime, 1993).

Therefore, investigation of alternative methods for increasing the protein content is required in optimizing Greek yoghurt production process. The current study was planned not only to increase total solids levels by fortification but also to apply concentrating milk proteins using an ultrafiltration technique prior fermentation. While the current approach would generate some milk permeate, which has a neutral pH and comprises mainly of lactose and minerals, this neutral stream can easily be processed by further downstream processing (Marwaha & Kennedy, 1988; Tsakali et al., 2010). For example, the industry can apply well known techniques for successful separation of mineral salts and lactose from such a stream and use the separated fractions as quality food ingredients (Alsaed et al., 2013; Chandrapala et al, 2015). Furthermore, the pure water stream obtained after the removal of salts and lactose can be used within the factory for purposes such as equipment washing. Therefore, this proposed study would potentially alleviate the issue of further processing acid whey and would aid sustainability of Greek yoghurt market. To ensure that the quality of the novel Greek yoghurt is comparable with currently marketed products, the final prepared Greek yoghurts and commercially available Greek yoghurts were evaluated in terms of their physio-chemical & structural properties and compared.

1.2 Research Aim and Objectives

The overall aim of this study was to propose novel strategies for production of Greek yoghurt of acceptable textural properties that would result in the release of reduced quantities of acid whey.

The objective of the project was thus to answer the following research questions;

- How would alterations of total solid levels in milk affect starter culture growth and their activity during the fermentation process?
- What would the chemical and rheological properties of Greek yoghurt be produced with these process alterations?
- What would the chemical and rheological properties of Greek yoghurt be if produced with the application of ultrafiltration technique to concentrate milk prior to fermentation?
- How would the chemical and structural properties of novel Greek yoghurts compare to commercially available Greek yoghurts?

1.3 Structure of the thesis

To achieve the main aim of proposing a process for production of Greek yoghurt with reduced acid whey generation, the proposed study was organized via several stages. The experiment was planned to develop three different Greek yoghurts, which were prepared using initial milk total solid levels at 15%, 20% and 23% w/w. Bacteriological, chemical and structural properties of developed products were investigated to answer the fundamental thesis objectives.

Following is a brief outline of each chapter that is contained in this thesis;

Chapter – 1

This chapter presents an introduction and highlights the significance of Greek yoghurt production. The industrial concerns regarding the acid whey issue are presented, followed by thesis objectives and thesis outline.

Chapter – 2

This chapter presents a literature review on Greek yoghurt production, classification of different production techniques and the fundamental theory behind yoghurt gel formation. Acid whey generation, whey related issues and their current usages are also discussed. Finally, the niche area requiring further research and development in Greek yoghurt production process is identified in this chapter.

Chapter – 3

In this chapter, chemical, bacterial and structural properties of Greek yoghurts are presented as a function of initial changes in milk composition during and after fermentation and compared to commercially available Greek yoghurts.

Chapter – 4

A novel method for Greek yoghurt production incorporating ultrafiltration to concentrate initial milk total solid is presented in this chapter. Chemical and structural properties of novel Greek yoghurts are presented and compared in terms of the properties of the yoghurts as well as the properties of generated acid whey.

Chapter – 5

This chapter presents conclusions and important findings drawn from the research project. The chapter also identifies and highlights the future directions for research, mainly on process moderations to minimize acid whey generation during Greek yoghurt production.

Chapter 2

Literature review

2.1 Evolution of Greek yoghurt

Fermentation is one of the oldest milk preservation methods, which can be traced back to around 10000 – 15000 B. C. (Tamime & Robinson, 1999; Litopoulou-Tzanetaki & Tzanetakis, 2014). Fermentation assists in extending a shelf life of perishable dairy products such as milk by increasing the acidity level, which thereby prevents the growth of most pathogenic microorganisms. In addition, fermentation creates a novel texture of milk by making it thicker and adding a unique flavour to milk. The products derived by fermented milk can be classified based on their physical and sensory properties (De Oliveira, 2014). Yoghurt is a variety of fermented dairy product, which gained high consumer popularity due to its specific nutritional, textural, flavour and rheological properties (Lee & Lucey, 2010).

Greek yoghurt is technically defined as a semi-solid product derived from regular yoghurt by straining away part of its water and water soluble components, mainly lactose and salts (Nsabimana et al., 2005). In accordance with this definition, Greek yoghurt production process is an extension of a regular yoghurt manufacturing. Concentration of insoluble parts in the yoghurt mixture and strengthening of the gel structure are some of the main processes taking place when converting regular yoghurt in to Greek yoghurt. Generally, Greek yoghurt is characterized by its white colour, soft and smooth body, good spreadability and a slightly acidic flavour (Nsabimana et al., 2005). These favourable characteristics place Greek yogurt in a highly competitive market position compared to a regular yogurt (Lange, 2013; Nsabimana et al., 2005).

Traditionally, Greek yogurt has been a major food item in the Middle Eastern countries (Al-Kadamany et al., 2002; Lange, 2013). Countries of Turkestan, the Balkans, the eastern Mediterranean, and the Indian subcontinent (Tamime & Robinson, 1999; Lange,

2013), all have a famous history in producing traditional Greek yoghurt. However, the exact processing methods traditionally used, and the name given to the product are reported to be different from country to country (Table 2.1).

Table 2. 1: Traditional names used for Greek yoghurt in different countries (Tamime et al., 2014)

Traditional name	Countries
Labneh	Eastern Mediterranean
Ta, than	Armenia
Laban zeer	Egypt, Sudan
Stragisto, sakoulas, tzatziki	Greece
Torba, suzme	Turkey
Syuzma	Russia
Mastou, mast	Iraq, Iran
Basa, Zimne, kiselo, mleko-slano	Yugoslavia, Bulgaria
Ititu	Ethiopia
Greek-style	United Kingdom
Chakka, shrikhand	India
Ymer	Denmark
Skyr	Iceland

In ancient times, Greek yoghurt was derived from regular yoghurt using an animal skin (Tamime & Robinson, 1999). Fermented milk was stored within an animal skin until it was consumed. During this process, a portion of the water soluble parts was absorbed by the animal skin. Another portion penetrated through the skin and evaporated. While this processing method resulted in Greek yoghurt with desired characteristics, it was considered unhygienic (Tamime & Robinson, 1999). Thus, continued evolution of Greek yoghurt processing has resulted in advancing it from this animal skin based method through to earthenware vessels to modern technological methods using ultrafiltration and reverse osmosis (Nsabimana et al., 2005). While technology has

provided new equipment, all these methods share the same basic principle of separating water soluble particles from water insoluble components of milk. The main intents of using more modern technologies have been in utilizing advantages such as shorter processing time, less labour requirement and increased product shelf life (Tamime & Robinson, 1999).

2.2 Milk as the Main Ingredient

Although milk of different animal species is used for yoghurt production in different regions of the world, cow's milk is the most prominent variant mostly used in industrial yoghurt production (Yıldız, 2010b). Milk is produced in the mammary glands of mammals during their lactation period. It is a nutritionally rich medium considered as the most complete food for infants (Park et al., 2013b). This heterogeneous liquid contains numerous nutrients including proteins, fat, carbohydrates, minerals and vitamins. Protein, fat and lactose are considered as macronutrients in milk. The minor compounds found in milk include enzymes, organic acids, nitrogenous compounds, and vitamins (Brisson & Singh, 2013). The milk compounds are present in either dissolved state (lactose and salt), emulsified state (fat globules) or as colloidal disperser (caseins and globular proteins). The average chemical composition of cow milk is given in Table 2.2. This composition may vary from time to time and breed to breed based on seasonal variations, breed and the stage of lactation (Dissanayake, 2011; Walstra et al., 2006c).

2.2.1 Proteins

Milk proteins are organic polymers which consist of twenty different amino acids. Different arrangements of amino acid sequences cause different proteins to have different functional properties. The average protein content of bovine milk is reported as

3.3% w/w. This protein content is subdivided into two fractions as casein (80% w/w) and whey proteins (20% w/w) (Kukovics & Nemeth, 2013).

Table 2. 2: Typical composition of bovine milk (Walstra et al., 2006c)

Component	Average content in milk (% w/w)
Water	87.1
Solid non fat	8.9
Lactose	4.6
Fat	4.0
Protein[a]	3.3
Casein	2.6
Mineral substances	0.7
Organic acids	0.17
Miscellaneous	0.15

[a] Non-protein nitrogen compounds not included.

2.2.1.1 Casein

The largest structural protein found in milk is a group of proteins termed caseins. Caseins account for 80% w/w of total milk protein content (Kukovics & Nemeth, 2013). These proteins create a complex structure, termed casein micelle, which has a spherical shape with 0.1 μm diameter. (Kukovics & Nemeth, 2013) Milk casein micelle is composed of four different types of caseins named as α-casein, β-casein, γ-casein and κ-caseins (Kukovics & Nemeth, 2013). These casein fragments arrange in a three dimensional network to form the complex casein micelle.

While the exact structure of the casein micelle is still unknown, several models have been proposed to interpret casein structure including the coat core model, submicelle model and internal structure model. The widely accepted casein submicelle structure is shown in Figure 2.1. This model shows that casein micelle consists of a number of

spherical submicelles which have a various diameter ranging between 12-15 nm. Each submicelle is made up of 20 – 25 casein molecules (Phadungath, 2005a). Due to the casein fragment arrangement in protein micelle, the micelles are divided into two groups. Hydrophobic inner core of the micelle is built with submicelles which contain α_s and β-caseins. Submicelles which contain α_s and κ-casein have hydrophilic nature at native stage and build the outer layer of casein micelle. The hydrophilic C terminal of κ-caseins is arranged near the outside of submicelle giving a hairy layer to the casein micelle.

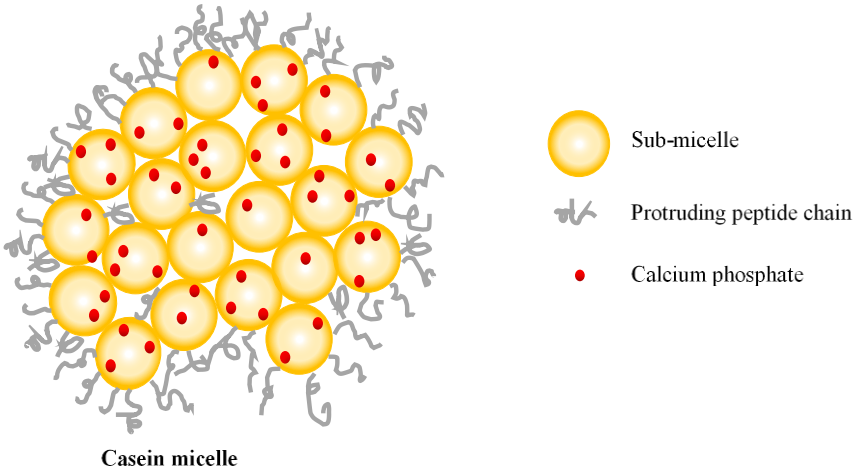

Figure 2. 1: The structure of casein micelle in the sub-micelle model showing the protruding C-terminal parts of κ-casein as proposed by Walstra (Phadungath, 2005a)

The casein sub micelles are bound with each other inside the casein micelle due to hydrophobic interactions between proteins. Also, the colloidal calcium phosphate builds cross link between the submicelles. However, casein micelles do not aggregate together in their native state due to the location of κ-caseins. The hairy layer prevents the casein micelle to aggregate with each other by steric and electrostatic repulsion. Therefore, casein micelles remain as independent particles and are colloidally suspended in water base fluid (milk serum) which surrounds them (Guzel-Seydim et al., 2010). Casein is

also the most heat stable complex molecule which resists up to 120 °C. However, it precipitates at its isoelectric point at pH 4.6 (Fox, 2003).

2.2.1.2 Serum proteins

These proteins compose about 20% w/w of whole protein content in milk. Most of the whey proteins are hydrophobic globular proteins with well-defined secondary and tertiary structures (Anema, 2009). There are different types of whey proteins found in milk such as, β-lactoglobuline, α-lactoalbumin, bovine serum albumin, immunoglobulin and lactoferrin. They are heat labile and gets denatured above 85 °C. β-lactoglobulin plays a major role at lower pH yoghurt gel formation due to this heat denaturation ability (Phadungath, 2005a).

2.2.2 Fat

Milk fats are the largest and the lightest particles found in bovine milk. It dissolves in milk serum as fat in water emulsion. It has a globular in shape with various sizes ranging between 1 – 10 µm (Tamime & Robinson, 1999). The fat globule is divided into two main types called fat globular membrane and core. About 70% w/w of fat globule membrane is made of proteins (especially enzymes) while the other 30% w/w is made of polar lipids like phospholipids, cerebrosides and cholesterol. The inner core of the fat globule consists of non-polar lipids such as tryglycerides (98% w/w), diglycerides and monoglycerides, fatty acids, sterols, carotenoids and fat soluble vitamins (Gordon, 2013; Gallier, 2010).

2.2.3 Carbohydrates – Lactose

Lactose is a disaccharide and the primary carbohydrate found in bovine milk which readily dissolves in water (Crisa, 2013; Bylund, 1995). In order to utilize the lactose as an energy source by the body, Lactose needs to be hydrolysed into its monomers D-glucose and D-galactose (Be Miller & Huber, 2008). In yoghurt processing, this hydrolysis phenomena takes place during the fermentation stage. Yoghurt starter culture bacteria convert lactose into D-glucose and D-galactose using bacterial enzymes called lactase (β-galactosidase) (Bylund, 1995). Thereafter it is further broken down into lactic acid via anaerobic fermentation (Be Miller & Huber, 2008). This lactic acid helps to reduce milk pH which govern yoghurt gel formation.

2.2.4 Minerals

Milk minerals are divided in to two groups; macro-elements (Ca, P, Mg, Na, K, Cl) and trace elements (Fe, Cu, Zn, Se, Mn, I, F, Cr, Pb, Cd, Co, Mo, As, Ni, Si, B) (Gaucheron, 2013). Calcium and Phosphorous are the most important macro-elements found in milk which stabilize protein micelle by making cross links between casein sub micelles. While average Ca content of cow milk is reported to be around 1200 mg/L, it is highly affected by the stage of lactation (Gaucheron, 2013). The 99% w/v of this Ca amount is distributed in casein phase and serum phase in 2:1 ratio. Ca which are in casein micelles, make granular structures "colloidal $CaPO_4$" with the incorporation of organic phosphate and inorganic phosphate (Gaucheron, 2013). In the milk serum phase, Ca is dispersed as free Ca^{+2} ions and also interact with Citrates and inorganic phosphates. Low milk pH at fermentation converts insoluble Ca in casein micelle phase into soluble Ca. This soluble Ca is transferred into the serum phase.

2.3 Greek yoghurt production process

The initial stages of Greek yoghurt production are similar to regular / set type yoghurt production. An additional filtration step at the end, converts the regular yoghurt into Greek yoghurt as shown in Figure 2.2.

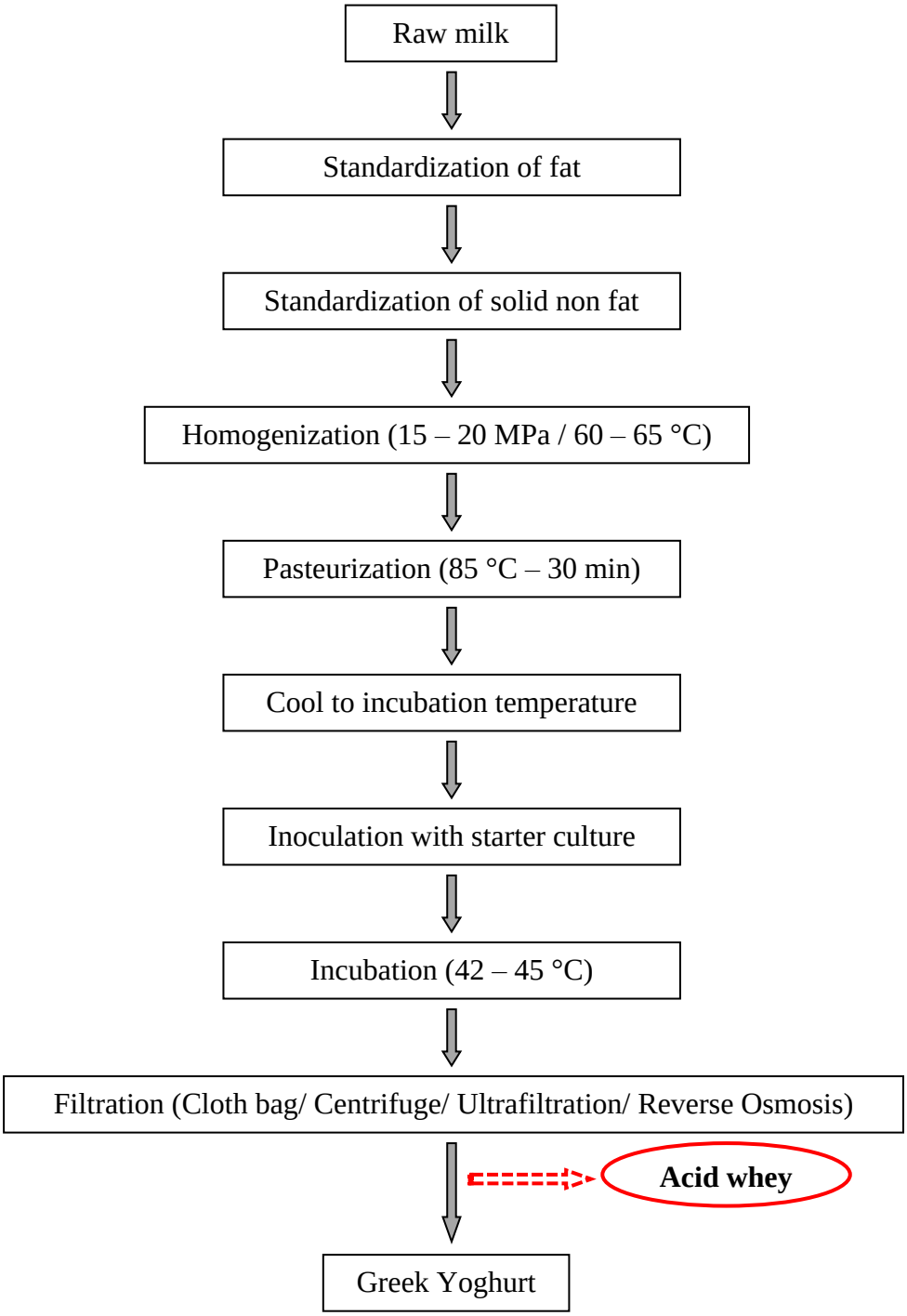

Figure 2. 2: Typical Greek yoghurt production process

2.3.1 Standardization

The milk standardization is carried out mainly to minimize the impact of compositional variations on the final product during the Greek yoghurt production. Furthermore, milk standardisation is performed to achieve a desirable texture profile of the yoghurt. Milk standardisation is generally performed on two aspects: standardization of milk fat and standardization of solids non-fat (Chandan & O' Rell, 2013). Solid non-fat portion comprises of lactose, proteins and minerals.

2.3.1.1 Fat standardization

The average fat content in bovine milk is between 3.2 - 4.2% w/w (Sfakianakis & Tzia, 2014). The fat content in bovine milk during lactation is mainly affected by the environmental temperature changes. For example, the fat content in milk has been observed to decrease with increasing temperature during summer (Sekerden, 1999; Lacroix et al., 1996; Ozrenk & Inci, 2008). In addition, the fat content changes from 3.1 to 2.3% w/w when seasons change from winter to summer (Ozrenk & Inci, 2008). In Greek yoghurt industry, the fat level is controlled within set limits to enrich the consistency and viscosity of the resultant yoghurt (Walstra et al., 2006a; Shaker et al., 2000). In addition, milk fat level significantly affects the rate of pH decline and the pH lag phase during milk fermentation (Soukoulis et al., 2007; Sfakianakis & Tzia, 2014). To obtain these desirable advantages in the final yoghurt, it is necessary to standardize the milk fat levels in the range of 8 – 10% w/w (Ozer & Robinson, 1999).

Cream and skim milk are commonly used to adjust the fat levels. The amount of cream or skim milk required for fat standardization can be calculated using the Pearson's square method as summarized in Figure 2.3. The corresponding equations to calculate

the amount of each raw material (A and B) for a given fat level are given by Equations 2.1 and 2.2. (Bird, 1993).

