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DEVELOPMENT AND PREDICTIVE MODELLING OF SET YOGHURT AS A MADE- IN-TRANSIT (MIT) PRODUCT

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ABSTRACT

The manufacture of food during distribution, a concept known as “made-in-transit” (MIT) manufacture, has the potential to expand the distribution reach, extend shelf-life, and provide the customer with the freshest possible product. Benefits for the manufacturer include maximising throughput while minimising manufacturing space and inventory. This concept is new, with mushrooms being the only MIT food developed so far. In this study, the feasibility of developing an MIT product from a fermented food was determined using set yoghurt as a model system. An MIT yoghurt was developed through the alteration of some of the yoghurt manufacturing parameters (e.g. milk base formulation, heat treatment, starter culture composition and fermentation temperature), extending the yoghurt fermentation time from about 4 h to 168 h (7 days). It was found necessary to fortify the milk base (reconstituted skim milk powder) with a suitable protein-containing dairy ingredient (sodium caseinate and milk protein concentrate were found best) to rectify the poor texture of the resulting yoghurt. Descriptive testing and acceptance testing using trained and consumer sensory panels, respectively, showed that MIT yoghurts were significantly different from conventionally made yoghurt, but were more acceptable. The yoghurts were found to have adequate shelf lives post fermentation, partly owing to the use of ultra-high temperature (UHT) sterilization of the yoghurt milk base prior to inoculation to ensure the absence of starter bacteria competitors. As the production of MIT yoghurt involves a relatively long, temperature dependent fermentation, it was considered useful to develop models for predicting the effects of both time and temperature on such dependent variables as starter bacteria count, pH, and firmness. A predictive microbiology approach was taken. The modified Gompertz equation was found to model adequately the time dependence of starter bacteria growth and firmness development during fermentation, while the modified logistic equation was found adequate for modelling pH decline. These two equations are primary models, whose parameters were then related to temperature using the square root (Ratkowsky) equation as a secondary model. Combination of the primary and secondary models provides means of predicting the effects of both time and temperature simultaneously. Comparison of predictions with data generated in extrapolation and interpolation experiments proved the efficacy of the models.

The work described in this thesis demonstrates the potential of the MIT concept for a fermented food. The concept could be applied to many fermented foods.

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ABBREVIATIONS

λ	Lag phase
μ_{\max}	Maximum growth
BMP	Buttermilk powder
CCP	Colloidal calcium phosphate
cfu	Colony forming unit
g	gram
h	hour
HTLT	High temperature long time
MIT	Made-in-transit
mL	millilitre
mmol	millimoles
MPC	Milk protein concentrates
NaCN	Sodium caseinate
SMP	Skim milk powder
STLA	<i>Streptococcus thermophilus</i> STM5 and <i>Lactobacillus acidophilus</i> LA5
STLB	<i>Streptococcus thermophilus</i> STM5 and <i>Lactobacillus delrueckii</i> subsp. <i>bulgaricus</i>
STLC	<i>Streptococcus thermophilus</i> STM5 and <i>Lactobacillus casei</i>
UHT	Ultra-high temperature
WMP	Whole milk powder
WPC	Whey protein concentrates
WPD	Whey protein denaturation

LIST OF PUBLICATIONS

Nor-Khaizura M-A-R, Flint SH, McCarthy OJ, Palmer JS, Golding M, Jaworska A (2012). Development of made-in-transit set culture yoghurt: effect of increasing the concentration of reconstituted skim milk as the milk base. *International Journal of Food Science & Technology* 47 (3):579-584. doi:10.1111/j.1365-2621.2011.02880.x

Nor-Khaizura M-A-R, Flint SH, McCarthy OJ, Palmer JS, Golding M, Jaworska A. The Effect of Milk Base Fortification and Heat Treatment on the Texture of Set Culture Yoghurt as a Made-in-Transit (MIT) Product. Submitted to *Food Science & Nutrition*