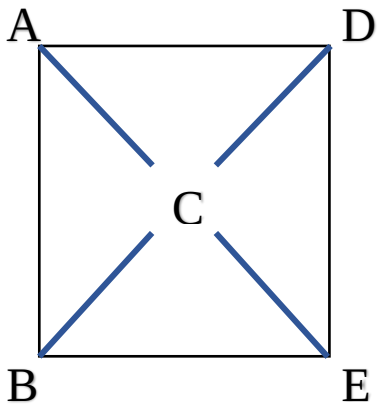

Figure 2. 3: The Pearson's square method for milk standardization

Where;

A – is the fat % of the first raw ingredient

B – is the fat % of the second raw ingredient

C – is the required fat % in finally standardized milk

D – is the parts of A required for milk standardization

E – is the parts of B required for milk standardization

F – is sum of D and E (total parts of A and B)

$$A = \frac{(B-C)\ or\ (C-B)\times 100}{F} \qquad \text{Equation 2.1}$$

$$B = \frac{(A-C)\ or\ (C-A)\times 100}{F} \qquad \text{Equation 2.2}$$

2.3.1.2 Solid non-fat standardization

The variation of solids non-fat, especially protein and lactose, is observed to be affected by different dietary supplements received by the cows, stage of lactation and season of the year (Gall, 2013; Park et al., 2013a). The solids non-fat content in milk governs the properties such as viscosity, texture, mouthfeel and syneresis of the final prepared

Greek yoghurt (Jaros & Rohm, 2003). Addition of solids non-fat ingredients also improves the structural properties and flavour in the end product (Tamime & Robinson, 1999). Added nutritional supplementary ingredients are also important during fermentation to supply the nutritional media that are essential for the growth of starter cultures. The legal standard for solid non-fat contents in yoghurt can vary between 8.2 - 8.6 g/ 100 g final product based on different legislations and codex regulations adopted across different regions (Tamime & Robinson, 1999). Yoghurt manufacturers mainly follow these standards during Greek yoghurt production to maintain the final Greek yoghurt quality.

Milk fortification with different nutritional rich ingredients is a commonly used successful method for milk solid non-fat standardization (Jaros & Rohm, 2003). Milk fortification can be achieved by adding powdered milk enriched with different ingredients such as proteins, lactose and minerals. According to a given requirement, milk powder is selected considering its included ingredients. Milk fortification is also reliant on the cost and availability of ingredients, scale of production and method of fortification (Jaros & Rohm, 2003). Full cream milk powder is one such ingredient which when added to the milk mixture can enhance the total solids level, while providing most of the milk constituents including protein, lactose, fat and minerals. In low fat or non-fat Greek yoghurt production, use of skim milk powder instead of full cream milk powder is recommended to avoid incorporation of fat to the milk mixture. Milk protein concentrate (MPC) is another kind of milk powder used by the industry to provide protein in large quantities to the final product (Tamime & Robinson, 1999). MPC powders are produced by ultrafiltration of milk which removes lactose. Therefore, it possesses the important ability to provide a Greek yoghurt processing method that addresses the requirements of lactose intolerant consumers. In addition to these main

ingredients, whey powder, casein powder and non-milk proteins such as soy milk and its protein derivatives can be added to fulfil nutrient requirements (Tamime & Robinson, 1999).

2.3.2 Homogenization

Milk can be considered as an emulsion of fat globules and a colloidal dispersion of casein micelles. These globular fat particles vary in size ranging between 1 – 10 µm (Tamime & Robinson, 1999). During homogenization these fat globules get disrupted to diameters below 2 µm (Tamime & Robinson, 1999). In addition, homogenization helps to improve the desirable structural properties in the final Greek yoghurt (Tamime et al., 1991b). The reduction of fat globule size (Figure 2.4B) positively contributes to prevention of cream layer separation during the fermentation. The increased surface area of fat globules during homogenisation significantly increases the binding of proteins (Lee & Lucey, 2010). Thus, the newly formed protein-fat particles act as building blocks in producing a strong gel network and increasing the water holding capacity due to increased hydrophilicity of fat-protein particles. In addition, the increased number of fat globules increases the light refraction and scattering, improving the white colour of the final product (Lee & Lucey, 2010; Walstra et al., 2006b).

The working principle of homogenizer is to force a liquid through a valve with a narrow opening (Figure 2.4A). This splits the particle size in milk components, converting high potential energy stored by milk to kinetic energy (Walstra et al., 2006b). Greater liquid velocity obtained using a narrow opening in the valve leads to an increased turbulence. Generally, energy less than 0.1% of the total kinetic energy is used for fat disruption. Several factors can affect the size of disrupted fat globule size (Walstra et al., 2006b). One such factor is the type of homogenizer utilized. Construction of the homogenization

valve is an important parameter which determines the resultant fat globule size. Differently built homogenization valves with same applied pressure show considerable variations in fat globule sizes due to different liquid passing times (Walstra et al., 2006b).

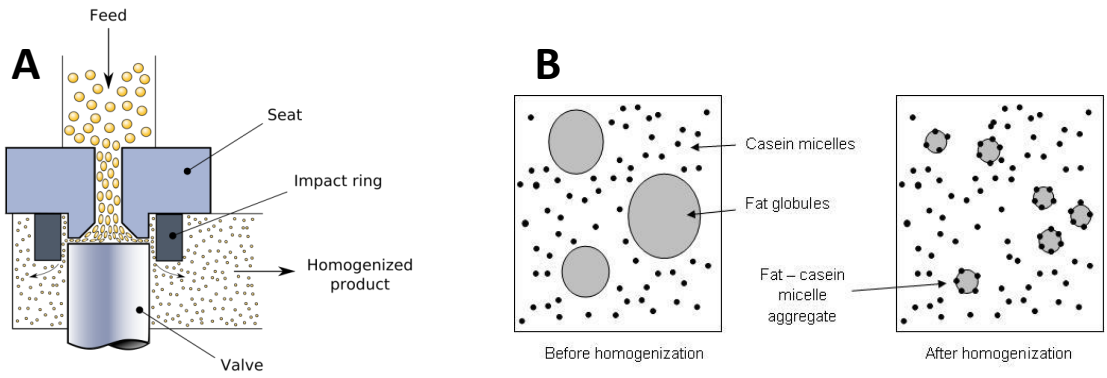

Figure 2. 4: (A) Principle of homogenization (B) Homogenization effect on the milk (Dhankhar, 2014)

Industrial homogenizers are mostly manufactured as using either as a single stage or a double stage design (Dhankhar, 2014; Kilara, 2013). Fat particle disruption takes place only once in a single stage homogenizer. This type of homogenizers is widely used with low fat liquids where a high viscosity is desired. The double stage homogenizer works in two stages to reduce the particle size (Walstra et al., 2006b). These machines are widely used for liquids which contain high fat levels, high solid contents and on occasions where low viscosity is desired.

The effectiveness of homogenization is dependent on the liquid temperature (Walstra et al., 2006b). Homogenisation efficiency decreases at low temperatures (below 40 °C) due to crystallization of fat. Typically, homogenization of milk is undertaken in the temperature region between 55 – 65 °C. Pressure control is also an important parameter during homogenisation to avoid unnecessary particle size reductions in milk. For

example, ultra-high pressure may cause whey protein denaturation and disruption of casein micelles (Lee & Lucey, 2010). Moreover, unsatisfactory homogenization could occur under situations such as pressure fluctuations, air inclusion or liquid contamination with solid particles such as dust (Walstra et al., 2006b). Typical homogenization pressure for milk varies between 15 MPa and 20 MPa (Lee & Lucey, 2010; Walstra et al., 2006b).

2.3.3 Pasteurization

In dairy industry, typical time / temperature combination used to pasteurize milk is 72 °C for 5 seconds (continues flow pasteurization) or 63 °C for 30 minutes (batch pasteurization) (Lewis, 1994; 2003). The main objective of pasteurisation is to reduce the number of harmful microorganisms present in milk down to a safe level under which they do not affect consumer health (Lewis, 2003). During yoghurt manufacturing, pasteurization is performed at 80 – 85 °C for 30 minutes or 90 – 95 °C for 5 minutes (Lee & Lucey, 2010). Apart from ensuring consumer safety, pasteurisation during yoghurt manufacturing is also aimed at obtaining the desirable chemical changes within milk (Lewis, 1994) and to create a microbial free media for starter culture to grow during fermentation.

The basic desired chemical change in milk under these pasteurization conditions is the denaturation of whey proteins (Singh, 2009). The denaturation of whey proteins takes place in two stages; unfolding followed by aggregation. When milk is heated to temperatures above 70 °C, 85 – 95% w/w whey proteins are denatured which then crosslinks with caseins. As an example, most of denatured β-lactoglobulins particularly interact with κ-caseins at the casein micelle surface and later help initiating protein coagulation before milk reach its isoelectric point of pH 4.6. (Anema, 2009;

Phadungath, 2005a). Therefore, pasteurization helps in achieving milk fermentation in a short time period.

Two methods are practiced by the industry (Jong, 2008) for milk pasteurisation. First method is batch pasteurization, which is performed in an enclosed tank. The milk is heated inside the tank to the recommended temperature and left for the required time period before releasing for further processing. This equipment is widely used in small scale yoghurt productions. In the second method, pasteurization is carried out as a continuous operation using a heat exchanger. The milk passing through a heated tube provides heating for the recommended time. Large scale yoghurt plants mostly adopt this type of pasteurization.

2.3.4 Fermentation

Fermentation is the process where milk is chemically and structurally converted into yoghurt. Selective microorganisms called “starter bacteria” mainly govern this phenomenon. Milk that has been subjected to standardization, homogenization and pasteurization provide the required nutrition supplement and a suitable media for the culture growth.

2.3.4.1 Activity of starter cultures

Two main functions are fulfilled by the starter culture microorganisms. The main function is to generate sufficient amounts of lactic acid in a short time period to reduce the milk pH and thus to promote protein coagulation (Anema, 2008b). The secondary function is to release desirable chemical components which enhance acceptable texture, viscosity and flavour of the final product (Gurakan & Altay, 2010; Stevens, 2003). Starter bacteria can be classified into two main groups according to their favourable

optimum temperatures: mesophilic and thermophilic (Vasiljevic & Shah, 2008). The mesophilic bacteria show maximum growth at temperatures around 26 °C. Strains such as *Leuconostoc mesenteroides* subsp. *cremoris* and *Leuconostoc lactis,* used in dairy industry are included in this group (Vasiljevic & Shah, 2008). Most of the culture strains used for yoghurt production belong to the thermophilic group (Vasiljevic & Shah, 2008). They are more heat stable than mesophilic cultures. The optimal growing temperature for thermophilic bacteria is around 42 °C. Thermophilic starter cultures such as *Lactobacillus* and *Streptococcus* strains are widely used in dairy fermentations. Industrial manufacturing of yoghurt relies greatly on the relationship between these two thermophilic lactic acid bacteria (Mihail et al., 2009). Common, well-balanced starter culture strains used by the industry consist of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lb. bulgaricus*) (Stevens, 2003). Other cultures which have been used in the yoghurt manufacturing include *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus acidophilus* and *Lactobacillus helveticus*.

2.3.4.1.1 *Lactobacillus delbrueckii* subsp. *bulgaricus*

Morphologically, *Lactobacillus bulgaricus* is a rod shaped bacterium with rounded ends (Figure 2.5A). They normally exist as either single or as a collaborated chain with 3 – 4 short rods (0.5 – 0.8 x 2.0 – 9.0 µm) (Teixeira, 1999). Long chains mostly appear at late stationary phase cells. Furthermore, internal granulation occurs when the *Lactobacillus bulgaricus* becomes older. The morphological characteristics of *Lactobacillus bulgaricus* also depend on the composition of growing media and impact of oxygen. *Lactobacillus delbrueckii* subsp. *bulgaricus* is a Gram-positive, catalase-negative and non-motile bacteria characterizing anaerobic/aero-tolerant, homo-fermentative behaviours (Gurakan & Altay, 2010). More than 90% of carbohydrate types used by *Lactobacillus bulgaricus* bacteria is composed of fructose, glucose and lactose

(Teixeira, 1999). Since this bacterium is homo-fermentative, converts these simple sugars almost exclusively into lactic acid as the end product. Depending on conditions of fermentation, acetaldehyde, acetone, acetoin and diacetyl are produced in small quantities as by-products (Teixeira, 1999).

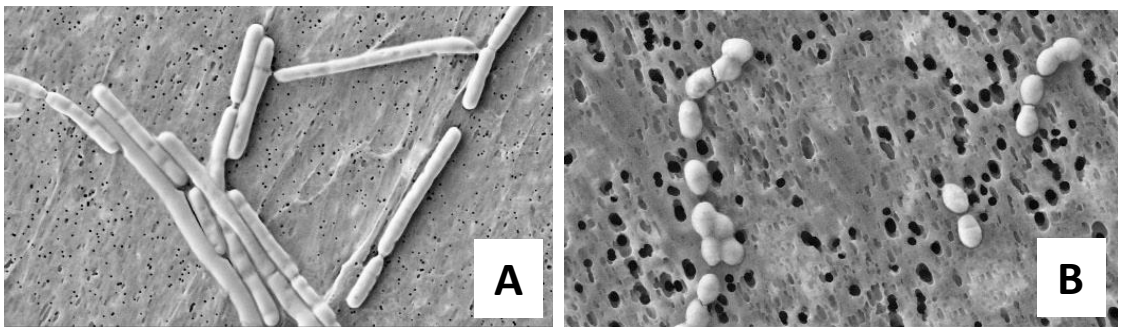

Figure 2. 5: Scanning Electron Microscopic images of (A) *Lactobacillus delbrueckii* subsp. *bulgaricus* (B) *Streptococcus thermophilus* (Chandan & O' Rell, 2013)

2.3.4.1.2 *Streptococcus thermophilus*

Streptococcus thermophilus is present in milk in pairs or long chains which consist of 10 – 20 cells (Figure 2.5B). This spherical shaped, 0.7 – 0.9µm long bacterium shows unique characteristics of Gram – positive, facultative anaerobic, non-motile and catalase negative behaviour (Zirnstein & Hutkins, 1999; Chandan & O' Rell, 2013). Similar to *Lactobacillus bulgaricus*, this culture is also homo-fermentative sucrose, glucose, galactose in addition to lactose, and sometimes use fructose as a carbon source instead of galactose (Chandan & O' Rell, 2013). Although this bacterium requires amino acid as a nitrogen source, its proteolytic activity is weak. Therefore, it depends on other symbiotic bacterial activity to provide these requirements for maximum functionality. The optimal growth temperature for *Streptococcus thermophilus* is between 40 to 45 °C although it can survive at 60 °C for about 30 minutes (Chandan & O' Rell, 2013).

2.3.4.1.3 Symbiotic growth

The quality of milk fermentation greatly relies on the co-operation between *Streptococcus thermophilus* and *Lb. bulgaricus*. Indirect positive interaction between these two bacteria types is called "proto-cooperation" (Driessen, 1981; Radke-Michell & Sandine, 1984; 1986). This bacterial association functionally accommodates bacterial growth, lactic acid production and aroma compound development in the final product. Functionally, both bacterial types depend on each other's performance. *Streptococcus thermophilus* produce pyruvic acid, formic acid and CO_2 during fermentation (Parisi, 2014; Gurakan & Altay, 2010), all of which stimulate the growth of *Lb. bulgaricus* which in return is a highly proteolytic bacterium thus produces peptides and amino acids that stimulate the growth of *Streptococcus thermophilus* (Parisi, 2014; Gurakan & Altay, 2010).

Several incubation conditions affect the bacterial synergetic reactions (Walstra et al., 2006a) such as;

- Incubation time – shorter incubation times deliver lower acid production. This is because *Streptococcus thermophilus* bacteria propagate in greater amounts at initial stage of fermentation. During long incubation runs, *Lb. bulgaricus* starts its progeny and takes over the fermentation process around mid-way (at pH around 5.5 – 5.6) of fermentation.
- Inoculums rate – increase in inoculation ratio of starter bacteria, the number of starter culture bacteria increases. It grows following a logarithmic pattern, doubling the numbers at each propagation cycle simultaneously accelerating the acid production.

- Incubation temperature – the incubation temperature varies between 43 – 45 °C. When the temperature exceeds 45 °C, *Lb. bulgaricus* growth is enhanced thus the product may be more acidic. However, lower temperatures (42 – 45 °C) are desirable for *St. thermophilus* growth.

Therefore, for efficient symbiotic starter culture activity during milk fermentation, it is required to balance and maintain the combined bacterial growth during the incubation time.

2.3.4.1.4 Sugar metabolism

The main sugar in milk, lactose is utilized as an energy source by LAB during fermentation (Elfahri, 2012). Starter cultures, *St. thermophilus* and *Lb. bulgaricus* (work symbiotically to generate lactic acid by breaking down lactose using their intracellular enzymes (Vasiljevic & Shah, 2008). Lactic acid as the final product of fermentation is excreted into milk where it governs pH reduction and imposes major physical changes in proteins. During the lactose metabolism process, lactose is translocated into the cell through the cell membrane assisted by an active transport system called lactose permease (Elfahri, 2012).

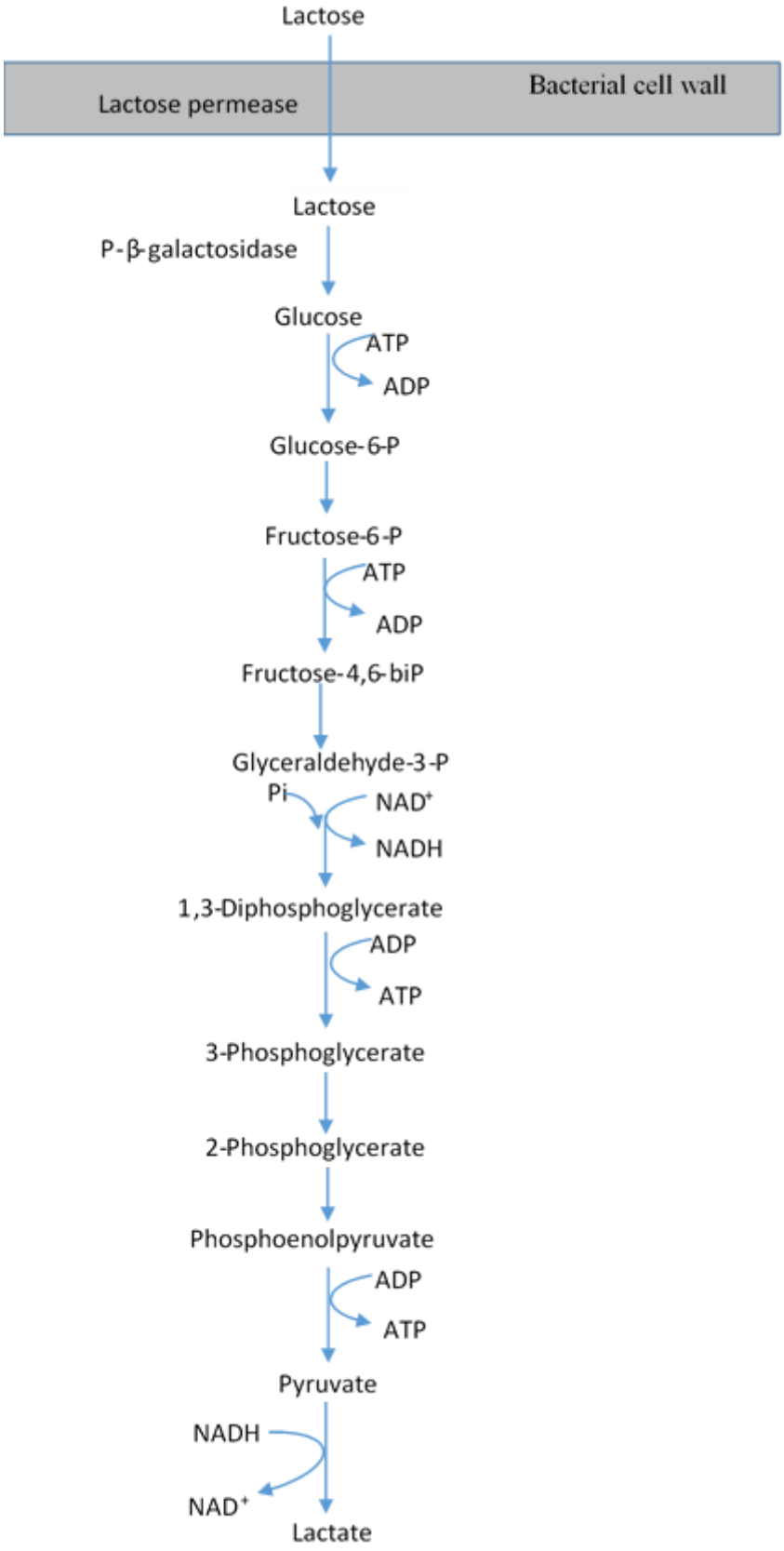

Figure 2. 6: Lactose metabolism by lactic acid bacteria Embden-Meyerhof-Parnas pathway (glycolysis) (Vasiljevic & Shah, 2008).

Once inside the cell, lactose is hydrolysed into glucose and galactose by a β-galactosidase enzyme. Liberated glucose is further metabolized into pyruvate via Embden – Meyerhof pathway as described in the Figure 2.6 (Vasiljevic & Shah, 2008; Donkor, 2007). In the final step, lactic dehydrogenase converts pyruvate into lactic acid, which is consequently released into milk. Most of yoghurt cultures do not possess ability to utilize galactose as the key enzymes of the phosphoketolase pathway are absent, thus galactose is also excreted and accumulated in milk. However, some strains of *Streptococcus thermophilus* possess galactokinase which activates galactose to galactose-1-P. This phosphorylated form of galactose is then transformed into either glucose-1-P or galactose-6-P, depending on the strain and further metabolized into lactic acid. Accumulated lactic acid lowers the pH level of milk which ultimately leads to destabilisation of the casein micelle structure (Vasiljevic & Shah, 2008). This loss of stability promotes protein coagulation which is one of the main goals of fermentation in yoghurt manufacturing.

2.3.4.1.5 Nitrogen metabolism

Other than using carbon sources to fulfil energy requirements, lactic acid bacteria (LAB) require a nitrogen source for essential cellular function including division and functional requirements during its propagation period. Lactic acid bacteria require various types of amino acids amounting from 4 to 14 amino acids based on the specific bacterial strains (Elfahri, 2012). Although milk is a rich nutrition source, it naturally lacks free amino acids essential for LAB growth (Vasiljevic & Shah, 2008). To accommodate this amino acid deficiency, LAB have developed a sophisticated proteolytic system that includes various proteinases and peptides to breakdown proteins, especially caseins. During this proteolytic activity, caseins are broken into free amino

acids and peptide fragments which LAB can effectively utilize during their growth (Donkor et al., 2007b).

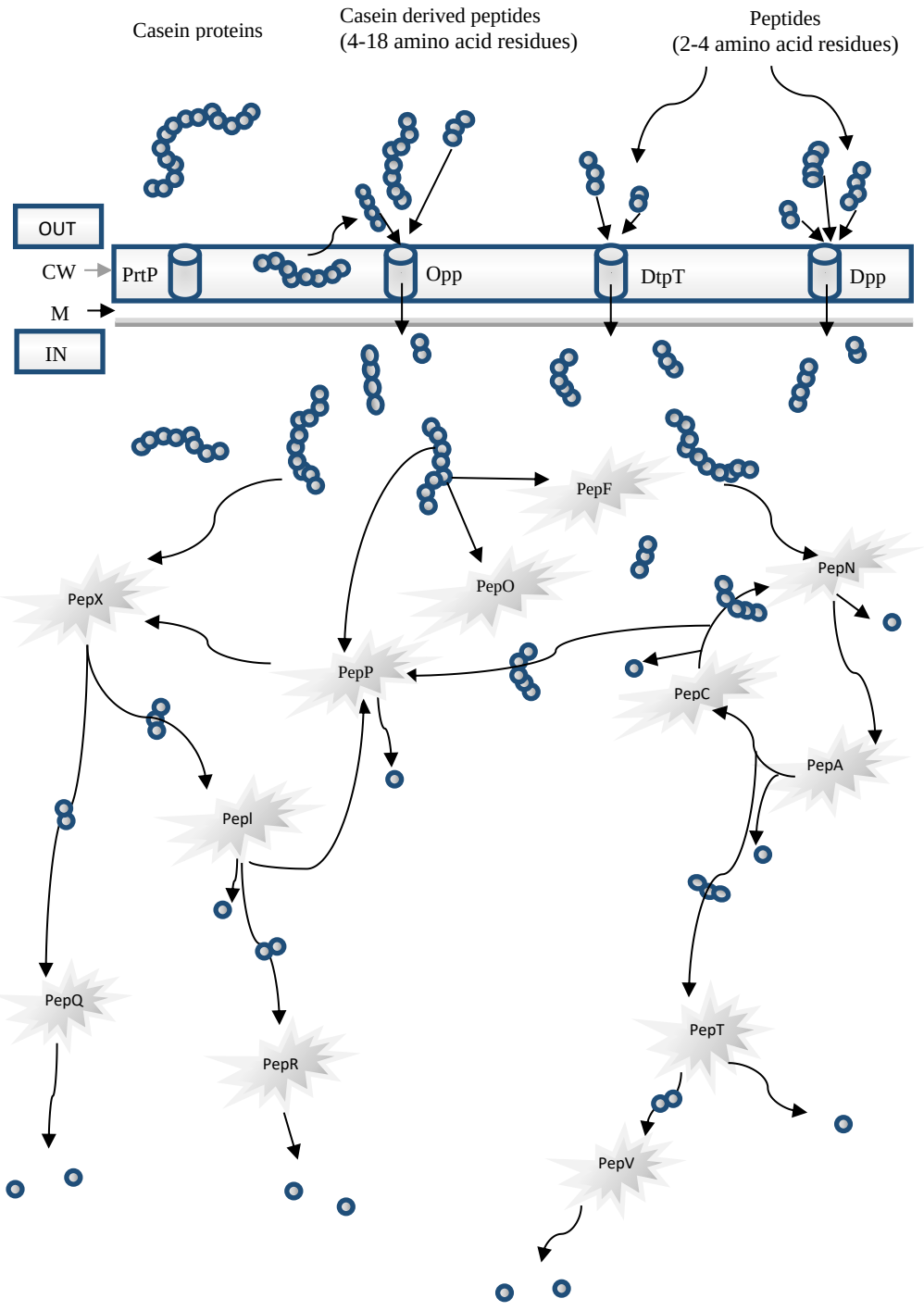

Figure 2. 7: Lactic acid bacteria Proteolytic system (Elfahri, 2012)

A number of steps is involved in this protein metabolism process as shown in Figure 2.7. Initially, LAB extracellular proteinases degrade casein into a large number of different oligopeptides (Elfahri, 2012). Then the transport systems translocate the

resulting smaller peptides and amino acids across the cytoplasmic membranes. The peptides which are generated through this process are then further broken into amino acids by the intracellular peptidase. Finally, these liberated amino acids are converted into various compounds by different enzymes which bacteria can use as structural building blocks (Elfahri, 2012; Donkor, 2007).

Some bacterial strains such as *St. thermophilus* have a weak proteolytic ability and therefore require additional peptides and free amino acids from an external source as a supplementary source for survival. *Lb. bulgaricus* is characterized by a high proteolytic activity and via symbiotic growth it assists *St. thermophilus* with nutritional requirements by generating adequate amount of essential amino acids for both of these cultures (Vasiljevic & Shah, 2008; Elfahri, 2012). Provision of these essential nutrients consequently leads to higher growth rates and more efficient lactose utilisation and ultimately lactic acid formation as the primary goal of fermentation.

2.3.4.2 Protein structural changes during fermentation

During fermentation, decrease of milk pH below 4.6 results in phase changes in milk proteins and forms a solid yoghurt gel. When milk pH starts to drop during fermentation, the net negative charge of casein micelle continues to get lowered. This results in a reduction of stability of colloidal $CaPO_4$ nano clusters and κ-casein hairy layer on the surface of casein micelle. At pH 5 – 6, κ-casein starts to shrink, resulting in decrease of both electrostatic repulsion and steric stabilization which largely affect casein micelle stability (Phadungath, 2005b; Lee & Lucey, 2010). In addition to this κ-casein dissociation, colloidal $CaPO_4$ clusters progressively start to solubilize and detach from the casein micelle. This results in release of α-caseins, β-caseins and κ-caseins to the milk serum phase (Lange, 2013). At pH 5.0, almost all the colloidal

$CaPO_4$ get solubilized and causes loosening of the internal structure of casein micelles and increases the electrostatic repulsion among the newly exposed phosphoserine groups. When milk pH becomes closer to its iso-electric point, the net charge of casein micelle becomes neutral and decreases the electrostatic repulsion between casein molecules (Phadungath, 2005b; Lee & Lucey, 2010). Ultimately this increase the hydrophobic and electrostatic interactions between caseins results in formation of a three dimensional network consisting of chains and clusters. The formation of heterogenic protein particles in milk during pasteurisation can lead to varying iso-electric points and thus can help to shift the aggregation and gelation to a higher pH level, causing aggregation to start before pH 4.6. For example, the formation of complexes between denatured β-lactoglobulin and κ-casein during pasteurization results in an isoelectric point around 5.2 – 5.4 for casein micelles (Lange, 2013).

2.3.5 Filtration

The main objective of filtration in Greek yoghurt production is to concentrate macro nutrients in the final yoghurt which enhances the total dry matter content while removing free water and soluble components termed as “whey”. The filtration step mainly contributes to establishing chemical composition and structural properties of the final Greek yoghurt. Several methods have been widely utilised including the traditional cloth bag method, use of ultrafiltration, reverse osmosis, centrifugation and direct reconstitution (Nsabimana et al., 2005).

2.3.5.1 Cloth bag

Traditionally, a bag made from a double layer of cheese cloth or similar material is used for the Greek yoghurt filtration process (Nsabimana et al., 2005). The bag is left

hanging in a cold room (4 °C) overnight to allow complete draining. This traditional Greek yoghurt production method results in excellent sensory qualities including good viscosity, pleasant mouthfeel, increased acidity up to 18 - 20g /kg and fat content raised to 100g/kg (Ozer & Robinson, 1999). Although this technique can achieve a high quality final product, it has two major drawbacks (Ozer & Robinson, 1999) limiting its fitness for widespread industrial use:

1) Reduced production capacity due to high labour involvement and production time

2) Difficulty in maintaining consistent product quality

Due to the increased production time and manual handling, this method can lead to unhygienic conditions, due to growth of undesirable microorganisms during processing (Litopoulou-Tzanetaki & Tzanetakis, 2014; Nsabimana et al., 2005). Shelf life of Greek yoghurt made with traditional cloth bag technique has been studied and reported to be limited due to deterioration by the growth of yeast and mould (Al-Kadamany et al., 2002). This quality deterioration is mainly governed by early stage flavour defection than textural changes.

2.3.5.2 Quarg-type separators

Modern commercial manufactory uses Quarg-type separators as an alternative to traditional cloth bag filtration for dry matter concentration. Quarg-type separator is similar in construction to centrifuges which are widely used for milk serum and fat separation (Spreer, 1995). The design of the equipment contains many nozzles which discharge concentrated yoghurt continuously. Therefore, when Quarg-type separators are to be used for yogurt production, a skimmed milk base is preferred over a full cream milk base (Tamime & Robinson, 1999). When using Quarg-type separators, an

additional increase of dry matter concentration be achieved by heating of curd prior whey separation (Spreer, 1995). Such heating can provide an added advantage to avoid possibility of over acidification in yoghurt gel and maximizing flavour attributes in final yoghurt. Process parameters associated with using Quarg-type processing can be adjusted based on measured the whey turbidity, allowing convenient optimization to achieve a desired set of properties. Quarg-type separators are more hygienic and effective whey separators compared to cloth bag whey separation. Due to structural differences during separation stage and the fermentation rate, yoghurts manufactured using Quarg-type separators may exhibit different compositions compared to those made using cloth bag. However, the resulting yoghurts have found to be organoleptically similar to each other (Tamime & Robinson, 1999).

2.3.5.3 Ultrafiltration

With the technological advancement, ultrafiltration has become the most suitable technique developed in dairy industry for selective concentration of dairy ingredients (Marcelo & Rizvi, 2009). Specific membranes with selective pore size around 0.01 µm are used. Application of pressure around 1000 kPa (Marcelo & Rizvi, 2009) drives the separation while pore size of the membrane selectively concentrates the final liquid mixture acting as a sieve. Milk is not subjected to any phase separation during ultrafiltration processing. With the growing demand for the ultrafiltration technique to be used during milk concentration, four types of ultrafiltration membrane designs have become widely available named as tubular membrane, flat membrane, spirally wound membrane and hollow – fibre design (Marcelo & Rizvi, 2009). When the milk is passed through the surface of a membrane, soluble milk particles which are smaller than membrane pores pass through into the permeate. Particles which are greater in

molecular sizes are rejected by the membrane and are left behind the membrane surface, creating a concentrated retentate (Figure 2.8).

In Greek yoghurt manufacturing, ultrafiltration can be applied either prior to or after milk fermentation. The chemical and physical properties of final Greek yoghurt are highly dependent on the process stage at which filtration is applied. During ultrafiltration of milk, lactose and minerals pass through the membrane while most macro molecules such as fat and proteins are retained by the membrane. Ca content in milk is reported to be about 117 mg/100g milk, while 68% w/w of total Ca is present in the colloidal phase with casein micelles (Looney, 2014). During ultrafiltration, colloidal Ca bound to caseins are retained in the retentate phase while soluble Ca accumulate in the permeate phase. However, when ultrafiltration is carried out after fermentation of milk (acidified milk), a portion of this colloidal Ca becomes solubilised and leads to a smaller proportion of Ca to be retained in the retentate by the membrane. The concentration of Ca in the retentate is therefore lower than that in non-acidified milk (Brule & Fauquant, 1980; Green et al., 1984; Davies & White, 1960). However, minor variations have been observed for the amount of lactose in the final product when ultrafiltration is applied prior and after fermentation.

Table 2. 3: Chemical component changes with concentration factor in skim milk (Bird, 1996)

Components	**Skim milk**	**Concentration Factor**				
		2	3	4	5	6
Lactose (g/100g milk)	4.8	4.8	4.78	4.77	4.76	4.76
Ash (g/100g milk)	0.73	0.73	0.73	0.72	0.72	0.71

Studies by Bird have shown that the concentration factor of milk during the ultrafiltration stage is the main driving factor for the compositional changes (Bird, 1996). Table 2.3 presents the relationship between lactose concentration and ash in skim milk relates to the concentration factor, as reported from this study. It is clearly observed that the lactose level changes from 4.8 g/100g milk up to 4.76 g/100g milk, and ash content of skim milk changes from 0.73 g/100g milk up to 0.71 g/100g milk as the concentration factor increases from zero to six.

Table 2. 4: Chemical composition of differently concentrated Greek yoghurt and permeates (Tamime et al, 1989)

Concentration Method		**Chemical composition (% w/w)**				
		Total solids	Protein	Fat	Ash	Lactose
Ultrafiltered milk	Retentate	20.10-21.94	6.36-7.22	8.75-9.58	0.96-1.05	4.03-4.09
	Permeate	5.38-5.85	0.28-0.32	–	0.42-0.46	4.68-4.77
Ultrafiltered yoghurt	Retentate	21.82-22.32	7.42-7.43	9.6-9.97	0.7-0.71	4.09-4.22
	Permeate	5.53-5.72	0.18-0.21	–	0.7	4.62-4.84
Traditional cloth bag	Retentate	23.5-24.96	8.07-8.38	10.53-11.55	0.68-0.7	4.2-4.35
	Permeate	6.13 – 6.28	0.28 – 0.32	0.02	0.76	5.05-5.22

In a separate study by Tamime et al, three Greek yoghurts prepared using ultrafiltered milk, ultrafiltered yoghurt and cheese cloth bag to get a desirable concentration (Tamime et al, 1989). The chemical composition of final Greek yoghurt retentate and permeates have been measured and reported as presented in Table 2.4. This study suggests that Ultrafiltered milk retains more minerals than filtering of yoghurt using Ultrafiltration and cloth bag. The retained lactose content is similar in Ultrafiltered milk

and Ultrafiltered yoghurt. This lactose content is higher than the lactose concentration retained using cloth bag.

The total solid elevation technique used during Greek yoghurt production has been found to affect the structure of the product. Fermentation of ultrafiltered retentate resulted in a greater firmness of formed protein gel compared to Greek yoghurt made with ultrafiltered yoghurt (Nsabimana et al., 2005). Also the structure of yoghurt gel made with ultrafiltered retentate has been found to be closer to the structure of Greek yoghurt made using the traditional cloth bag filtering (Nsabimana et al., 2005).

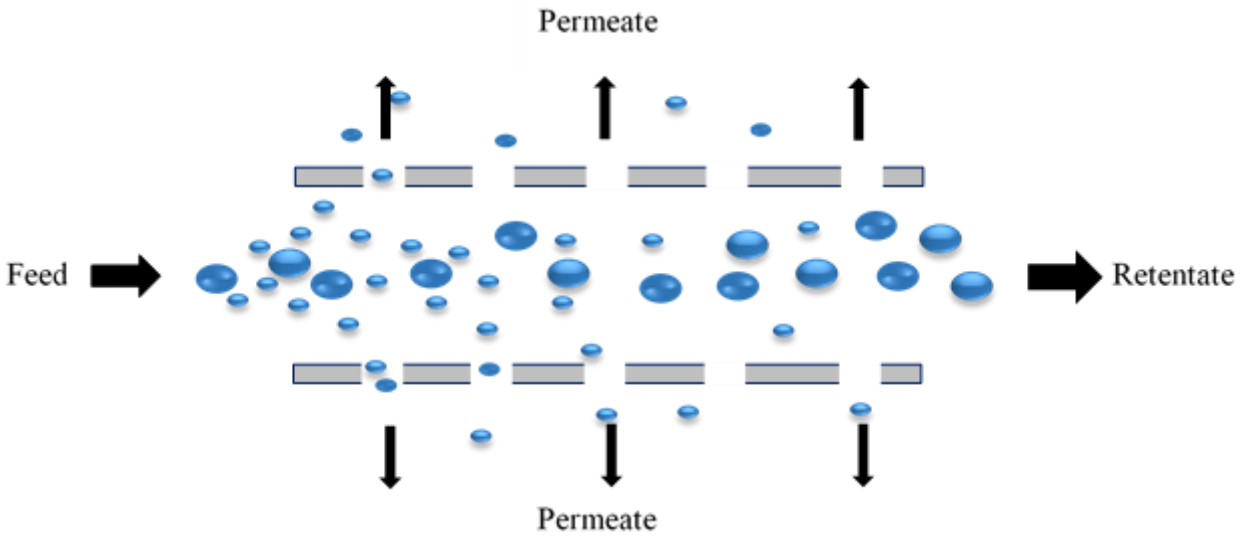

Figure 2. 8: Particle separation during ultrafiltration

The main drawback of using ultrafiltration technique prior and after fermentation in Greek yoghurt manufacturing is the loss of some compounds with whey. As mentioned previously, lactose is one major compound that is lost during ultrafiltration. However, lactose plays an important role in yoghurt processing as it helps in yoghurt fermentation as the major energy source used by the starter cultures. Similarly, soluble Ca is lost during ultrafiltration, but is an essential component in yoghurt gel formation. Calcium ions influence the degree of electrostatic attraction or repulsion between β-lactoglobulin and κ-casein by providing an ionic environment around the interacting molecules

(Lange, 2013). This promotes the association of β-lactoglobulin with casein micelles, which helps in protein coagulation in higher pH values (Lange, 2013). When ultrafiltration is carried out prior to fermentation, lactose and Ca are removed with the permeate due to their high solubility in water. This loss may negatively affect the fermentation and yoghurt gel strength. Therefore, it is necessary to have a sufficient amount lactose and Ca in milk during fermentation for efficient fermentation and strong gel development. When ultrafiltration is carried out after fermentation, the chemical composition of coagulum changes. Fermentation results in lactose conversion into lactic acid and increase of solubilised Ca in the serum due to the acidic environment. The ultrafiltration of yoghurt gel therefore results in acid whey which is higher in lactic acid and soluble Ca.

2.4 Acid whey

Whey is defined as a cloudy, slightly opaque liquid that is released as a by-product from yoghurt and cheese coagulum (Anand et al., 2013; Alsaed et al., 2013). Whey is generally classified into two groups in dairy processing based on the process which release them as, acid whey (which has pH 3.57 – 5.1) and sweet whey (which has pH 6.02 – 6.58) (Alsaed et al., 2013). The whey generated during typical Greek yoghurt manufacturing is acid whey.

2.4.1 Acid whey composition

In general, whey contains reasonable amounts of valuable nutrients including water (~ 95% w/w), minerals (~ 0.7% w/w), lactose (~ 5% w/w) and proteins (~ 1% w/w) (Tsakali et al., 2010), especially β-lactoglobulin (~ 57.9%), α-lactoalbumin (~ 24.6%) and immunoglobulins (~ 4.5%) (Anand et al., 2013). However, sweet whey and acid

whey streams are compositionally different (Table 2.5). The main differences between these two whey types are found in lactic acid content, lactose level and ash contents.

2.4.2 Acid whey issue

In commercial scale, production of four pounds of Greek yoghurt creates approximately three pounds of acid whey (Elliott, 2013). Production of Greek yoghurt has tripled over the last five years due to the higher consumer demand resulting 1.6 billion litres of acid whey per year (Chandrapala et al. 2016a; Chandrapala et al., 2015). This large amount of acid whey generation has become an issue from industrial point of view as acid whey cannot be dumped into water ways due to its environmental pollutant properties. Higher nutritional levels in acid whey, especially lactose and proteins, increase the Biological Oxygen Demand (BOD) in water and lowers pH level, negatively affecting the aquatic life (Elliott, 2013). In theory, applicable techniques for addressing the acid whey problem can be broadly categorized into two main approaches. Firstly, technological advancements in the production process may be implemented for lowering the quantity of the resulting whey. Secondly, the resulting whey may be converted to useful by-products.