Nor-Khaizura M-A-R, Flint SH, McCarthy OJ, Palmer JS, Golding M, Jaworska A. Fermentation Conditions to Enable the Manufacture of a Long Life Set Yoghurt as Made-in-Transit Product. Submitted to *International Journal of Dairy Technology*

Conferences

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Nor-Khaizura M-A-R, Flint SH, McCarthy OJ, Palmer JS, Golding M (2012). The Effect of Fortification and Heat Treatment on the Texture of Set Culture Yoghurt as a Made-in-Transit Product. New Zealand Institute Food Science and Technology (NZIFST) Conference 2012, Claudelands Events Centre, Hamilton, 26-28 June 2012. (Oral and poster presentation)

Nor-Khaizura M-A-R, Flint SH, McCarthy OJ, Palmer JS, Golding M (2012). Development and Predictive Modelling of Set Yoghurt as a Made-in-Transit (MIT) Product. Food Symposium 2012, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, 16 November 2012. (Oral presentation)

CHAPTER ONE : INTRODUCTION

1.1 Introduction

Shelf-life loss during the distribution of food is a growing problem for the food industry as manufacturers centralize production into large manufacturing units and expand their markets. Jaworska (2007) revolutionized the transportation of food from merely relocating products to a productive system through her innovative Made-in-Transit (MIT) concept. The MIT research that she conducted was in growing agriculture produce, particularly mushrooms, which are grown in a special package that provides suitable conditions for mushroom growth. Growth occurs during transportation instead of on the farm. The benefits of MIT are a shorter manufacturing time and the delivery of a fresher product simply by making use of the transportation time for the manufacture of the product. Adaptation of this concept to a food manufacturing system, which would permit production during distribution, is one potential solution to maximizing product shelf-life.

1.2 The rationale and importance of the study

The MIT concept could create an opportunity for New Zealand manufacturers to expand their product market since New Zealand is a long way from world markets. To investigate the feasibility of applying the MIT concept in food manufacturing, this project required a suitable food product for which at least some of the processing occurs during distribution. Potential foods most suited to MIT are fermented products like cheese, salami, fermented drinks and yoghurt. Yoghurt has a worldwide market as a fermented product yet has a short shelf-life. As yoghurt has a rapid fermentation time, it was selected as a suitable food for this feasibility study as results could be obtained in a short time frame.

To apply the MIT concept to yoghurt, fermentation needs to be carried out during distribution. Standard yoghurt is manufactured using pasteurized milk inoculated with 2 to 3% by volume of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* as the starter culture (at a total concentration of about 10^8 cfu mL⁻¹). Generally, the fermentation lasts for 6 to 12 h in the processing plant, which, for the MIT concept, would only allow only a short distribution of the product. Manipulation of crucial factors including milk base composition, heat treatment, starter culture composition and inoculum size (Tamime & Robinson, 2007) could extend the fermentation to give a yoghurt that could be used to test the feasibility of the MIT concept. Yoghurt with an extended fermentation time of up to seven days could be produced during

transportation from New Zealand to Australia or the Pacific Islands. No research on extending yoghurt fermentation time to several days has been reported.

Manipulation of the yoghurt fermentation time could be expected to affect other properties of the yoghurt, including texture. Yoghurt gelation is closely dependent on the pH-time profile during fermentation. Extending the fermentation time will extend this profile and hence yoghurt gelation. However, there are possible solutions to problems with yoghurt texture. Among them are increasing the total solids by fortification with skim milk powder (Damin, Alcântara, Nunes, & Oliveira, 2009; Guzmán-González, Morais, Ramos, & Amigo, 1999), buttermilk powder (Trachoo & Mistry, 1998), whey protein concentrates (Damin, et al., 2009; Guzmán-González, et al., 1999), whey protein isolates (Isleten & Karagul-Yuceer, 2006), milk protein concentrate (Guzmán-González, et al., 1999) and sodium caseinate (Damin, et al., 2009; Isleten & Karagul-Yuceer, 2006). The addition of bacteria producing exopolysaccharides as adjunct starter cultures has also been reported to influence yoghurt texture (Amatayakul, Sherkat, & Shah, 2006; Hassan & Frank, 1997; Hassan, Frank, Schmidt, & Shalabi, 1996; Hassan, Ipsen, Janzen, & Qvist, 2003; Sodini, Remeuf, Haddad, & Corrieu, 2004). Other approaches, such as the addition of stabilizers including gelatin in plain yoghurt, is not permitted in some countries (Tamime & Robinson, 2007).