Table 2. 5: Composition of Sweet whey and Acid whey (Alsaed et al., 2013)

Component	Sweet whey (% w/w)	Acid whey (% w/w)
Total solids	6.35	6.50
Moisture	93.7	93.5
Total protein	0.80	0.75
Lactose	4.85	4.90
Ash	0.50	0.80
Lactic acid	0.05	0.40

The industry has reported several limited uses of acid whey as a by-product. For example, manufactory of fertilizers by mixing acid whey with animal manure has been attempted. However, the higher acidity level limits the use of acid whey in this manner. While mixing of acid whey with silage to feed cattle has also been investigated (Elliott, 2013), it has also been found to have limited applicability as greater amounts of lactose badly can affect the animals' digestive system. A study on adding an enzyme to recover lactose from acid whey has been reported (Gonzalez & Smith, 2014). In this method, lactose and galactose are converted into galacto-oligosaccharides and the resultant soluble fibre is incorporated into food products such as cereal, baked goods and snack bars. In addition, acid whey has been used as a baby formula ingredient and treated as a bio-digester to generate electricity (Elliott, 2013; Prazeres et al., 2012). Neutralizing acid whey using a base solution such as calcium hydroxide and potassium bicarbonate to pH 7.0 has also been attempted in a patented technique for treating acid whey (Smith et al., 2014b). The neutralized acid whey is further processed into a neutralized acid whey concentrate by incorporation of either filtration method such as ultrafiltration, reverse osmosis, and nanofiltration or evaporation techniques (Watson, 2014) followed by drying using techniques such as drum drying, oven drying or freeze drying to obtain an acid whey powder (Smith et al., 2014a). Although above methods for using acid whey as a by-product are proposed, the processing is difficult in practice due its chemical and physical nature.

Presently, sweet whey, which is a by-product of cheese manufacturing having a closer to neutral pH, is commercially utilized in powder form to make valuable products including protein supplementary for energy boosting, lactose supplementary for bakery industry and fertilizers for farming (Chandrapala et al., 2015). Spray drying is the common method used for sweet whey processing as shown in Figure 2.9. Lactose

crystallisation is utilized to separate lactose prior to spray drying. This step is important to avoid spray drier nozzle blockages at whey powder processing. In acidic environments (pH below 5.0) lactose remains in amorphous form without crystallization (Wijayasinghe et al., 2015) and therefore causes difficulties in use of spray driers. As shown in Table 2.5, the composition of lactic acid is different in two whey types. The higher amount of lactic acid (0.4% w/w) present in acid whey is largely responsible for prevention of effective spray drying of acid whey.

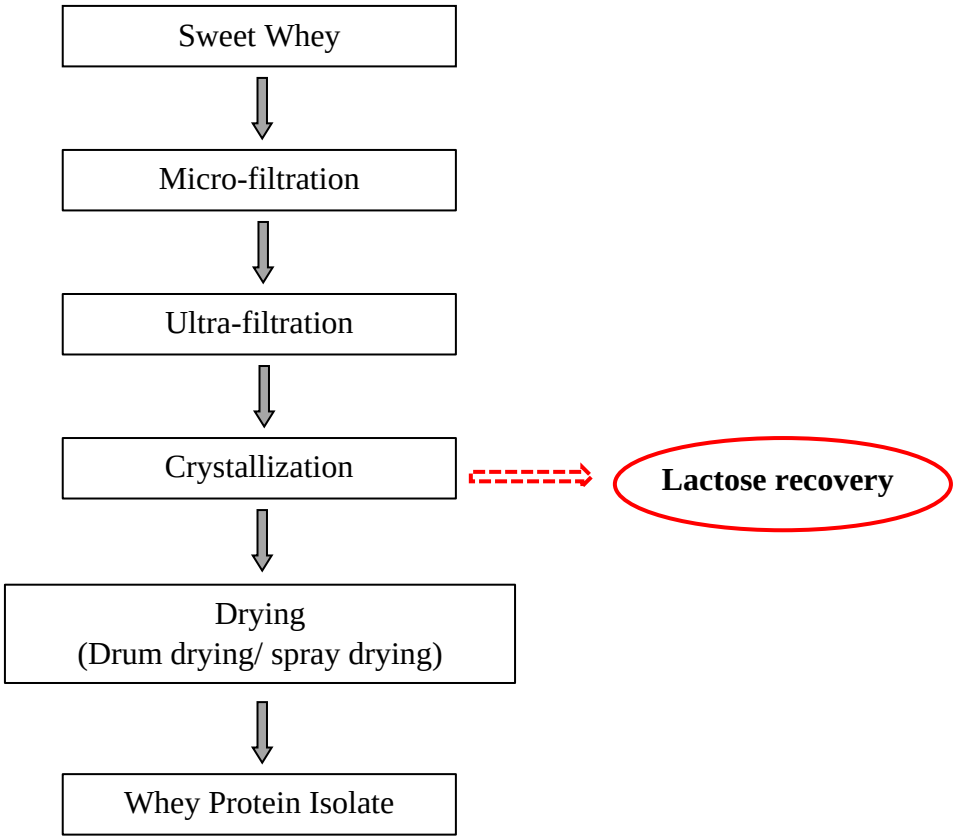

Figure 2. 9: Processing of Sweet whey to produce whey powder

Apart from use of acid whey as a by-product, modifying Greek yoghurt production process to reduce acid whey generation at the point of origin, can be a viable approach to address the acid whey problem from an industrial point of view. Several studies have been carried out to minimize the acid whey generation during the Greek yoghurt production. One such study has investigated incorporation of micellar casein

concentrate to increase the total solid level of the final Greek yoghurt (Bong & Moraru, 2014). This study has found that the added protein content positively affects the final yoghurt gel strength, while reducing acid whey generation. In a separate study, Merrill (2014) has reported a method which mixes a casein-containing ingredient to milk base prior fermentation. There findings indicate that this approach produce a yoghurt with a higher protein content, and that the resulting composition leads to a lower acid whey discharge after fermentation. This study further included a method for making a suitable casein-containing ingredient by micro filtering milk into a whey-containing permeate and a casein-containing retentate. This casein containing retentate leads to a ingredient with casein-to-whey protein ratio of 82:18 or greater. Although findings of these milk fortification studies have revealed new paths to develop Greek style yoghurt with lower acid whey generation, the production process significantly deviates from the traditional Greek yoghurt manufacturing process due to exclusion of the unique whey draining stage.

Although such different approaches have been attempted to minimize the production of acid whey and to use acid whey as a by-product, the feasibility of these methods remains limited due to many factors including the volume of production at present. The significance of the acid whey problem remains a major consideration in proportion to the increased popularity of Greek yoghurt. This large quantity of acid whey presents a challenge for the sustainable growth of the Greek yoghurt industry, as the disposal of acid whey is not trivial. Hence, further investigations to develop novel techniques for producing Greek yoghurt with desirable functionalities while minimizing acid whey production is of utmost importance to combat this acid whey issue. Milk filtration such as use of ultrafiltration prior fermentation results in higher total solid level in milk base while generating sweet whey with pH 7.0, which can be further processed using

currently known techniques. However, the quality attributes of final Greek yoghurt products made from such a process, and the attributes of resultant acid whey still remain unknown.

Chapter 3

Minimizing Generation of Acid Whey during Greek Yoghurt Manufacturing by use of MPC Fortified Milk

3.1 Introduction

Greek yoghurt is a popular product which has resulted from a long history of evolution of yoghurt industry. Straining of yoghurt gel after fermentation is the unique step which differentiates Greek yoghurt manufacturing from regular yoghurt manufacturing. This straining generates whey with high lactic acid content termed “acid whey”, which can cause serious environmental problems unless properly disposed. Currently, commercial production of four pounds of Greek yoghurt creates approximately three pounds of acid whey (Zuber, 2012; Elliott, 2013). Additional processing is required to allow proper disposal of this whey stream. Production of Greek yoghurt has tripled over the last five years and thus, industries have been struggling to find solutions for the generated 1.6 billion litres of acid whey per year (Chandrapala et al., 2015). As an attempt to address this problem, inclusion of acid whey into manure to be used as an organic fertilizer has been trialled. Using acid whey as a supplementation to cattle feed has also been tested. Additionally, converting whey into bio-gas has been investigated, and found to require expensive anaerobic digesters to be installed underground, which has been deemed unfeasible at present (Chandrapala et al., 2016a). Therefore, the necessities of developing strategies to reduce the acid whey production are importantly emphasized by the dairy industry.

Although the straining of yoghurt gel after fermentation results in acid whey, this step remains as the critical step which enhance the dry matter content in final product. In comparison to regular yoghurt, this elevated solid directly contribute to remarkable increment in viscous body, while improving creaminess, and moderate acidic flavour (Chandan, 2006; Tamime & Robinson, 1999). Thus, higher dry matter content in yoghurt base is important for quality yoghurt production. In addition to filtration,

different varieties of milk powders are widely used in yoghurt industry to increase total solid content in initial milk base. Addition of milk solid non-fat such as milk protein concentrate (MPC) powder or skimmed milk powder to the initial milk base increases the total solid level in final product. Milk Protein Concentrate is a product obtained from skim milk by a series of processes which includes UF, evaporation and drying. MPC contains a large amount of casein and whey proteins. The level of protein, lactose and minerals present in the powder can vary depending on the degree of concentration. The composition of MPC is largely determined by the ultrafiltration process while the main role of evaporation and drying is to remove water from the powder (Patel & Patel, 2014).

In addition to increasing the total solid level in the final product, protein based additions also affect the nutritional value and yoghurt structure development specially improving texture, viscosity and mouthfeel (Tamime & Robinson, 1999). However, increasing total solids to greater than 22% w/w by addition of skim milk powder hinders the LAB growth due to the development of higher osmotic pressure in yoghurt base (Tamime & Robinson, 1999). Apart from increasing total solid level, it is also important to have best starter culture growth for efficient fermentation process. The performance of starter culture is affected by numerous factors including milk composition, bacterial strain, amount of inoculum, incubation temperature, incubation duration and milk cooling time (Mahdian & Tehrani, 2007; Bong & Moraru, 2014).

A few studies on Greek yoghurt production have reported using milk fortification techniques for reduction of acid whey generation. For example, fortification of the milk base using micellar casein concentrates to enhance milk protein levels has been discussed (Bong & Moraru, 2014; Merrill, 2014). However, these reported work have been based on production process for Greek style yoghurt which obtains comparable

chemical and physical quality of Greek yoghurt through additions of different ingredients, instead of whey drainage. Although these studies show an attractive strategy for Greek style yoghurt production without generating acid whey, they do not follow the production process for real Greek yoghurt production which incorporate draining of whey after fermentation. Furthermore, incorporation of both milk fortification and straining at Greek yoghurt manufacturing to reduce acid whey generation has not been reported in open literature.

The main objective of the study presented in this chapter is to establish a production method closely following the traditional Greek yoghurt manufacturing process, together with fortification of milk base prior fermentation to enhance initial milk total solid level. Use of this approach has potential to minimize the amount of straining at the end of the fermentation, which would result in lower acid whey generation. The performance of starter culture was monitored during fermentation as LAB is sensitive on total solid level. The properties of acid whey were measured along with the chemical and structural properties of produced novel Greek yoghurts. This study provides important information for understanding the effect of milk fortification on release of acid whey and quality of final yoghurt. Laboratory manufactured end products were compared to three commercially available Greek yoghurt products to establish a benchmark in terms of composition and structure.

3.2 Materials and methods

3.2.1 Materials

Raw milk, cream, milk protein concentrate (MPC) and skim milk powders (SMP) were obtained from Murray Goulburn (Laverton, Victoria, Australia). In order to depict seasonal variations of raw milk composition, two batches of raw milk were obtained in

a time frame of three months. Their average composition is presented in Table 3.1. A commercially available starter culture (FD–DVS YC–380), which is a blend of non-exopolysaccharide (EPS) producing pure *Streptococcus thermophilus* (*St. thermophilus*) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lb. bulgaricus*) strains were obtained from Chr. Hansen (Bayswater, Victoria, Australia). The culture was stored in a freezer at -80 °C until being used. The culture was diluted by adding 50 units of freeze dried culture to 500 mL of sterile milk as per manufacturer's guidelines.

Table 3. 1: Proximate composition of Raw milk, Cream, SMP, MPC and Greek yoghurt samples at different processing stages with the corresponding acid whey resulted during Greek yoghurt production by incorporating milk fortification with SMP or MPC

Sample[a]	Total solid	Ash	Fat	Protein	Lactose	Lactic acid	Whey (%)[b]	pH
Raw milk	13.14 ± 0.46	0.73 ± 0.10	3.81 ± 0.60	3.68 ± 0.50	4.17 ± 0.17	0.03 ± 0.01	-	-
Cream	47.87 ± 2.05	0.39 ± 0.04	43.92 ± 0.40	1.75 ± 0.04	1.25 ± 0.06	0.04 ± 0.01	-	-
SMP	97.55 ± 0.35	8.32 ± 0.08	-	36.20 ± 0.01	51.25 ± 0.18	-	-	-
MPC	93.7 ± 0.40	4.27 ± 0.02	-	85.80 ± 0.03	3.80 ± 0.09	-	-	-
RY-1	14.55 ± 0.51	0.90 ± 0.03	3.95 ± 0.22	4.74 ± 0.20	3.80 ± 0.03	0.68 ± 0.02	-	-
RY-2	20.05 ± 0.38	0.82 ± 0.02	7.99 ± 0.24	6.95 ± 0.20	2.84 ± 0.34	0.79 ± 0.01	-	-
GY-1	22.81 ± 0.28	0.79 ± 0.01	8.61 ± 0.35	9.98 ± 0.09	2.44 ± 0.16	0.47 ± 0.02	-	-
GY-2	23.01 ± 0.48	0.78 ± 0.01	10.24 ± 0.20	8.74 ± 0.03	2.69 ± 0.18	0.60 ± 0.01	-	-
GY-3	23.67 ± 0.06	0.91 ± 0.03	8.30 ± 0.26	9.28 ± 0.07	3.28 ± 0.50	0.90 ± 0.08	-	-
AW-1	7.09 ± 0.20	0.98 ± 0.01	-	0.22 ± 0.07	4.99 ± 0.19	0.86 ± 0.13	113.93	4.30
AW-2	6.48 ± 0.14	0.97 ± 0.01	-	0.66 ± 0.01	3.37 ± 0.10	1.40 ± 0.01	28.04	4.46

[a] Samples are named as follows;
SMP and MPC are the skim milk powder and milk protein concentrate, respectively.
RY-1 and RY-2 are the fermented yoghurt mix prior to the concentration step made with 15 and 20 g/100g initial milk total solid, respectively.
GY-1 and GY-2 are the Greek yoghurt concentrated from RY-1 and RY-2, respectively.
AW-1 and AW-2 are the acid whey expelled during the concentration step during GY-1 and GY-2 manufacturing, respectively.
GY-3 is the Greek yoghurt made with 23 g/100g initial milk TS level.
Values are means of at least 4 independent observations ($n \geq 4$); the results are presented as means ± standard deviation (SD)
[b] The amount of acid whey removed is expressed as a percentage relative to Greek yoghurt yield.

3.2.2 Preparation of milk samples

A yoghurt mix with 15% w/w total solids (TS) was prepared by adding calculated amounts of SMP to raw milk and was used as the control. Two yoghurt mixtures with 20% and 23% w/w TS were prepared by adding calculated amounts of cream and MPC to raw milk using the Pearsons Square method (Tamime & Robinson, 1999). Fat standardization was carried out to maintain 8% w/w fat level.

3.2.3 Production of Greek yoghurt

Three milk samples prepared as described above were homogenized using a homogenizer (110Y - Microfluidics, Newton, Massachusetts, USA) at 60 – 65 °C, followed by a batch pasteurisation using a water bath at 85 °C for 30 min (Lucey, 2004) and were subsequently cooled down to 42 °C. The mix was then inoculated with 0.1% w/w of active starter culture and incubated at 42 °C for 6 h. All batches were chilled to 4 °C followed with straining of the fermented bases containing 15% and 20% w/w TS at 4 °C using the cloth bag method to a standardized 23% w/w TS level. The final 23% w/w of TS was obtained by following Equation 3.1;

$$TS\%GY = \frac{(TS\%RY \times WRY) - TS\%AW \times WAW}{WGY} \qquad \text{Equation 3.1}$$

Where;

WRY is the weight of initial regular yoghurt, g;

TS%RY is the total solid level of initial regular yoghurt;

WAW is the weight of repelled whey, g;

TS%AW is the dry matter content of acid whey;

WGY is the weight of the Greek yoghurt, g.

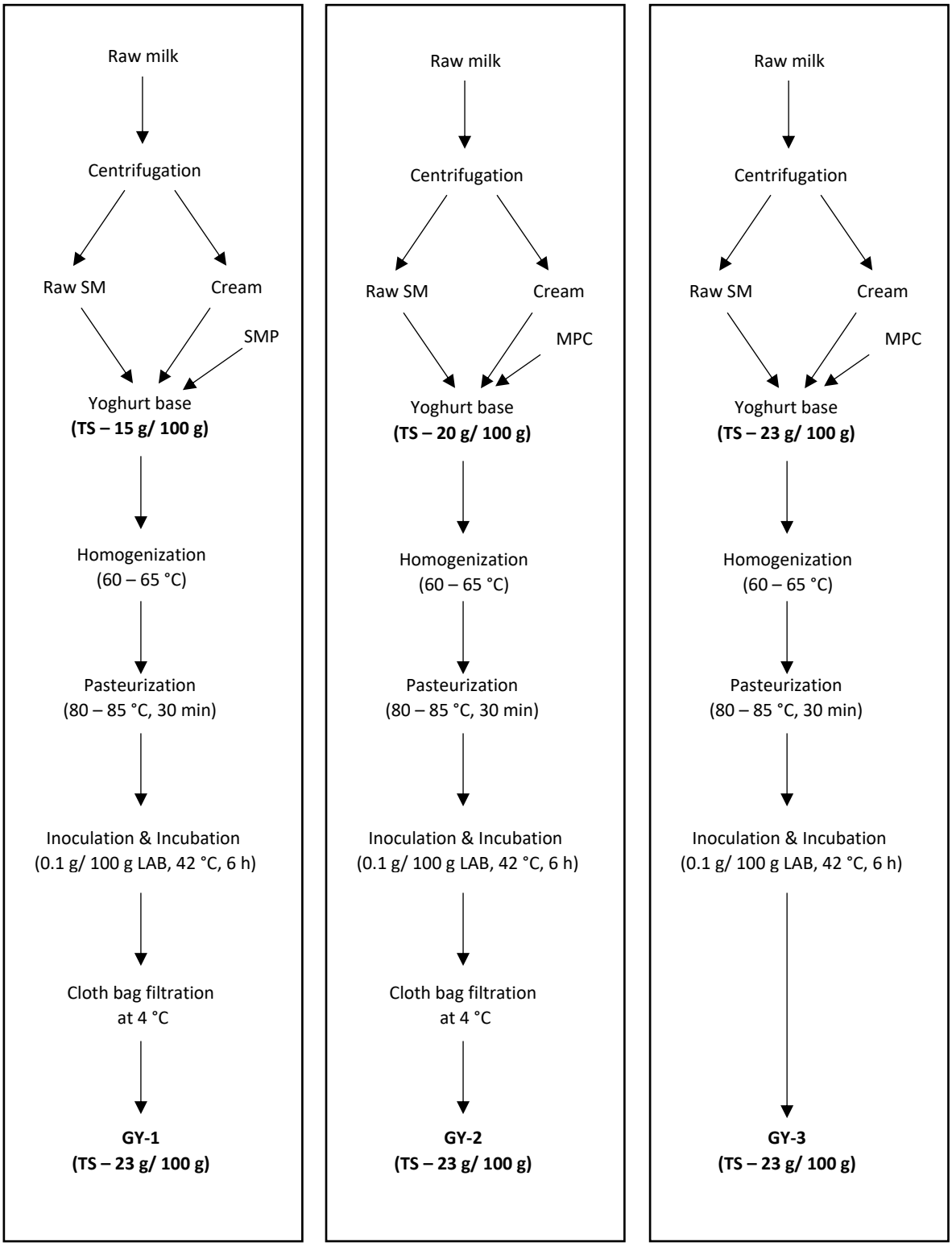

Figure 3. 1: Experimental design and coding employed in manufacturing of Greek yoghurt samples by incorporating milk fortification with SMP or MPC

The whey draining was terminated at the stage where Greek yoghurt showed the standardized 23% w/w TS level. The yoghurt with 15% w/w total solid required a drainage period 12 h 30 min while the yoghurt with 20% w/w total solid required 3 h 15 min to reach this level. A third yoghurt mix containing 23% w/w TS was treated and fermented without the straining step as described in Figure 3.1.