Applying MIT conditions successfully requires careful control of product parameters, and this is best achieved through predictive mathematical modelling. This can assist in predicting the outcomes of the fermentation process. In addition, models can be used to identify the conditions that need to be manipulated to yield a product for a specific destination. Some research has been reported on the application of predictive modelling to standard yoghurt fermentation (De Brabandere & De Baerdemaeker, 1999; Soukoulis, Panagiotidis, Koureli, & Tzia, 2007). The modified Gompertz model provided information on the pH and texture profile of yoghurt during fermentation. Due to the complexity of the fermentation process, empirical models function better than existing mechanistic models (Soukoulis, et al., 2007).

1.3 Hypothesis and Objectives

The hypothesis underlying this research work was that yoghurt processing and fermentation conditions (e.g. milk base composition, fermentation temperature) could be altered to extend the fermentation time to 168 h (7 days) to develop yoghurt as an MIT product, and that empirical predictive models could assist in designing yoghurt fermentation conditions and predicting the effects of variation in conditions.

In order to verify this hypothesis, the following aims were defined:

1. To investigate the effect of starter culture composition, inoculum size and fermentation temperature on fermentation time (Chapter 4).
2. To investigate the effects of increasing the concentration of skim milk powder in the yoghurt milk base on the texture, and fermentation time extension, of set culture yoghurt as an MIT product (Chapter 5).
3. To determine if the texture of MIT set yoghurt could be improved by fortification of the milk base by testing five different dried dairy ingredients (skim milk powder (SMP), buttermilk powder (BMP), whey protein concentrate (WPC), milk protein concentrate (MPC) and sodium caseinate (NaCN)) in combination with a single heat treatment (ultra-high temperature (UHT) sterilization) or a double heat treatment (a high temperature long time (HTLT) heat treatment followed by UHT sterilization) (Chapter 6).
4. To determine the optimum fermentation temperature, starter culture composition and inoculum size for producing set yoghurts as MIT products using skim milk bases fortified with either sodium caseinate (NaCN) or milk protein concentrate (MPC) (Chapter 7).
5. To evaluate the microstructure, sensory characteristics and storage stability of MIT yoghurts produced using skim milk bases fortified with either MPC or NaCN (Chapter 8).
6. To model yoghurt acidification (pH change), starter bacteria growth and firmness development as functions of fermentation time and temperature, and to validate the developed models using interpolation and extrapolation data (Chapter 9).

1.4 Thesis outline

This thesis covers the background information from previous research related to the thesis subject (Chapter 2); the development of the set yoghurt with an extended fermentation (168 h) and shelf-life as an MIT product (Chapter 3 to 8) to fulfil the objectives 1-5; the development of an empirical model and its validation to describe and predict the yoghurt fermentation in term of acidification, starter culture growth and firmness (Chapter 9) to fulfil the objectives 6 (Figure 1.1).

Chapter 2 provides background information about the MIT concept, its definition and benefits. The suitability of a food as an MIT product is generally a fermented food therefore yoghurt fits this category. The yoghurt processing and factors that might influence yoghurt fermentation are also included. Finally, the predictive model with the ability to describe fermentation is discussed.

Chapter 3 provides the general materials and methods applied in most chapters of this thesis.

Chapter 4 describes the alteration of yoghurt processing and fermentation conditions in order to extend the fermentation to 168 h. The effect of three different starter culture combinations (STLB, STLA and STLC) is examined at various inoculum sizes and fermentation temperatures, investigated initially in a microbiological impedance assay system (BacTrac™ 4300) and extended to the manufacture of set yoghurt.