3.2.4 Culture performance

The culture performance was monitored hourly during the 6 h fermentation period. The following parameters were assessed: culture concentration, pH, titratable acidity and bacterial proteolytic activity.

Pour plate counting method (Donkor et al., 2007a) was used to enumerate culture bacteria *(Lb. bulgaricus, St. thermophilus).* MRS agar with anaerobic incubation at 42 °C for 48 h and M17 agar with aerobic incubation at 37 °C for 48 h were used for culturing of *Lb. bulgaricus* and *St. thermophilus*, respectively.

Change of pH in milk base was monitored with a pH meter (WTW inoLab 720, Germany). Titratable acidity was established using a 0.1 mol/L NaOH solution (AOAC, 2005d) using phenolphthalein as an indicator.

Bacterial proteolytic activity during growth was measured using the O-phthaldialdehyde (OPA) method as described by Donkor (2007). The absorbance was measured using an UV spectrophotometer (Libra S12 – Biochrom Ltd, Cambridge, England) at 340 nm. The yoghurt mix before inoculation was used as the control. The relative degree of proteolysis was determined as the difference between the free amino groups in fermented milk to that of the control.

3.2.5 Chemical composition of raw materials and yoghurt samples

Total solid, ash and lactose contents were measured as described previously (AOAC, 2005a; Chandrapala et al., 2015; 2016b).

The fat content was quantified using the Rose-Gottlieb method (AOAC, 2005c). The weight of the extracted fat was taken and represented as a percentage to the dry matter basis.

Total nitrogen content of all samples was determined using the Kjeldhal method (AOAC, 2005b). Total protein contents were calculated by multiplying the total nitrogen content by the conversion factor of 6.38.

Concentration of lactic acid was determined using the same HPLC fitted with Aminex HPX 87H, 300 X 7.8 mm ion exchange column (Biorad Instruments, Gladesville, NSW, Australia) (Chandrapala et al., 2015) maintained at 65 °C. A degassed 0.01 mol/L H_2SO_4 solution was used as the mobile phase. The flow rate, injection volume and wavelength were selected as 0.6 mL/min, 20 µL and 220 nm respectively.

3.2.6 Physical properties of Greek yoghurt

All Greek yoghurt samples were stirred using a one stage hand mixer (Adesso, HB 1908K - SA) for 2 min and stored in 100 mL plastic containers overnight at 4 °C. This approach was applied to erase the effects of processing history on the properties of yoghurt gels. The new structure was therefore totally dependent on the composition of yoghurt mixture. (Purwandari et al., 2007).

For the textural analysis, 50 g Greek yoghurt samples were dispensed into 60 mm diameter cylindrical containers and stored overnight at 4 °C prior to testing. Textural attributes of Greek yoghurt samples were established using a large-strain approach in a penetration mode by a TA XT Plus Texture analyzer (Stable Micro Systems Ltd., Surrey, UK) fitted with 5 kg load cell and a 20 mm diameter stainless steel cylindrical probe (model no: P/20). Double compression test (TPA) was performed for Greek yoghurt samples at 5 °C to identify gel firmness, cohesiveness and adhesiveness. Speed of the probe and penetration depth was pre-set as 0.5 mm/s and 15 mm, respectively (Ozer et al., 1997).

Viscoelastic and flow properties were measured using a cone (CP50-1, 1°, 50 mm) and plate (P-PTD200/56) geometry attached to a MCR 301 Rheometer (Anton Paar GmbH, Graz, Austria) at 5 °C. Prior to testing, samples were stirred using a constant shear rate of 500 1/s for 30 s to diminish structural differences among samples during processing conditions (Purwandari et al., 2007). Samples were then allowed to equilibrate for 10 min to rebuild yoghurt structure dependant on the composition of sample. Afterwards, a dynamic frequency sweep between 0.1 – 100 Hz at a constant shear strain of 0.5% was performed to obtain G' and G". A measurement of dynamic rheology as a function of shear rate in the range between 0.1 and 100 1/s (upward and downward sweeps) was performed over 20 min to measure variations in viscosity against shear rate.

Gel syneresis was determined as the amount of whey expelled from Greek yoghurt samples using a centrifugation technique as described by Zisu et al., (2011) and expressed as the ratio of weight of whey to the initial Greek yoghurt weight.

3.2.7 Comparison with commercial available products

Understanding the novel product quality compared to the commercially available Greek yoghurts is important to maximize potential retail success. Therefore, chemical composition, texture and rheological properties of three commercially available Greek yoghurts were measured using the same methods used for quantifying novel Greek yoghurts prepared during the study, as presented above in section 3.2.5 & 3.2.6.

3.2.8 Statistical analysis

All experiments were replicated two times independently in a randomized split plot block design with two sub-samplings to provide at least 4 independent observations ($n \geq 4$). Total solid content served as the main variable with the replication as blocks. The data was analysed using a GLM procedure of the statistical analysis system (SAS, 1996) with the statistical level of significance pre-set at 0.05 ($P < 0.05$).

3.3 Results and Discussion

3.3.1 Fermentation process

Required chemical and structural attributes of Greek yoghurt are heavily dependent on the nature of the fermentation process and thus the performance of starter culture bacteria. The primary goal of an efficient fermentation process in yoghurt manufacturing is to reduce the pH of milk below isoelectric point of casein (pH 4.6) to facilitate protein aggregation. The rate of pH reduction affects the visco-elastic nature of yoghurt gel. This is important as the pH value reduction needs to be achieved in shortest possible time to acquire a rigid gel structure (Anema, 2008b). Therefore, pH profile of milk during fermentation is important in understanding the culture performance on

yoghurt gel development (Figure 3.2A). All samples followed the typical pH reduction profile while having a slow and linear pH decrease at the beginning having no significant difference with total solid changes ($p > 0.05$) and a steady steep decrement after 2 hours at incubation. The target pH of 4.6 was achieved after 5 h 33 min, 5 h 16 min and 5 h 45 min for 15%, 20% and 23% w/w TS levels ($p > 0.05$), respectively, highlighting negligible impact of TS levels on the time of fermentation (Brabandere & Baerdemaeker, 1999). Yoghurt base containing 23% w/w TS has taken longer than the other two samples to reach pH 4.6, attributed to the buffering capacity of milk (Ozer et al., 1998b). Approximately at pH 5, colloidal calcium phosphate which is bound to casein micelles solubilizes into the milk serum phase and contributes to the development of milk buffering capacity (Peng et al., 2009). In our study, TS levels were adjusted by MPC which largely contains micellar casein.

The amount of lactic acid production gives a better indication of effective fermentation than pH reduction as it is governed by the buffering capacity of milk. In addition, the rate of acid development is essential for the desirable aroma, texture and flavour development in Greek yoghurt. During the present study, the rate of acid generation was increased significantly ($p < 0.05$) with elevation of TS levels (Figure 3.2B). Indicatively the lactose metabolism of the starter culture bacteria was directly linked to increase in the TS level and likely attributed to the presence of free amino acids and smaller peptides in the medium (Figure 3.2C) as their content was at least three times higher ($p < 0.05$) than that in other two samples at the start of fermentation.

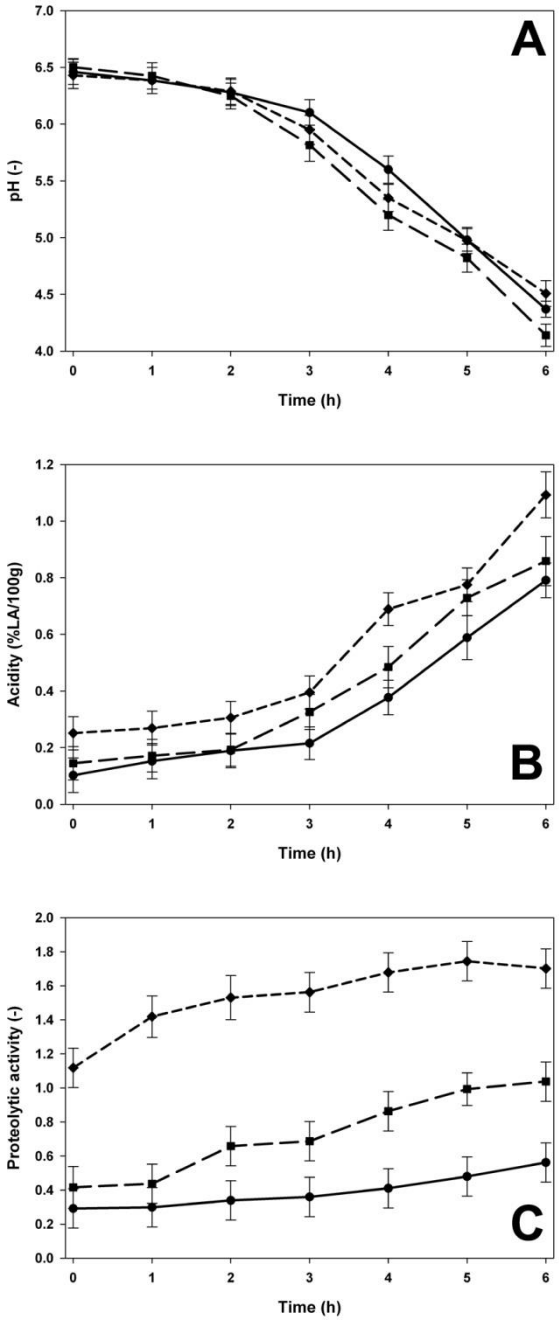

Figure 3. 2: Starter culture performance as depicted by pH change (A), acidity (B) and proteolytic activity (C) during fermentation of a yoghurt mix standardized to 15% (Solid line with Circle), 20% (Dash line with Square) or 23% (Dash line with Diamond) total solids by addition of skim milk powder (SMP) or milk protein concentrate (MPC). Values are means of at least 4 independent observations ($n \geq 4$); the error bars represent ± standard deviation (SD)

The growth of individual strains (*St. thermophilus* and *Lb. bulgaricus*, Figure 3.3A and 3.3B, respectively) are linked to increase in TS level in growth medium. Both strains reached fairly high concentrations, almost a log cycle higher than it is usually reported for yoghurt (Vasiljevic & Shah, 2008). However, the supplementation was achieved by a protein rich product – MPC, which contained elevated amounts of amino acids and peptides (Figure 3.2A). Both strains are highly fastidious organisms requiring numerous nutrients for their growth including lactose, amino acids, peptides, salts, nucleic acid derivatives and vitamins (Vasiljevic & Jelen, 2001), which could have been provided by supplementation instead of usually observed symbiotic growth. Interestingly longer lag phase and hindered growth rate of *St. thermophilus* was noted when the TS level increased from 15% to 23% w/w TS (Figure 3.3A), similar to a previous report (Mahdian & Tehrani, 2007). The present study resulted 1h, 2h and 3 h lag phase durations respectively for 15%, 20% and 23% w/w TS. This growth retardation was attributed to rise osmotic pressure in milk base due to higher concentration of TS, especially lactose and minerals (Tamime & Robinson, 1999).

For example, 20% w/w TS sample exerted an osmotic pressure of ~1338 MPa, while 23% w/w TS sample exhibited an osmotic pressure of ~1548 MPa. However, it is not clear whether this could be one of the reasons for growth hindrance of *St. thermophilus* as MPC mainly contains proteins with a small quantity of minerals or it was also due to metabolic activity of *Lb. bulgaricus* which has shown a completely different behaviour compared to *St. thermophilus* (Figure 3.3B). With increasing TS level, the growth rate of *Lb. bulgaricus* has increased with concomitant shortening of its lag phase. Especially, *Lb. bulgaricus* in milk base with 23% w/w TS started their log phase at the beginning of fermentation which is agreement with Figure 3.2C, depicting the highest free amino

acid content in the sample containing 23% w/w TS sample at the beginning of fermentation.

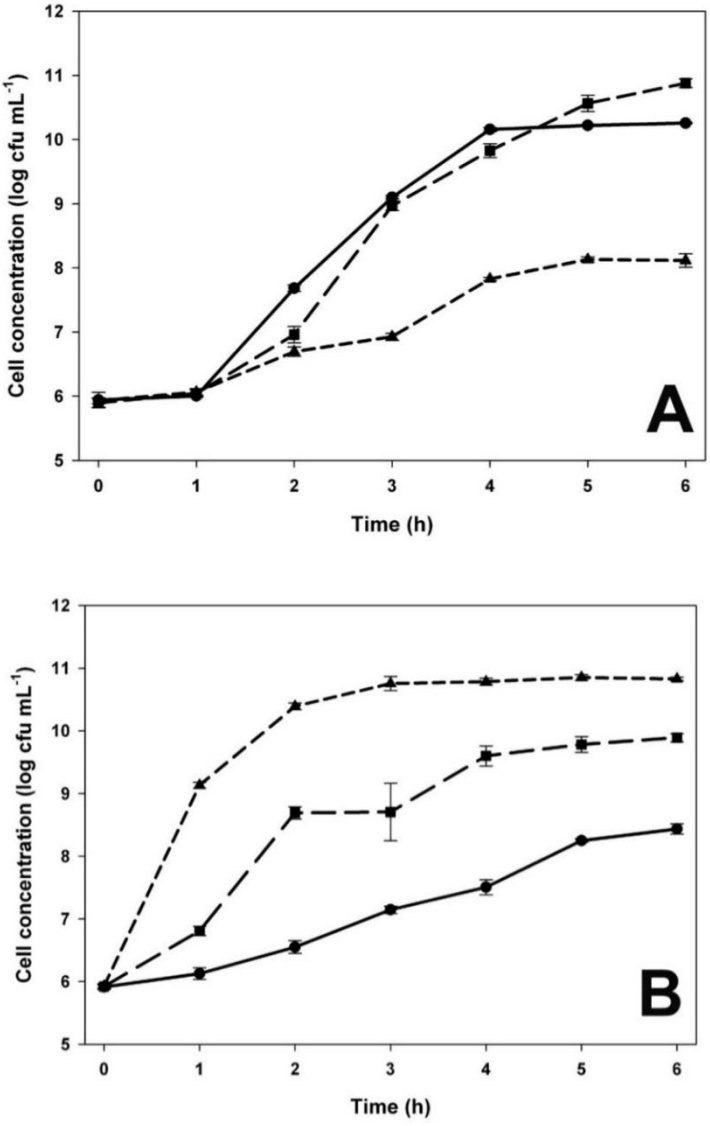

Figure 3. 3: Starter culture growth involving (A) *Streptococcus thermophilus* and (B) *Lactobacillus delbrueckii* subsp. *bulgaricus* during fermentation of a yoghurt mix standardized to 15% (Solid line with Circle), 20% (Dash line with Square) or 23% (Dash line with Triangle) total solids by addition of skim milk powder (SMP) or milk protein concentrate (MPC). Values are means of at least 4 independent observations (n ≥ 4); the error bars represent ± standard deviation (SD)

Presence of free amino acids and peptides in milk serum is important for starter bacteria to fulfil their essential nitrogen requirements (Bong & Moraru, 2014). Usually *St. thermophilus* initially releases shorter peptides and amino acids required by *Lb. bulgaricus* during yoghurt manufacturing (Vasiljevic & Shah, 2008). In other complex media this requirement may be met by supplementation in the form of yeast extract, whey proteins or even skim milk powder (Vasiljevic & Jelen, 2001). Both strains appear dependent on the conditions of the environment and usually reach 10^8 - 10^9 cfu /mL during fermentation of yoghurt base (Vasiljevic & Shah, 2008). However, cell concentrations in the current study may be attributed to presence of free and available amino acids (Figure 3.2C) and enhanced buffering capacity (Figure 3.2A) as it fulfils nitrogen requirement for bacterial cell growth.

3.3.2 Properties of acid whey

Apart from the starter culture performance, the study of generated acid whey quantity and quality is vital as is the primary goal of the present study. According to Table 3.1 results, Greek yoghurt produced with 23% w/w initial milk TS level (GY-3) did not generate acid whey during the production as whey drainage stage was omitted. GY-1 and GY-2 released acid whey as drainage was incorporated to increase final yoghurt dry matter content up to 23% w/w TS. As expected, GY-1 released significantly ($p < 0.05$) higher amount of acid whey (113.93% w/w) compared to GY-2 (28.04% w/w) simply owing it to a different initial solid content. Acid whey generation during commercial Greek yoghurt production is approximately 75% w/w of initial yoghurt mix (Zuber, 2012). Therefore, raising the initial total solid content of the yogurt mix as in case of GY-2 results in lower amount of acid whey generation showing 86% and 47% w/w lower the acid whey compared to GY-1 and industrially reported values (Zuber, 2012)

respectively. This consequently leads to lower requirements for downstream processing. Lactic acid content is a major factor, which determines the properties of acid whey. It also appears as an obstacle for lactose recovery during acid whey processing as it acts as an inhibitor of lactose crystallization (Chandrapala et al., 2016a; 2016b; Wijayasinghe et al., 2015). In GY-1 production, about 70% w/w of lactic acid contained in the intermediate regular yoghurt (RY-1) passed into the whey stream. On the other hand, only about 40% w/w of lactic acid accumulated in acid whey during GY-2 manufacturing due to the minimal whey drainage.

3.3.3 Chemical composition of Greek yoghurt

Chemical composition of Greek yoghurt and expelled acid whey rely mainly on the extent of the concentration step applied during manufacturing. The GY-3 was higher in lactose, lactic acid and ash compared to other two Greek yoghurt samples (Table 3.1), which can clearly be attributed to the omission of the concentration step. On the other hand, GY-1 and GY-2 composition differed substantially from their corresponding fermented yogurt mixes (referred to as RY-1 and RY-2, respectively). The straining step increased the protein content by retaining almost all of the proteins while allowing passage of lactose into the whey stream (Table 3.1). At the same time, fat was concentrated along with the proteins likely due to even distribution throughout the protein gel resulting from the homogenisation step, which in turn may increase the creamy mouthfeel in the final product (Ozer & Robinson, 1999).

Table 3. 2: Proximate composition, textural properties and syneresis of three commercially available Greek yoghurt samples

Characteristic	Sample		
	X	**Y**	**Z**
TS (g/100g)	14.42	17.67	19.95
Protein (g/100g)	9.56	3.05	4.78
Fat (g/100g)	0	10	9.7
Na (mg/100 g)	47	78	62
Ca (mg/100 g)	114	130	166
Hardness (g)	12.4 ± 0.70	14.95 ± 0.05	14.16 ± 1.34
Cohesiveness (%)	0.74 ± 0.03	0.69 ± 0.01	0.71 ± 0.01
Adhesiveness (g.sec)	-77.39 ± 61.14	-189.81 ± 3.20	-174.88 ± 29.54
Syneresis (g/100g)	49.07 ± 0.15	14.36 ± 0.27	10 ± 0.01

Values are means of at least 4 independent observations ($n \geq 4$); the results are presented as means ± standard deviation (SD)

It is essential to match these gel characteristics with commercially available Greek yoghurts for better chance of retail success. Therefore, compositional information, texture and rheological properties of three different brands of commercial Greek yoghurts were reviewed and compared against the Greek yoghurts produced during this study (Table 3.2). Our samples contained elevated levels of milk solids and proteins but similar fat levels to some of these commercial products. This suggest that Greek yoghurt produced in the present study gives a higher amount of solids especially protein per unit amount of consumed Greek yoghurt compare to commercially available products. Furthermore, these substantial compositional differences influence the structural changes of Greek yoghurt as protein is the backbone of yoghurt gel structure.

3.3.4 Impact of initial solid content on structural properties of Greek yoghurt

The structural properties of yoghurt gel are important parameters governing the consumers' preferences. Increasing initial TS content alone is not enough to achieve appropriate structural properties (Table 3.3). The significantly different ($p < 0.05$) protein contents were gained in Greek yoghurts showing 9.98%, 8.74% and 9.28% w/w for GY-1, GY-2 and GY-3, the concentration step appears a key step increasing the hardness by 28% and 33.6% for GY-1 and GY-2 samples respectively, as compared to GY-3 (14.9%). Also, the hardness of these concentrated samples was double that of the commercial products (Table 3.2).

The greatest hardness was noted for GY-2 which can be attributed to the role of MPC. The casein network is the backbone of the yoghurt gel, influencing the perceived hardness in a concentration dependent manner (Bong & Moraru, 2014). The

concentration step apparently reinforces it, bringing casein aggregates closer by removing whey and enhancing non-covalent bonds. The role of water (whey) appears obvious in GY-3; although it contained highest levels of TS, the formed gel exhibited a remarkably lower hardness value. GY-3 never regained its original properties after stirring. This is reflected in very low measured hardness (Table 3.2). Removal of whey during GY-1 or GY-2 at straining decreases the pore size of the yoghurt gel while increasing the gel strength (Bong & Moraru, 2014). Furthermore, the whey removal leads to lower free water content and consequently lower syneresis. GY-2 had the lowest syneresis (Table 3.3) compared to other two manufactured samples, within a range of the commercial products (Table 3.2). When the casein to whey protein ratio is lowered, the number of whey proteins associated with casein micelles are increased and thus act as a bridging material to increase strength and number of bonds between proteins in yoghurt gel network (Phadungath, 2005b; Vasbinder et al., 2003). This reduces the movement of free water by reducing the pore size in the gel network (Puvanenthiran et al., 2002). GY-2 also showed the highest degree of acidification during the fermentation (Figure 3.2B). This would allow for a stronger casein gel at fermentation due to arrangement of cross links between casein and whey proteins (Vasbinder et al., 2003) minimizing movement of free water (Bong & Moraru, 2014). Furthermore, higher ratio of protein (especially casein) and fat content as in GY-2 increases the hydrophilic nature in the yoghurt gel structure, maximizing the water binding ability (Lucey et al., 1998a). The greater water holding capacity indicates the strong micro structure of yoghurt gel, capable of holding a bulk amount of water (Abu-Jdayil et al., 2002).

Table 3. 3: Textural properties and syneresis of Greek yoghurt samples prepared from the yoghurt mix containing 15% (GY-1), 20% (GY-2) or 23% w/w (GY-3) initial total solids obtained by incorporating milk fortification with SMP or MPC

Sample	**Hardness (g)**	**Cohesiveness (%)**	**Adhesiveness (g.sec)**	**Syneresis (g/100g)**
GY-1	28.37 ± 1.62	0.67 ± 0.01	-364.83 ± 44.39	16.38 ± 0.49
GY-2	33.6 ± 1.31	0.62 ± 0.01	-407.55 ± 20	13.68 ± 0.06
GY-3	14.86 ± 0.77	0.78 ± 0.06	-229.34 ± 8.14	29.52 ± 1.43

Values are means of at least 4 independent observations ($n \geq 4$); the results are presented as means ± standard deviation (SD)

The microstructure of yoghurt is mainly reflected by the rheological properties of yoghurt gel, mainly its storage (G') and loss moduli (G"). During the study, all samples exhibited a greater storage than loss modulus throughout the frequency range (Figure 3.4A, B), depicting and confirming their solid like behaviour (Ozer et al., 1997). GY-2 has exhibited a greater storage modulus compared to the other two manufactured samples and the commercially available Greek yoghurt (Figure 3.4A, B, C), indicating a stronger gel strength. This may be due to the incorporation of MPC enhancing the casein concentration and ultimately strengthening of casein network due to greater non-covalent interactions. The draining removes most of the free water content while reducing built of kinetic energy at given forces. Furthermore, the cloth bag draining has apparently allowed enough time for establishment of a strong gel matrix (Ozer et al., 1999a). Removal of whey is, far more important than the casein content to achieve the required viscoelastic behaviour (Bong & Moraru 2014). Our results supported this observation as the greatest MPC supplementation (GY-3) resulting in required protein content exhibited lowest G' compared to both GY-1 and GY-2 due to absence of the straining step.

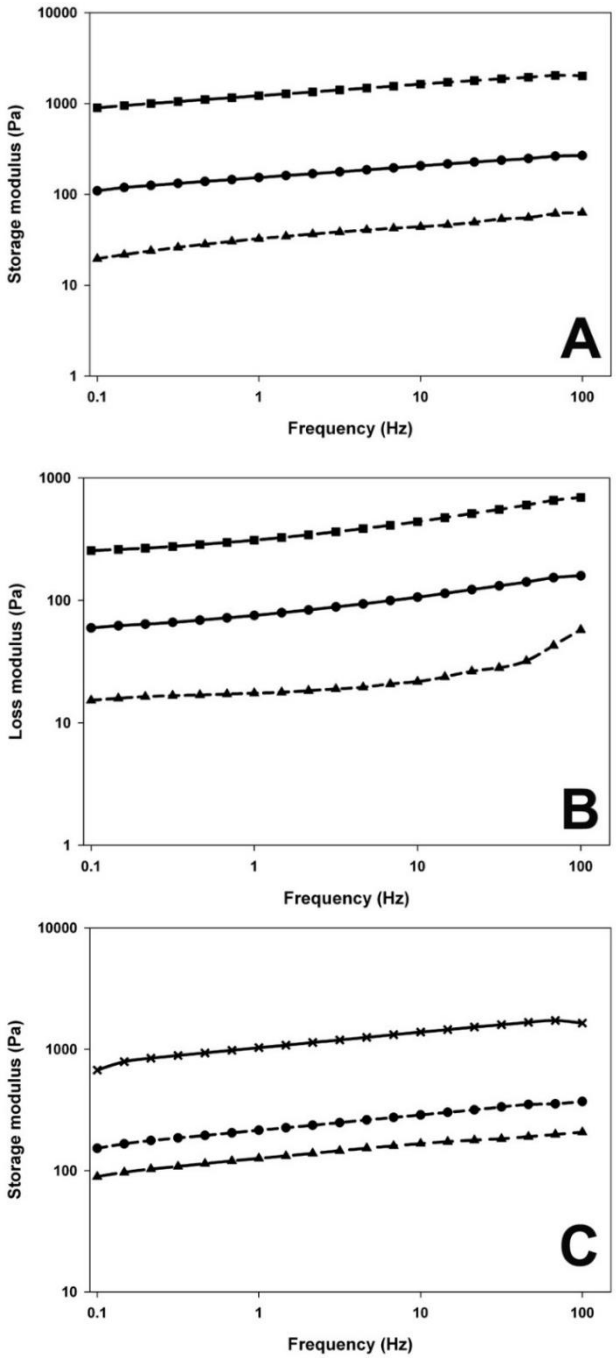

Figure 3. 4: Viscoelastic properties of Greek yogurt represented as (A) storage modulus - G' and (B) loss modulus – G" produced by fermentation at 42°C for 6 h of a yoghurt mix standardized to 15% (Solid line with Circle) (GY-1), 20% (Dash line with Square) (GY-2) or 23% (Dash line with Triangle) (GY-3) total solids by addition of skim milk powder (SMP) or milk protein concentrate (MPC) in comparison to storage modulus (G') of some available commercial Greek yoghurt samples (C) due to confidentiality identified as yoghurt X (Solid line with Cross), yoghurt Y (Dash line with Triangle) and yoghurt Z (Dash line with Circle).Values are means of at least 4 independent observations ($n \geq 4$)

Apart from the viscoelastic nature, flow behaviour of Greek yoghurt is important for establishing an efficient industrial scale production especially pumping, filling or stirring. Shear thinning (pseudoplastic) behaviour is more dominant for yoghurt with increasing shear rate (Abu-Jdayil et al., 2002). During the experiment, all Greek yoghurt samples exhibited typical non Newtonian flow behaviour, with immediate and greater reduction of viscosity under increasing shear rates (Figure 3.5A) due to break down of gel network and further reduction of particle size (Bong & Moraru, 2014). However, GY-3 sample had a lower apparent viscosity than GY-1 or GY-2 over the examined shear rate range.

Although GY-3 sample contained elevated casein content, created casein network likely contained larger pores and thus weaker non-covalent interactions between the strands of the network due to greater distances (Purwandari et al., 2007). Draining whey from the samples also increased casein to whey protein ratio lowering the viscous nature of the yoghurt gel (Sauer et al., 2012). Whey draining shorten the intra and intermolecular interactions in GY-1 and GY-2 samples augmented weak interactions, enabling greater absorption of an applied force by flexing without breaking the intra-particle cross-links. This leads to greater gel strength and a higher retained force (Purwandari et al., 2007). While manufactured Greek yoghurt samples had significantly different ($p < 0.05$) flow behaviour compared to the commercial Greek yoghurt (Figure 3.5B) they resulted in far less whey release.

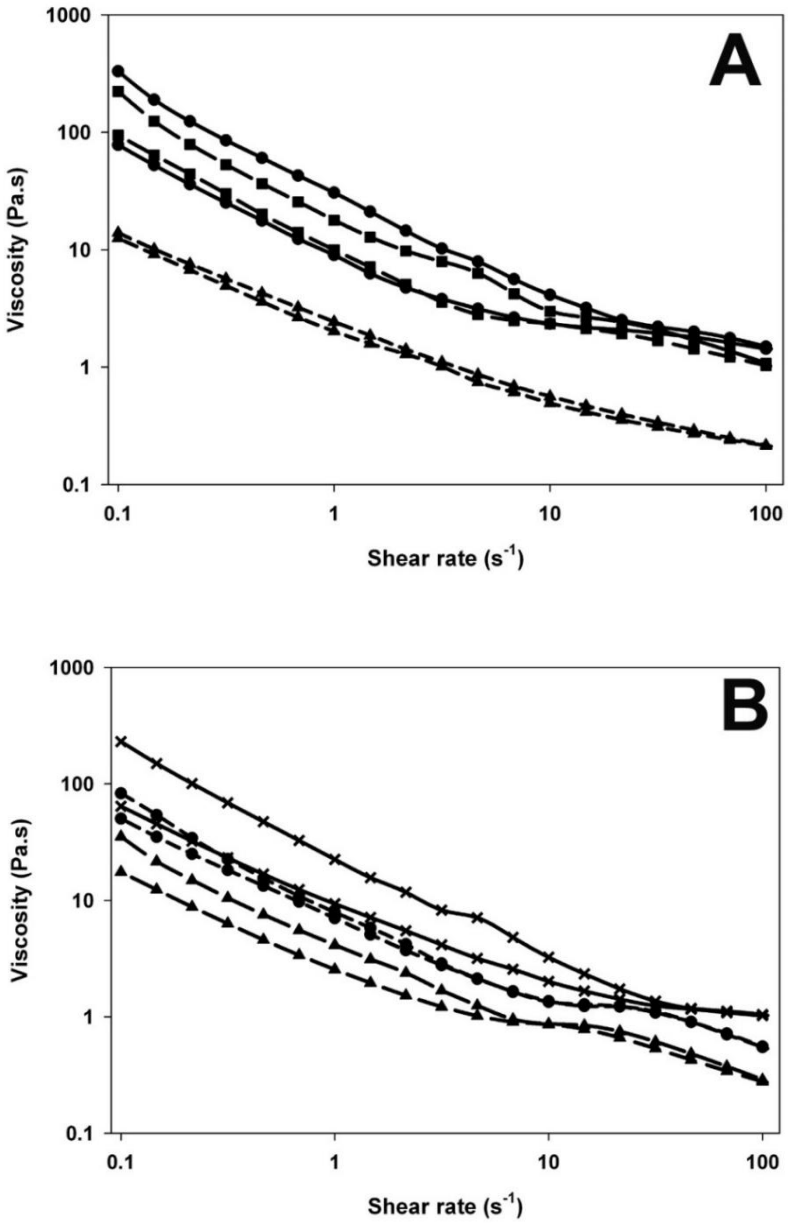

Figure 3. 5: Flow behaviour of Greek yogurt samples (A) produced by fermentation at 42°C for 6 h of a yoghurt mix standardized to 15% (Solid line with Circle) (GY-1), 20% (Dash line with Square) (GY-2) or 23% (Dash line with Triangle) (GY-3) total solids by addition of skim milk powder (SMP) or milk protein concentrate (MPC) in comparison to flow properties of some available commercial Greek yoghurt samples (B) due to confidentiality identified as yoghurt X (Solid line with Cross), yoghurt Y (Dash line with Triangle) and yoghurt Z (Dash line with Circle).Values are means of at least 4 independent observations ($n \geq 4$).

3.4 Conclusion

The study has shown that it is possible to manufacture Greek yoghurt by manipulating the initial TS levels of the yoghurt base up to 23% w/w with milk protein concentrate. Increasing initial milk TS from 15% to 23% w/w positively affected performance of the starter culture, although the growth of *St. thermophilus* appeared to be compromised by developed osmotic pressure. This is due to solubilisation of colloidal calcium phosphate from the casein micelle and likely lactic acid production due to enhanced metabolic activity of *Lb. bulgaricus*. All three novel Greek yoghurt samples exhibited greater protein content and enhanced textural and rheological properties compared to commercially available Greek yoghurts. GY-2 sample appeared superior in many parameters with the highest gel strength due to a combined effect of draining and elevated protein content and minimized syneresis. Most importantly, this production led to substantially lower acid whey generation due to low drainage requirement without comprising yoghurt quality. This novel processing approach may assist in minimizing acid whey generation and thus downstream processing issues associated with acid whey processing issue and promote sustainable Greek yoghurt production.

Chapter 4

Pre-concentration of yoghurt base by ultrafiltration for reduction of acid whey generation during Greek yoghurt manufacturing

4.1 Introduction

Greek yoghurt is a popular dairy product among many from infants to adults due to its favourable properties. One such property is its higher dry matter level (Nsabimana et al., 2005). High dry matter content in Greek yoghurt is gained by incorporating a whey draining step in the production process. Additionally, initial milk fortification with protein based milk powders such as MPC and SMP are widely practised in the dairy industry (Tamime & Robinson, 1999). During the previous chapter, Greek yoghurt produced using milk fortification with MPC to increase initial milk total solid levels up to 20% or 23%, generated low amounts of acid whey compared to a control sample. Furthermore, greater starter culture growth with enhanced acidification rate was concomitant with increase in milk total solids. Additionally, Greek yoghurt produced with 20% initial milk TS achieved harder gel structure with lower syneresis compared to other two Greek yoghurts. These results suggested that enhancing initial milk TS based on protein concentration may deliver Greek yoghurt with less acid whey generation, while achieving favourable chemical and structural properties at the same time. However, use of MPC as an ingredient may not be suitable for large scale production due to the high cost of powder ingredients (Robinson & Tamime, 1993). Therefore, further investigation on alternative methods for increasing TS content (protein content) is required for optimizing Greek yoghurt production process.

Presently, several well-known filtration techniques are widely used in dairy industry to achieve dry matter enhancement. These techniques range from the traditional method using a cloth bag to the modern technical methods such as the use of ultrafiltration, nanofiltration and reverse osmosis. The main motivations of using these modern technologies are to reduce the processing time, labour requirement and to improve the

product quality (Abu-Jdayil et al., 2002). Among them, ultrafiltration technique is widely used in the industry as it leads to high protein concentration and better yoghurt gel strength compared to other filtration methods (Tamime & Robinson, 1999). The working principle of the ultrafiltration technology is based on concentration of milk that takes place when passed through a membrane having a pore size around 0.01 μm under applied pressure (Marcelo & Rizvi, 2009).

Most work to date on Greek yoghurt have been conducted by following the traditional processing method, which drains whey after fermentation (Tamime et al., 1991a; Ozer et al., 1997; Ozer & Robinson, 1999; Ozer et al., 1999a) with the use of ultrafiltration. While a few studies have focussed on applying ultrafiltration prior fermentation (Ozer et al., 1997; Ozer & Robinson, 1999; Ozer et al., 1999a), no study has specifically focussed on the properties of acid whey released during Greek yoghurts manufactured with ultrafiltered milk. In addition, those studies have not focussed on evaluating the detailed structural and textural attributes of Greek yoghurts produced which may influence the consumer perception. During ultrafiltration of milk prior fermentation, lactose and minerals are passed through the membrane while most macro molecules such as fat and proteins are retained by the membrane. These chemical compositional changes in the initial milk base may mainly impact the properties of the final yoghurt product (Mahdian & Tehrani, 2007; Sha et al., 2009; Bird, 1996; Domagała, 2012).

The main objective of the study presented in this chapter is to introduce a milk ultrafiltration stage prior to milk fermentation to enhance the milk total solid content. The study evaluated the physio chemical and rheological properties of the Greek yoghurts produced with the use of ultrafiltered milk to interpret the quality of the end product. UF was applied to increase the total solid levels to 13.8% and 17.5% w/w prior

fermentation and subsequently filtered through a cloth bag after fermentation where necessary to finally achieve a 23% w/w TS traditional Greek yoghurt.

Use of ultrafiltration of milk prior fermentation will generate majority of sweet whey which then can easily be processed successfully using currently known technologies. Furthermore, this milk thickening process prior fermentation will ultimately reduce the amount of acid whey generation after fermentation due to lower draining requirements. Hence, the effect of use of ultrafiltered milk prior fermentation on the amount of acid whey generated during the manufacture was also investigated. This study is aimed at addressing the significant environmental issue that arise from generation of large quantities of acid whey and would aid sustainability of the Greek yoghurt market.

4.2 Materials and methods

4.2.1 Materials

Raw milk, cream and skim milk powder (SMP) were obtained (Devondale Murray Goulburn, Laverton North, Australia) on two separate time intervals to represent seasonal variations of chemical composition of raw milk. The chemical composition of raw milk and cream were tested prior preparation of yoghurt milk bases (Table 4.1). The commercial starter culture FD – DVS YC – 380 (Chr. Hansen, Bayswater, Victoria, Australia) was prepared following the method described in previous chapter, section 3.2.1.

4.2.2 Preparation of milk samples

Raw milk was skimmed by centrifugation at 3000 × g for 30 min at 4 °C to remove the fat (Wen et al., 2012). Milk base with 20% or 23% TS levels was prepared by

incorporating both UF step and fat standardization. The amount of UF skim milk and cream required to maintain 8% w/w fat level in the yoghurt base was calculated using the Pearson square method. Afterwards, the amount of TS required through the ultrafiltration of skim milk was calculated using following Equation 4.1;

$$\text{TS\% of UF milk} = \frac{(\text{TS\% of final milk base} \times \text{weight of final milk base}) - (\text{TS\% of cream} \times \text{weight of cream})}{\text{weight of UF milk}}$$

Equation 4.1

Based on this calculation, skim milk was concentrated to 13.8% or 17.5% w/w separately using an ultrafiltration membrane with a 10 kDa molecular weight cut off and a membrane area of 0.18 m^2 (Sterlitech, USA). The ultrafiltration was performed at 50°C with a transmembrane pressure of 2 bar (Ozer et al., 1997). Application of ultrafiltration to achieve concentrations of 13.8% and 17.5% w/w resulted in generation of 363.5 g and 543 g of permeate respectively. The control sample was prepared separately by adding SMP to raw milk to achieve a 15% w/w total solid level.

4.2.3 Production of Greek yoghurt

As shown in Figure 4.1, Greek yoghurt was manufactured to 23% w/w final total solid from yoghurt base containing 15%, 20% or 23% w/w total solids. Milk treatments of homogenization, pasteurization, inoculation with 0.1% w/w of active starter culture and incubation at 42 °C were performed following method described in the section 3.2.3. During the previous chapter, isoelectric point of pH 4.6 was reached during 5 and 6 h of incubation time for all three yoghurt bases. Therefore, all three yoghurt samples were incubated for 6 h at 42 °C to complete gel formation and final pH was measured in yogurt samples after 6 h of incubation. The fermented milk samples containing 15%

w/w and 20% w/w total solids were subjected to straining using the cloth bag method at 4 °C following method as described in the section 3.2.3.

4.2.4 Chemical analysis

Total solid, fat, protein, lactose and lactic acid levels of Greek yoghurt produced and regular yoghurts that produced at the intermediate stage of Greek yoghurt production process were determined as described previously in Chapter 3, section 3.2.5.

Incorporation of ultrafiltration stage mainly change on the mineral content of milk and therefor, Calcium and Phosphorus content were measured as presented in the following section.

Inductively coupled plasma atomic emission spectrometry (ICP-AES) measurements were made using a sequential plasma spectrometer ICPE-9000 system (Shimadzu Corporation, Japan) for simultaneous determination of Ca. Residual ash samples obtained from muffle furnace, were dissolved with 10 mL HNO_3 acid (1.0 M) and further diluted with milli Q water to achieve < 1g L^{-1} dry matter contents.

The Phosphate concentrations were determined using a colorimetric method as described in International IDF Standard 33C:1987. Ash samples were combined with 2 g of pre-prepared 0.5% w/w ammonium molybdate and 2.0% w/w ascorbic acid mixture. Resting periods of 15 minutes at 45 °C were used to develop blue colour complexes. The absorbance of the mixtures was measured at 820 nm wavelength. Standard solutions were prepared and treated similarly as test samples.