Chapter 5 provided the first work on improving the yoghurt texture after the fermentation was extended, increasing the percentage of skim milk base (from 12 to 20% (w/v)) to produce set yoghurt.

Chapter 6 provides the second series of trials to improve the yoghurt texture using five different dried dairy products including skim milk powder, buttermilk powder, whey protein concentrate, milk protein concentrate and sodium caseinate. The fortified milk bases were further subjected to two methods of heat treatment; single (ultra-high temperature) and double (high temperature long time and followed by ultra-high temperature) heat treatment.

Chapter 7 defines the optimum processing and fermentation conditions based on the fermentation temperature, starter culture composition and its inoculum size to produce yoghurt with an acceptable final pH when fortified with milk protein concentrate and sodium caseinate.

Chapter 8 describes the MIT set yoghurt characteristics in terms of the microstructure, sensory evaluation and storage stability for comparison between two yoghurt processing conditions.

CHAPTER ONE: INTRODUCTION

Two conditions that resulted from work in Chapter 7 are 1) milk bases fortified with milk protein concentrate, inoculated with 0.2% (v/v) of *S. thermophilus* and *L. acidophilus* (STLA) and 2) milk bases fortified with sodium caseinate, inoculated with 0.002% (v/v) STLA. Both conditions were fermented at 25°C for 168 h.

Chapter 9 describes the development of yoghurt acidification, starter cultures growth and firmness models. Three empirical primary models are used to describe the experimental data. Best primary model is selected based on the statistical indices and estimated growth parameters were used to develop the secondary model. The secondary model is developed as function of fermentation temperature. The predictive model is then validated using the interpolation and extrapolation of fermentation temperature.

The final Chapter 10 provides the summary of the main achievements of this research and provides recommendations for future studies.

Development and Predictive Modelling of Set Yoghurt for Made-in-Transit (MIT) Product

This thesis is driven to fill the central gap overlapping between the three major topics (I, II and III)