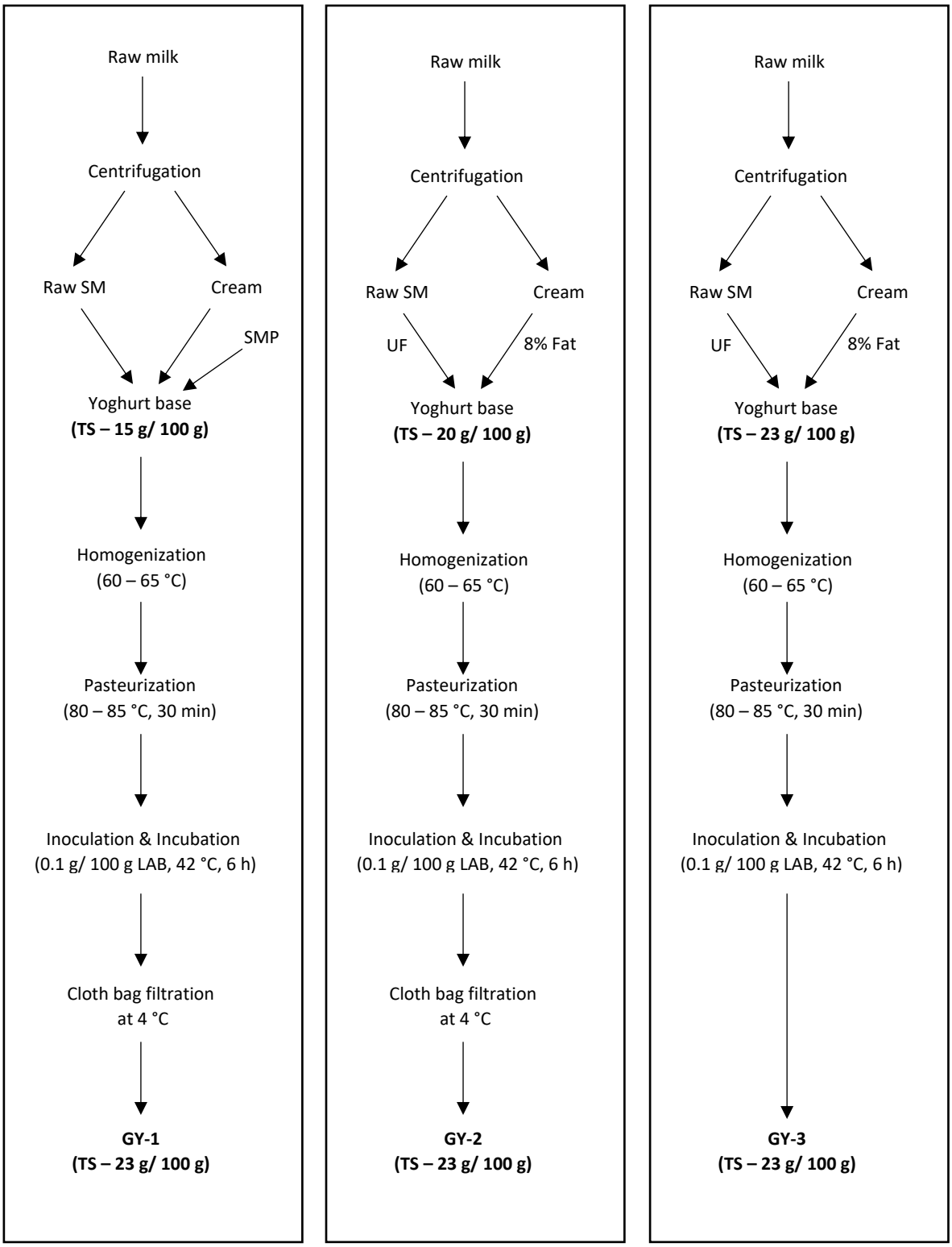

Figure 4. 1: Experimental design and coding employed in manufacturing Greek yoghurt samples by incorporating milk fortification with SMP or ultrafiltration

4.2.5 Structural analysis

Structural properties including texture attributes, rheological properties and syneresis were measured in Greek yogurts following the methods described in chapter 3, section 3.2.6.

4.2.6 Statistical analysis

The whole experiment was replicated independently twice in a randomized split plot block design with two sub-samplings ($n \geq 4$). Total solid content was selected as the main variable while replication served as blocks. Where appropriate, statistical analysis system (SAS, 1996) was employed to analyse the data. The statistical level of significance was preset at 0.05 ($P < 0.05$).

4.3 Results and Discussion

4.3.1 Chemical composition of Greek yoghurt

The chemical composition of Greek yoghurt is one of the main factors, which drives its high consumer demand (Yildiz, 2010a). The chemical analysis results obtained from the study presented in chapter three showed that different processing steps applied during yoghurt production such as whey draining and milk fortification highly affect the chemical composition of the final yoghurt. The present study has applied ultrafiltration instead of milk fortification to pre-concentrate the milk base. UF technique is a widely used in many dairy processing lines, which can be readily carried out during large scale yoghurt production. Traditional Greek yoghurt is widely considered as being rich in protein, lactic acid and mineral concentrations, which gives it a creamy white colour, a soft smooth body and acidic flavour, which attracts consumer appeal (Lange, 2013).

However, application of UF in Greek yoghurt manufacturing would affect the composition of soluble components of milk such as minerals and lactose, which may have an impact on the culture performance and structural properties of yoghurt.

Milk, which was subjected to UF for GY-2 or GY-3 production, contained significantly higher ($p < 0.05$) amount of proteins (Table 4.1) due to their retention by the UF membrane. Most of milk proteins including caseins and whey proteins possess molecular weight larger than >10kDa, which is a nominal cut off of the membrane and thus they are retained by the UF membrane under lower operational pressure. Apart from UF, cloth-bag whey draining also concentrates protein in the last stage of the processing. Since proteins are retained during both UF (~99.8%) and cloth bag filtering (~99.7%) (Yildiz, 2010a; Nergiz & Seckin, 1998), all three Greek yoghurts created in this study achieved required high protein content (Table 4.1). Although the overall protein content has an impact on the properties of the milk coagulum, the formation of yoghurt gel matrix is mainly reliant on the functional properties of casein fraction largely governed by the starter culture performance (Tamime & Robinson, 1999).

The efficient starter culture activity drives the pH reduction in milk during the fermentation. This reduction of milk pH affects the dissociation of casein micelle and reformation of three dimensional protein network. During the present study, GY-1 and GY-2 had a lower ultimate pH value in the final sample compared to GY-3, which indicates the higher rate of starter culture activity in GY-1 and GY-2 compared to GY-3. This observation is somewhat in agreement with results from work described in chapter three of this thesis, in which the Greek yoghurt produced with 23% initial milk total solid level experienced imbalanced starter culture activity likely due to rise in osmotic pressure due to dissolution of micellar casein during acidification and release of

soluble calcium phosphate, and presence of smaller peptides and free amino acids available in MPC. On the other hand, this should not be an underlying reason for hindrance of the starter culture activity as most of solutes contributing to osmotic pressure, including lactose and minerals, would have been removed during pre-concentration step. Therefore, a possible reason for slower growth would be related to availability of essential nutrients required for the proper culture activity. In the study presented in chapter three, the culture performed extremely well initially in a dose dependant manner indicative of constant supply of amino acids and peptides, which could have been removed by ultrafiltration in the study presented in this chapter. The mixed yoghurt culture, consisting of *St. thermophilus* and *Lb. bulgaricus*, is very fastidious with various growth requirements (Sah et al., 2014), some of which are provided through a symbiotic growth or supplementation. Another reason for slightly higher ultimate pH of GY-3 lies in buffering capacity of the micellar casein, which was concentrated by UF. The casein micelle releases colloidal calcium phosphate upon acidification counteracting the pH change (Lucey et al., 1993).

The reduction of milk pH is an important parameter in yoghurt gel formation as it affects solubility of minerals such as Ca and P. The physical nature of colloidal $CaPO_4$ changes at lower pH levels and plays a major role in yoghurt structure formation. During the present study, Ca and P contents were lower in yoghurt RY-2 and GY-3, which were prepared by ultrafiltration compared to RY-1 which was made without ultrafiltration (Table 4.1). This can be attributed to mineral removal through the UF membrane. Minerals in yoghurt are generally found in a colloidal or a free form in the liquid phase (Nergiz & Seckin, 1998). In milk, two thirds of the Ca content is bound to the casein micelle as $CaPO_4$ bridges. The remaining portion of the milk Ca is dissolved in the milk serum. During membrane processing, this soluble Ca passes through the

membrane into permeate. During fermentation of yoghurt, the pH level starts to decline, which leads to solubilisation of colloidal calcium phosphate and greater proportion of ionic forms in the soluble phase. The maximum dissociation of colloidal $CaPO_4$ occurs between pH 5.6 - 5.1 (Lee & Lucey, 2010). Almost all casein becomes completely free of minerals when the pH value reaches 4.6 - 4.7 (Nergiz & Seckin, 1998). A large proportion of solubilised minerals is removed along with the whey during the draining process. Although UF removed most of the soluble Ca and P in intermediate yoghurts RY-2 and GY-3, compared to RY-1; the final Greek yoghurt GY-1 contained a lower level of Ca and P compared to GY-2 and GY-3. This was likely due to the excessive amount of soluble Ca in milk serum phase which is removed through the clothbag whey draining. The lower pH level of 4.37 indicates complete dissolvent of Ca in milk serum at fermentation. Therefore, the longer whey draining of yoghurt gel required to reach the 23% w/w final dry matter content results in higher mineral losses in GY-1.

Lactose is the major sugar compound in milk, which is metabolized by the LAB during fermentation, and thus is important in yoghurt gel formation. Yoghurts which are made using ultrafiltered skim milk before acid whey draining (RY-2 and GY-3) contained lower amounts of lactose compared to RY-1 (Table 4.1). This certainly can be attributed to low molecular weight of lactose (342 Da), which is to pass freely through the UF membrane during the milk concentration process (Namvar-Mahboub & Pakizeh, 2012). Although greater concentration effect was achieved in GY-3, the lower lactose and higher lactic acid contents were observed in GY-1 and GY-2 compared to GY-3. During fermentation, the starter culture bacteria *St. thermophilus* and *Lb. bulgaricus* consume lactose as an energy source (Elfahri, 2012). Furthermore, they are homofermentative and metabolize lactose into lactic acid via Embden-Meyerhof-pathway (Vasiljevic & Shah, 2008; Donkor, 2007). The generated lactic acid thus causes the pH reduction

below 4.6 in milk (Vasiljevic & Shah, 2008). The lower pH values observed in GY-1 and GY-2 compared to GY-3 indicate efficient fermentation in GY-1 and GY-2. Therefore, the observed lower lactose and higher lactic acid contents indicates efficient fermentation and lactose metabolism in GY-1 and GY-2 compared to GY-3.

During the present study the yoghurts produced using milk with 15% w/w initial total solid (GY-1) and 23% w/w initial milk total solid did not have any significantly different fat content ($P > 0.05$). However, the yoghurt produced using 20% w/w initial milk total solid (GY-2) had a significantly higher fat content compared to both above yoghurts ($P < 0.05$). The fat level is important for maintaining thickness and the creamy sensation of yoghurts (Alting et al., 2009; Desai et al., 2013) and therefore an important indicator of yoghurt sensory attributes. The increased fat level in GY-2 can be attributed to the combined effect of fat standardization and cloth bag filtration. Although fat standardization was carried out for both GY-2 and GY-3 production, cloth bag whey draining was applied to GY-2 only. Fat filtration through cloth bag is reported to be limited to approximately 0.8% (Nergiz & Seckin, 1998). This improved fat level in GY-2 is a significant contributor to favourable textural properties in the yoghurt as detailed in a later part of this paper.

Table 4. 1: Proximate composition of Raw milk, Cream and Greek yoghurt samples at different processing stages with the corresponding acid whey resulted during Greek yoghurt production by incorporating milk fortification with SMP or ultrafiltration

Sample[1]	Characteristic							
	Total solid	Fat	Protein	Lactose	Lactic acid	Ca (mg/100 g)	P (mg/100 g)	pH
Milk	13.14±0.46	3.81±0.60	3.68±0.50	4.17±0.17	0.03±0.01	116.18±8.04	93.95±12.42	
Cream	47.87±2.05	43.92±0.40	1.75±0.44	1.25±0.26	0.04±0.01	58.54±3.52	40.78±6.04	
UF milk for GY-2	13.88±0.24	-	8.82±0.80	3.82±0.14	0.02±0.01	265.38±4.86	125.33±3.28	
UF milk for GY-3	17.53±0.62	-	12.14±0.10	3.64±0.56	0.02±0.01	254.29±7.22	148.70±3.88	
RY-1	14.55±012	3.95±0.10	4.74±0.24	3.8±0.38	0.68±0.24	244.62±16.28	121.88±8.20	
RY-2	20.20±0.11	8.10±1.30	8.29±0.48	2.15±0.26	0.66±0.03	228.66±14.55	109.91±3.04	
GY-1	22.86±0.48	8.33±0.11	10.24±0.51	1.22±0.18	0.7±0.22	169.5±23.44	68.84±10.84	4.37
GY-2	23.23±0.09	10.13±1.56	10.37±0.24	1.56±0.15	0.68±0.07	213.63±5.20	89.52±3.32	4.30
GY-3	22.81±0.09	8.61±0.42	9.98±0.13	2.44±0.20	0.47±0.02	218.63±15.19	128.04±6.83	4.52

[1]Samples are named as follows;

RY-1 and RY-2 are the fermented yoghurt mix prior to the concentration step made with 15% and 20% w/w initial milk total solid, respectively.

GY-1 and GY-2 are the Greek yoghurt concentrated from RY-1 and RY-2, respectively.

GY-3 is the Greek yoghurt made with 23% w/w initial milk TS level.

Values are means of at least 4 independent observations ($n \geq 4$); the results are presented as means ± standard deviation (SD)

4.3.2 Chemical composition of acid whey

In response to the remarkable consumer demand, the Greek yoghurt industry has tripled the production over last five years, generating 1.6 billion litres of acid whey per year (Chandrapala et al., 2015; 2016a). The reported typical figure for acid whey generation during commercial Greek yoghurt production is approximately 300 g/ 100 g of Greek yoghurt (Elliott, 2013). This large quantity of acid whey presents a challenge for the sustainable growth of the Greek yoghurt industry, as the disposal of the acid whey is not trivial. During the present study, the production of 100 g of GY-2 released 25 g of acid whey (AW-2) while production of 100 g of GY-1 (AW-1) resulted in 114 g of acid whey (Table 4.2). Therefore, the use of UF prior to fermentation of GY-2 has lowered the amount of acid whey draining by 78% and 92% w/w compared to GY-1 and the commercially available figure (Elliott, 2013), respectively. While a lower amount of acid whey is generated compared to commercial values, it is important to investigate the chemical composition of these two whey streams to evaluate their further processability (Wijayasinghe et al., 2015).

The chemical composition of GY-1 and GY-2 (Table 4.1) shows that the ultimate pH value around 4.3 and higher LA content in RY-1 and RY-2 indicates efficient fermentation in these samples, and thus dynamic lactose metabolism. Although the similar ($p > 0.05$) higher concentration of LA was attained for RY-1 and RY-2, the lactic acid concentration in acid whey increased significantly ($P < 0.05$) with increased whey drainage (Table 4.2). AW-2 contained a lower amount of lactic acid (0.55% w/w) compared to AW-1 (0.86 % w/w). The degree of acid whey draining was greater for RY-1 than that for RY-2, passing higher lactic acid content into AW-1 compared to AW-2 (Nergiz & Seckin, 1998). The higher lactic acid presence in AW-1 whey stream

directly affects lactose crystallization (Wijayasinghe et al, 2015) as it acts as an inhibitor and hinders lactose crystallization process (Jelen & Coulter, 1973; Ganzle et al., 2008). Lactose remains in its amorphous form during concentration of the acid whey stream and hinder its further processing (Dec & Chojnowski, 2006). Therefore, the lower lactic acid concentration in AW-2 should make it to easier further process compared to AW-1.

Apart from lactic acid, presence of minerals such as Ca and P is also detrimental for further downstream processing of the acid whey using membranes, as they affect membrane performance due to their deposition on the membrane surface thus directly affecting the flux (Chandrapala et al., 2015). Furthermore, the presence of large amounts of Ca restricts the lactose crystallisation downstream (Wijayasinghe et al., 2015). As a result of removal of soluble minerals during UF prior fermentation in RY-2, lower the Ca and P contents in 6.52% and 9.82% respectively compared to RY-1. However, the clothbag whey draining resulted 20.66% and 23.1% w/w lower Ca and P contents in GY-1 compared to GY-2. This suggest that, Ultrafiltered milk retains more minerals than filtering of yoghurt using cloth bag, which is also in agreement with work reported in literature (Tamime et al., 1989). Furthermore, the amount of soluble minerals was higher in yoghurt serum phase after fermentation thus resulted in a greater amount of mineral removal during the clothbag whey draining, which was performed after fermentation. Apart from mineral solubility, the mineral loss during the whey draining is mainly dependent on the factor of concentration (Bird, 1996). During the present study the degree of ultrafiltration performed in RY-2 is lower compared to cloth bag whey drainage performed on RY-1. Therefore, the resulting Ca and P concentrations were lower in AW-2 compared to AW-1. The lower mineral composition in AW-2 would assist in minimizing whey processing difficulties due to lower mineral sedimentation during membrane processing (Chandrapala et al., 2015).

Table 4. 2: Proximate composition of acid whey resultant during Greek yoghurt manufacturing by incorporating milk fortification with SMP or ultrafiltration

Sample[2]	Characteristic						
	Total solid	Protein	Lactose	Lactic acid	Ca (mg/100 g)	P (mg/100 g)	Acid whey %
AW-1	7.09±0.20	0.22±0.20	4.99±0.23	0.86±0.12	310.55±10.49	205.98±2.91	113.93
AW-2	8.12±0.55	0.92±0.01	4.5±0.01	0.55±0.10	288.43±13.06	191.18±4.41	25.08

[2]Samples are named as follows;

AW-1 and AW-2 are the acid whey expelled during the concentration step during GY-1 and GY-2 manufacturing, respectively.

Values are means of at least 4 independent observations ($n \geq 4$); the results are presented as means ± standard deviation (SD)

Table 4. 3: Textural properties and syneresis of Greek yoghurt samples prepared from the yoghurt mix containing 15% (GY-1), 20% (GY-2) or 23% w/w (GY-3) initial total solids obtained by incorporating milk fortification with SMP or ultrafiltration

Sample	Characteristic			
	Hardness (g)	Cohesiveness (%)	Adhesiveness (g.sec)	Syneresis (%)
GY-1	16.90±1.27	0.55±0.04	-149.14±12.95	19.66±0.12
GY-2	24.85±2.68	0.71±0.11	-338.19±52.32	8.83±0.40
GY-3	8.10±0.95	0.70±0.10	-74.66±11.28	24.91±0.32

Values are means of at least 4 independent observations ($n \geq 4$); the results are presented as means ± standard deviation (SD)

4.3.3 Structural properties of Greek yoghurt

The results shown above indicate that application of ultrafiltration as a pre-processing step resulted in remarkable differences in both the chemical composition of Greek yoghurts and the resultant acid whey. However, consumer perception of a product is a combination of compositional differences and the structural properties of yoghurts which include syneresis, rheological properties and textural attributes (Yazici & Akgun, 2004; Domagała, 2012).

The micro structure of a yoghurt gel is reflected by its water binding ability. Appearance of separated whey on top of the yoghurt occurs due to the decrease of water holding capacity by the gel structure. This whey separation on the yoghurt gel surface is termed syneresis and considered a negative attribute in quality considerations by consumers (Lee & Lucey, 2010; Lucey & Singh, 1998; Lucey et al., 1998b). Therefore, minimal whey separation is recommended for a better consumer appeal. During the present study, GY-2 showed significantly ($P < 0.05$) lower syneresis than GY-1 and GY-3 (Table 4.3). This observation could be attributed to greater covalent interactions between caseins and whey proteins denatured during heat treatment influenced by concentration step as well as reinforcing this structural organization during straining. During fermentation, when milk pH starts to decline, stability of casein micelle becomes compromised by solubilisation of colloidal calcium phosphate which dissolves into milk serum. When milk pH reaches iso-electric point of caseins 4.6, stability of the κ-casein brush on the surface of the casein micelles diminishes. Consequently, steric repulsions are minimized and van der Waals attractions prevail leading to flocculation of the casein micelles. Heat treatment of UF concentrated milk prior to fermentation likely enhanced molecular movement of proteins and facilitated covalent interactions

between whey proteins and mainly κ-casein, which ultimately led to greater gel hardness and higher storage modulus and less susceptibility to syneresis. It also appears that thiol groups of whey proteins are activated during heating but do not fully engage with other proteins in the system. During acidification and likely during straining these thiol groups may be involved in thiol group/disulfide bond interchange reactions and have an additional effect on gel strength (Vasbinder et al., 2003). In addition to this rearrangement of the protein network, straining after fermentation in GY-2 has removed most of the free water content further compacting the gel structure (Ozer et al., 1999a) with evenly distributed pores with a reduced pore size, which lower the water movement inside the gel network, thus increase the water holding capacity (Zayas, 1997).

Yoghurt gel hardness observed in this study agreed well with the syneresis data, by which GY-2 showed the hardest gel structure compared to both GY-1 and GY-3 (Table 4.3) due to concentration effects of both, ultrafiltration and straining. The casein micelles function as the primary building blocks of the yoghurt structure. Greater amount of proteins assists in building a more complex protein network (Ozer et al., 1999a). Although GY-3 was prepared by greater concentration of solids using UF than GY-2, presence of free water in the gel structure likely obstructed hydrophobically driven interactions among casein molecules by maintaining intermolecular distances preventing formation of a stronger network. This was avoided during GY-2 production, as the free water was removed by straining, which subsequently facilitated casein aggregation via non-covalent interactions (Phadungath, 2005b; Lange, 2013). In addition, casein aggregation was likely further promoted by greater concentration of whey proteins and fat. Since whey proteins preferentially position themselves on the surface of fat globules, such complexes may further develop additional cross links such

as disulphide bonds within the gel matrix (Lange, 2013) resulting in a harder gel structure.