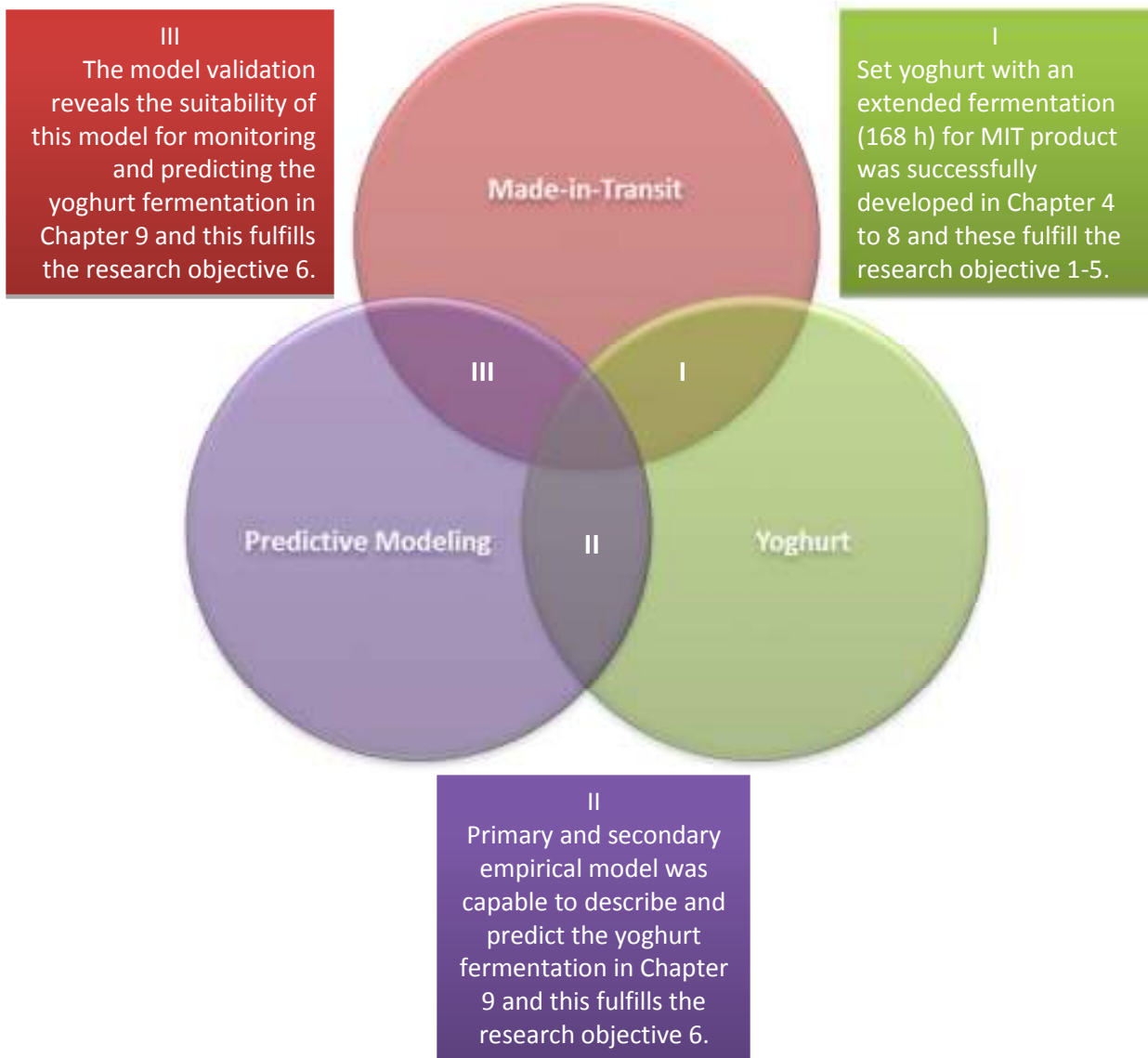


Figure 1.1: Overview of the thesis

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CHAPTER TWO : LITERATURE REVIEW

2.1 Fundamental features of MIT

Made-in-transit (MIT) is defined as a supply chain concept in which the production or manufacture of a perishable food occurs partially or completely during transportation (Jaworska, 2007a). The MIT concept transforms manufacture where manufacture is merged with distribution. This has the potential to change the role of transportation from simply relocating material to include manufacture (Jaworska, 2007a). Jaworska (2007b) described transportation as a productive creator of value by taking a total chain perspective from the outset and skipping, merging or reversing the order of events. The MIT concept is an example of convergent technology where two or more activities are combined into one. MIT and the traditional manufacture and distribution systems are compared in Figure 2.1. Traditionally, product shelf-life is reduced by the time taken for transportation (Figure 2.1a). In Figure 2.1b, the MIT concept avoids this loss of shelf-life and provides an opportunity for the consumer to harvest the fresh end product themselves.