Apparently, a combined effect of UF and straining results in a firm gel structure in GY-2 indicating a strong solid gel. The rheological properties of yogurt gel are affected by its dry matter content and the type of protein present in the yoghurt gel matrix (Barreto et al., 2006; Jumah et al., 2001; Oliveira et al., 2001). Figure 4.2 (A) and (B) shows the storage modulus (G') and loss modulus (G") over a frequency sweep between 0.1 and 100 Hz for all Greek yoghurt types under constant shear strain of 0.5%. According to the results, all the samples showed a predominantly elastic character over their viscous behaviour (G'>G''), indicating that non-relaxing protein bonds dominated over rapidly breaking and reforming weak bonds (Ozer et al., 1998a). During the study presented in chapter three, MPC fortification and straining increased the casein content strengthened the gel network in GY-2 as a result of establishment of non-covalent interactions. The higher amount of non-relaxing protein bonds forms a much denser and stronger gel structure (Ozer et al., 1998a; Anema, 2008a). The same phenomena are observed during the present study, in which use of UF and straining enhanced the casein content in GY-2, showing a greater G' than that of other two samples. During the work presented in chapter three it was noted that increase of casein content alone was not sufficient for increasing the G' and G". Straining after fermentation was also an important consideration to obtain a solid-like behaviour. During the present study, although GY-3 had an increased amount of protein content due to UF, the absence of straining after fermentation resulted in a significant amount of free water content, thus leading to a weak gel structure.

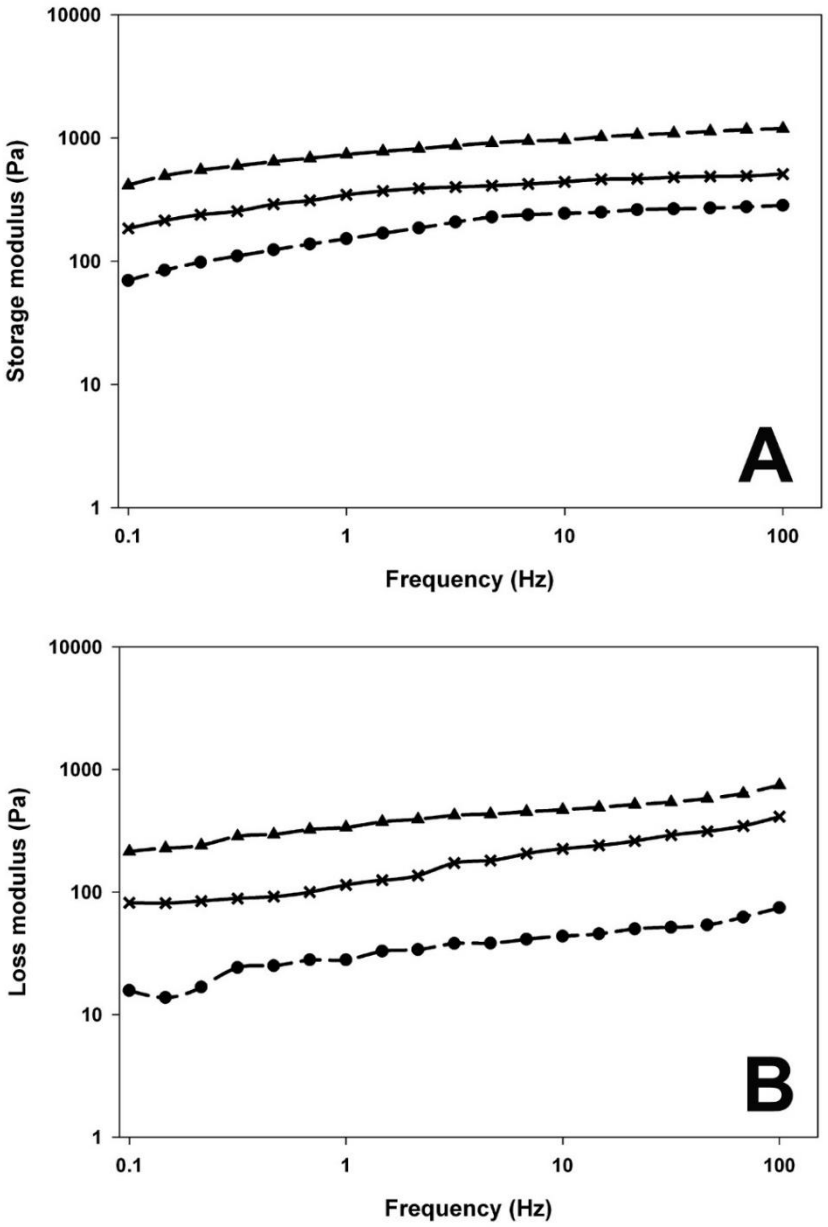

Figure 4. 2: Viscoelastic properties of Greek yogurt represented as (A) storage modulus - G' and (B) loss modulus – G" produced by fermentation at 42 °C for 6 hours of a yoghurt mix standardized to 15% (Solid line with Cross mark), 20% (Dash line with Triangle) or 23% (Dash line with Circle) total solids by addition of skim milk powder (SMP) or skim milk ultrafiltration. Values are means of at least 4 independent observations ($n \geq 4$).

Although the Greek yoghurts produced in the study showed considerable differences in gel strengths under small oscillations, they exhibited non Newtonian flow and shear thinning behaviour (Figure 4.3), illustrated by immediate and greater reduction of viscosity under increasing shear rates due to break down of gel network (Bong & Moraru, 2014). At the reversed decreasing shear rate, all samples were able to re-build the structure to a certain extent showing an irreversible time dependent effect or irreversible thixotropic behaviour. GY-2 showed higher viscosity values range throughout the shearing process due to presence of higher fat content, which allowed for absorbance of applied force (Shaker et al., 2000). At the same time, the shortening of the intramolecular and intermolecular interactions in GY-2 due to whey draining enabled flexing without breaking the intra-particle cross-links (Purwandari et al., 2007) also contributing to enhancement of viscosity.

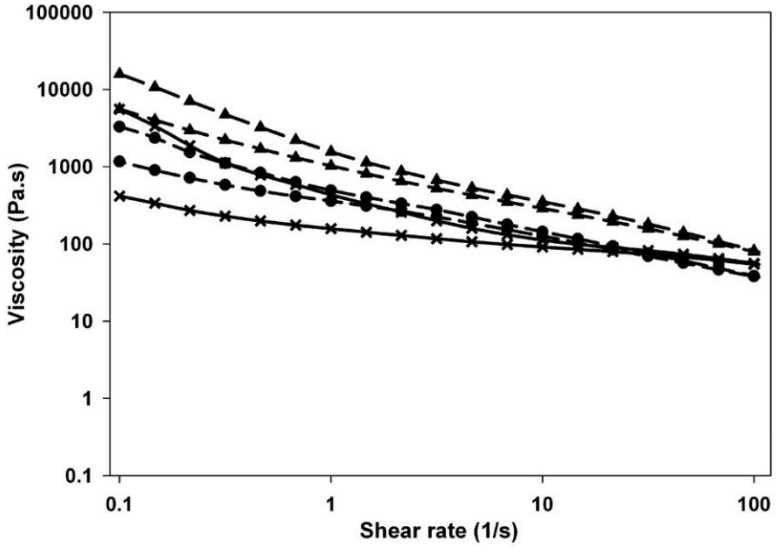

Figure 4. 3: Flow behaviour of Greek yogurt samples produced by fermentation at 42 °C for 6 hours of a yoghurt mix standardized to 15% (Solid line with Cross), 20% (Dash line with Triangle) or 23% (Dash line with Circle) total solids by addition of skim milk powder (SMP) or skim milk ultrafiltration. Values are means of at least 4 independent observations ($n \geq 4$).

The resultant chemical and structural properties obtained from this study strongly suggest that incorporation of skim milk ultrafiltration prior fermentation efficiently lower the acid whey generation while whey draining after fermentation strengthened the final yoghurt gel structure.

4.4 Conclusion

Minimising the quantity of acid whey generation at the point of origin and optimization of the chemical composition of generated whey are important considerations for sustainable Greek yoghurt production. Incorporation of an ultrafiltration stage prior to fermentation positively reduced the requirement for acid whey draining after fermentation. Accumulation of lactic acid and minerals in resulted acid whey has significantly increased with application of straining. While the use of UF enhanced the protein content in milk base, it removed soluble milk components such as lactose and minerals into the permeate. Concentration of milk to 17.8% by in GY-3 impeded the fermentation process due to loss of nutrients and developed osmotic pressure in milk base. Apart from UF, straining appeared to be an important processing step to achieve a rigid yoghurt gel structure. GY-2, which production incorporated UF as well as straining, was characterized with a harder gel structure, lower syneresis and maximum rheological properties compared to other two Greek yoghurts. Thus, the present study showed that producing Greek yoghurt applying both, UF and straining, was possible as products with required structural attributes were manufactured, and at the same time the amount of released acid whey was substantially reduced.

Chapter 5

Conclusions and Future Directions

5.1 Conclusions

Greek yoghurt is among the most demanded dairy products from infant to adult consumers around the world due to its unique sensory and functional properties. These favourable properties include higher concentrations of protein, lactic acid and minerals compared to regular yoghurt. Additionally, the product has a creamy white colour, a soft and smooth body, good spreadability with little syneresis and a flavour that is clean and slightly acidic. Although Greek yoghurt possesses these superior properties, the processing of Greek yoghurt is environmentally challenging. The straining applied after fermentation in Greek yoghurt manufacturing results in acid whey which contains higher amount of lactic acid. Other than lactic acid, acid whey composes of a significant amount of nutrients such as lactose, minerals and whey proteins. Acid whey cannot be dumped into water ways or land as it damages the aquatic life and crops. Therefore, proper disposal methods are mandatory if it is to be released. Therefore, as attempts to improve sustainability of Greek yoghurt industry a handful of methods to use of acid whey as a by-product has been attempted. This includes use of acid whey as animal feed supplementary, fertilizer and use to produce bio-gas. However, aspects such as the extra cost of production and high acid level in the whey stream has limited practical use of acid whey as a by-product. Therefore, new strategies are needed to resolve the acid whey problem.

Apart from post production treatment for generated acid whey, addressing the acid whey problem during Greek yoghurt manufacturing by lowering acid whey generation at its point of origin is an effective method. Elevation of milk total solid prior fermentation has been the subject of many studies including milk fortification with protein base supplementary and milk draining prior fermentation. These studies have

followed the production process for Greek style yoghurt production, which is different from the application of draining after fermentation unique to Greek yoghurt manufacturing. The present study was aimed to enhance milk total solid level prior fermentation while retaining the filtration stage after fermentation. Milk enhancement prior fermentation reduces the degree of straining after fermentation, and thus minimizes the acid whey generation during Greek yoghurt manufacturing while obtaining quality Greek yoghurt that can compete with commercially marketed products.

The present study has been based on elevation of initial milk total solid level up to 15%, 20% and 23% w/w prior fermentation and strained after fermentation to produce Greek yoghurts which contained 23% w/w total solid level. The study was conducted in two phases. In the first phase of the experiment, fortification of milk with MPC and SMP was used to increase initial milk total solid level. Initial milk bases of 20% and 23% w/w were prepared by adding MPC while maintaining 8% w/w fat level by incorporating cream. 15% w/w initial milk base prepared by adding SMP directly to raw milk and feed as the control. All three yoghurts were concentrated using cloth bag filtering after fermentation which was necessary to obtain Greek yoghurts with 23% w/w final TS level. The chemical composition of acid whey resultant during this cloth bag filtering was measured to understand the effect of alterations performed during the yoghurt processing on the resultant whey. As a preliminary investigation, this model of experiment measured the starter culture activity to understand how culture performed under three different total solid levels. In addition, chemical and structural properties of resultant Greek yoghurts were examined. Finally, novel products were compared chemically and structurally against well-known commercial products to evaluate aspects for retailer success.

The elevation of milk total solid level to 15%, 20% and 23% w/w using MPC and SMP observed positive changes in starter culture performance. All milk samples showed typical pH profile showing slow pH reduction at the beginning and steady increments after 2h of time at fermentation. Milk samples reached target pH 4.6 after 5 h 33 min, 5 h 16 min and 5 h 45 min for 15%, 20% and 23% w/w respectively. Milk with 23% w/w TS level spent longer time to obtain pH 4.6 due to the milk buffering capacity. The LA development increased with the elevated milk TS level. The growth of *St. thermophilus* and *Lb. bulgaricus* was higher compared to values reported in open literature due to the protein base milk supplementations (MPC and SMP) in this experiment. The development of higher osmotic pressure in milk with 23% w/w TS hindered the growth of *St. thermophilus* indicated with lower growth rate. The study found 1h, 2h and 3 h lag phase durations respectively for 15%, 20% and 23% w/w TS. However, *Lb. bulgaricus* showed increased growth with TS following the LA profile. The protein base milk supplementary provides sufficient nutrients (free amino acids and peptides) for *Lb. bulgaricus* propagation.

The use of milk fortification with MPC and SMP during this phase increased the dry matter content in milk base prior fermentation exhibiting lower requirement of acid whey draining after fermentation. Moreover, the Greek yoghurt produced with 23% w/w initial milk total solid level avoided whey draining due to already achieving required TS level in final yoghurt base by MPC fortification prior to whey draining stage. Additionally, Greek yoghurt produced with milk adjusted to 15% or 20% w/w total solid levels resulted in 113.93% and 28.04% w/w acid whey, respectively, during the clothbag whey draining to achieve 23% TS in the finished products. Approximately 30% w/w lower release of LA was achieved along with 86% w/w lower amount of acid whey in GY-2 production compared to acid whey generated in GY-1 production and

47% w/w compared to a typical commercial figure respectively due to the lower degree of draining requirement.

Apart from acid whey properties, whey draining also affected chemical and structural properties of resultant Greek yoghurts. MPC fortification increased protein content up to 6.95% and 9.28% w/w in milk bases which were used to produce GY-2 and GY-3, respectively. However, the clothbag filtering in GY-2 increased fat content to 10.24% w/w in final Greek yoghurt. Therefore, combined effect of MPC fortification and clothbag whey draining in GY-2, resulted in higher protein fat combination which assisted in achieving a rigid gel structure.

The removal of whey after fermentation in GY-1 and GY-2 minimized the size of pores in yoghurt gel structure compared to GY-3 increasing hardness by 28% and 33.6% for GY-1 and GY-2 samples, respectively, as compared to GY-3 (14.9%). Furthermore, removal of free whey in GY-2 resulted in lower syneresis compared to other two yoghurts due to higher amount of fat, which increased hydrophilic nature on yoghurt gel surface maximizing water binding sites. The best storage and loss modulus was observed in GY-2 due to higher protein content in the former gel as a result of MPC fortification and lower retainment of free whey. The GY-1 and GY-2 showed higher apparent viscosity compared to GY-3 at high shear rates due to the increased casein to whey protein ratio at whey draining.

While the use of milk powders during this first phase showed favourable qualities in resultant Greek yoghurts, cost of powders adds a considerable amount to total production cost which can be a challenging for Greek yoghurt production at a reasonable price. Therefore, in the second phase of the experiment, the UF technique was introduced instead of milk fortification to enhance milk TS level prior to

fermentation. The selection of UF as a concentration technique was also based on ability of UF to achieve comparable results with traditional cloth bag filtration with improved efficiency. During this phase the chemical and structural properties of resultant Greek yoghurt were evaluated and compared to acid whey generation to understand the effect of UF on the Greek yoghurt properties and resultant acid whey.

During this phase of the study, the application of UF to enhance TS up to 17.5% w/w (GY-3 production) prior fermentation resulted in zero acid whey generation due to complete exclusion of cloth bag filtering after fermentation. The initial milk bases with 20% TS and 15% w/w TS resulted of acid whey release of 25 g and 114 g, respectively, for 100 g of GY-2 and control GY-1 production. Therefore, milk base with 20% TS showed a reduction in acid whey generation by 78% w/w compared to the control (GY-1) yoghurt. Apart from the amount of acid whey, the lactic acid concentration in acid whey also increased significantly ($P < 0.05$) with a degree of draining showing 0.55% LA in AW-2 compared to 0.86 % w/w in AW-1. In addition, the Ca and P contents were lower in AW-2 compared to AW-1 due to applied UF in GY-2 production. This lower amount of LA and mineral composition in AW 2 can help to minimise whey processing difficulties.

Apart from changes in acid whey properties, use of UF affected chemical and structural properties of resultant yoghurts. GY-1 (pH 4.37) and GY-2 (pH 4.30) showed lower pH value in final Greek yoghurts compared to GY-3 (pH 4.52). This was likely due to removal of valuable and readily available nutrients such as peptides and free amino acids as well as development of higher osmotic pressure in GY-3 which consequently hindered the starter culture activity negatively affecting on pH reduction. The lowest pH which resulted in GY-2 indicated efficient liberation of excessive Ca and P into milk

serum during fermentation. In addition to minerals, a remarkably higher protein content and highest fat content was observed in GY-2 compared to other two yoghurts. These properties positively interacted towards formation of a strong gel structure.

The lowest syneresis was observed in GY-2 ($P < 0.05$) compared to others due to the lower degree of UF. Due to this lower filtration, the amount of Ca remained in RY-2 is higher compared to others. This results in a more compact structure with lower pore sizes. The UF and cloth bag whey draining in GY-2 enhanced the amount of casein and whey proteins. This lower casein to whey protein ratio in GY-2 results in a higher number of crosslinks in protein network resulting in a harder gel structure (24.85 %) compared to both GY-1 (16.9 %) and GY-3 (8.10 %). Furthermore, since GY-2 was rich in casein, it exhibited a higher G' than other two yoghurts during small oscillation rheological testing. The absence of whey draining after fermentation in GY-3 resulted in higher free water content, and thus lowered withholding for a given force. At increasing shear rates, all Greek yoghurts showed non Newtonian flow behaviour exhibiting immediate and greater reduction of viscosity due to break down of gel network. However, GY-1 and GY-2 showed greater absorption of applied force without breaking inter-particle cross-links when compared to GY-3. This may be due to the draining after fermentation making a closer arrangement in the gel networks in GY-1 and GY-2.

In this study two approaches to produce Greek yoghurt were investigated. In the approach presented in chapter 3, MPC fortification was used to increase the TS content while, in chapter 4 ultrafiltration was used. A comparison between the quantities of acid whey repelled in each method revealed that the amounts are similar in both methods. This can be attributed to the fact that both method lead to the same amount of TS level and hence the similarity in required whey removal. On the other hand, the qualities of

final Greek yoghurts were found be different in the two approaches, which can be attributed to compositional differences due to fortification techniques used. Milk base fortification with MPC added 6.95% and 9.28% w/w protein contents in GY-2 and GY-3 preparation at the fermentation respectively, while ultrafiltration increased 8.29% and 9.98% w/w of protein in milk bases at the fermentation of GY-2 and GY-3. This higher protein content in milk base from the ultrafiltration process maximize the number of crosslinks that build during fermentation, leading to a strong and rigid yoghurt gel structure.

In summary, the use of UF technique prior fermentation resulted in native whey which can be further processed successfully using presently well-known techniques. Thickening of milk prior fermentation reduced the degree of straining required after fermentation and therefore reduced the acid whey generation. Thus this study revealed helpful insights for reducing the large amounts of acid whey that is generated during the Greek yoghurt manufacturing, as a practical solution for the acid whey issue.

In addition to increasing initial milk dry matter content, use of whey draining after fermentation positively attributed in concentration of protein and fat in yoghurt to build a strong gel structure. The use of whey draining stage after fermentation was also found to be an important step in production process which builds a compact gel structure with favourable chemical and structural properties. Thus, the present study concludes that, combined effect of UF and filtration through a cloth bag can result in reduced generation of acid whey with a positive compositional content and a well-accepted Greek yoghurt structure.

5.2 Future Directions

To date, most of the solutions practiced for addressing the acid whey issue have attempted to use acid whey to produce by-products. The present study introduced an alternative strategy to minimize acid whey generation at the point of origin by incorporating dry matter enhancement prior milk fermentation. The use of an UF stage prior to milk fermentation minimized whey draining after fermentation and thus reduced acid whey generation effectively. The UF also positively contributed to developing a quality Greek yoghurt exhibiting good chemical and structural properties.

For extension of this laboratory level findings to an industrial production process, it is mandatary to investigate viability of each production stage in a bulk production environment. It is also important to investigate the customer satisfaction via a sensory evaluation. In addition, monitoring of shelf life is important as yoghurt is rich nutrition media for pathogenic microbes' growth.

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