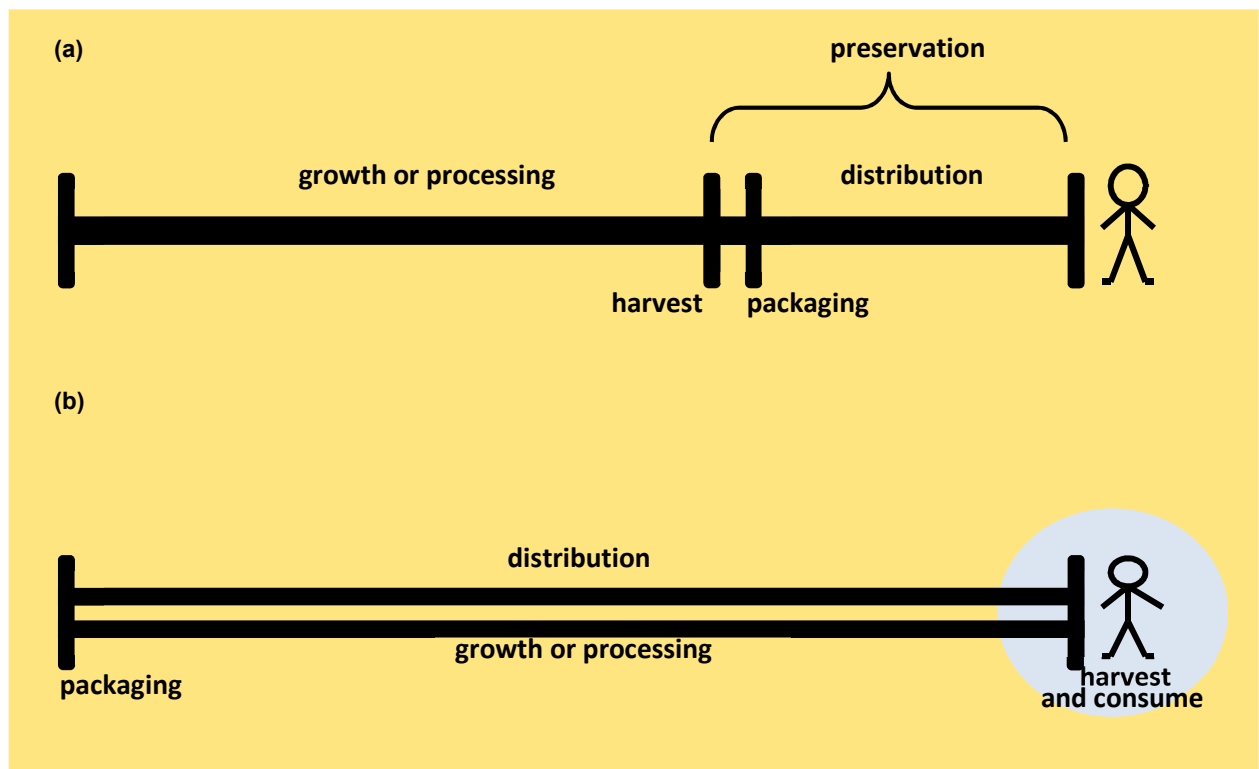


Figure 2.1: Description of Made in Transit Food (Jaworska, 2007a), with some modification. Agata Jaworska’s Master Thesis (with permission)

2.2 Application and advantages of MIT

One application of this concept is the growth of mushrooms which can occur in packs within 5 to 7 days during transportation. If packages arrive prematurely at the retailer, the last part of growth could happen there (Jaworska, 2008). Consumers may wish to purchase before the “ready by” date and pick one that suits their need, based on their planned time of consumption. The MIT concept has the potential to make use of a ‘ready by’ date in preference to a “use by” date. This ensures that the consumer receives a fresh product ideal for consumption. There are several other benefits of MIT (Jaworska, 2007a, 2007b);

- a) Reduced factory manufacturing and inventory space
- b) Growth-enabling technology replacing post-harvest technology for plant produce
- c) Expanding the distribution of product by making use of the manufacturing time for transportation
- d) Extending the shelf-life as the product arrives at the retailer in a fresher state than would be possible if manufacture and distribution occurred in the normal sequence.
- e) Preventing overproduction and consequent waste, when applied to on-demand supply chains, and
- f) Providing higher quality, freshness and nutrient-rich products to the consumer.

2.3 Challenges of MIT

Jaworska (2007b) mentioned that experts have raised a concern about the ability of the product to stand the vibration of transport. In the case of mushrooms, one concern was that the bodies of the mushrooms during growth may be too brittle and this may result in damage during the transportation. It was hypothesized by another researcher that any damage may depend on the stage of development. The diverse species of mushroom mean the some may be more tolerant than others to vibration during transportation (Jaworska, 2007b).

Other challenges of producing MIT product are; producing a consistent product, applying special packaging to ensure ideal conditions for manufacture, controlling the conditions surrounding the package (i.e. environment) and changing the standard system of production and distribution. Many of the systems are in place but not being used in the right way for MIT. For example, containers are available with the capacity to control the environment (humidity, temperature, carbon dioxide levels) to preserve freshness of product rather than being set to enable manufacture (Jaworska, 2008).

As MIT is considered a new concept, research on potential applications is limited. Yet, some of the issues facing manufacturers for product distribution could possibly be resolved through applying the MIT concept. For instance, New Zealand is a long way from world markets and this is a challenge for NZ manufacturers to market their product outside New Zealand. The MIT concept would allow food manufacturers to make use of the time a product is in transit to distant markets. As much food is discarded as it exceeds the 'best before' date in the market (Kleijnen & Vorst, 2005) and home (Julian Parfitt, Barthel, & Macnaughton, 2010), product manufactured using the MIT concept may avoid some wastage of food.

2.4 Potential of MIT in food system

To the authors' knowledge, the concept of MIT is currently only applied to mushrooms. There is potential to apply the MIT concept to many foods. Such foods need to be capable of transformation or maturity during transport. Fermented foods are ideally suited to MIT development as fermentation can occur during the transportation.

The types of food that depend upon fermentation include dairy, meat, soy, vegetable and cereal products (Table 2.1). Among all fermented products, yoghurt and cultured dairy products are the fastest growing dairy categories worldwide (O'Neil, Kleyn, & Hare, 1979). In one article the Fonterra Brands Managing Director (Anonymous, 2005) mentioned that yoghurt is not only well received locally but has a potential to be applied in markets outside New Zealand. Hence, yoghurt could be a suitable model of fermented product to be manufactured using the MIT concept, extending the distribution of yoghurt from New Zealand.

CHAPTER TWO: LITERATURE REVIEW

Table 2.1: Examples of Fermented foods: substrate, cultured microorganism(s) and country

Product	Substrate	Cultured Microorganism(s)	Country
Yoghurt	Milk	<i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	Worldwide
Acidophilus milk	Milk	<i>Lactobacillus acidophilus</i>	Several countries
Cheese	Milk	Lactic acid bacteria (<i>L. lactis</i> , <i>S. thermophilus</i> , <i>L. shermanii</i> , <i>Propionibacterium</i>) sometimes moulds (<i>Penicillium</i> spp.)	Worldwide
Fermented sausages	Meat	Lactic acid bacteria (lactobacilli, pediococci) Catalase positive cocci (<i>S. carnosus</i> , <i>S. xylosus</i> , <i>M. varians</i>) sometimes yeasts and/or moulds	Europe and United State
Soy sauce	Soybeans and wheat	<i>Aspergillus oryzae</i> or <i>A. soyae</i> , <i>Lactobacillus</i> , <i>Zygosaccharomyces rouxii</i>	The Orient (Japan, China, Philippines)
Bread	Wheat, rye, other grains	<i>Saccharomyces cerevisiae</i> , other yeasts, lactic acid bacteria	Worldwide
Sauerkraut	Cabbage	Lactic acid bacteria <i>Ln. mesenteroides</i> , <i>L. brevis</i> , <i>L. plantarum</i> , <i>L. curvatus</i> , <i>L. sake</i>	Worldwide
Kimchi	Cabbage, vegetables, sometimes seafood, nuts	Lactic acid bacteria	Korea

Source: Adapted from Beuchat (1997) and Jay (2000)

2.5 Yoghurt

Yoghurt is one of the best known and most popular cultured milk products internationally. Data provided by the USDA reveal that yoghurt consumption in the US gradually increased from 1954 to 2005 (Figure 2.2). Various factors influence the consumption of fermented milk, particularly yoghurt. These include the availability of milk, food habits, level of income, advertising, range of fermented milk products available in the market, distribution system and general acceptability of other dairy products (Kurmann, 1984).

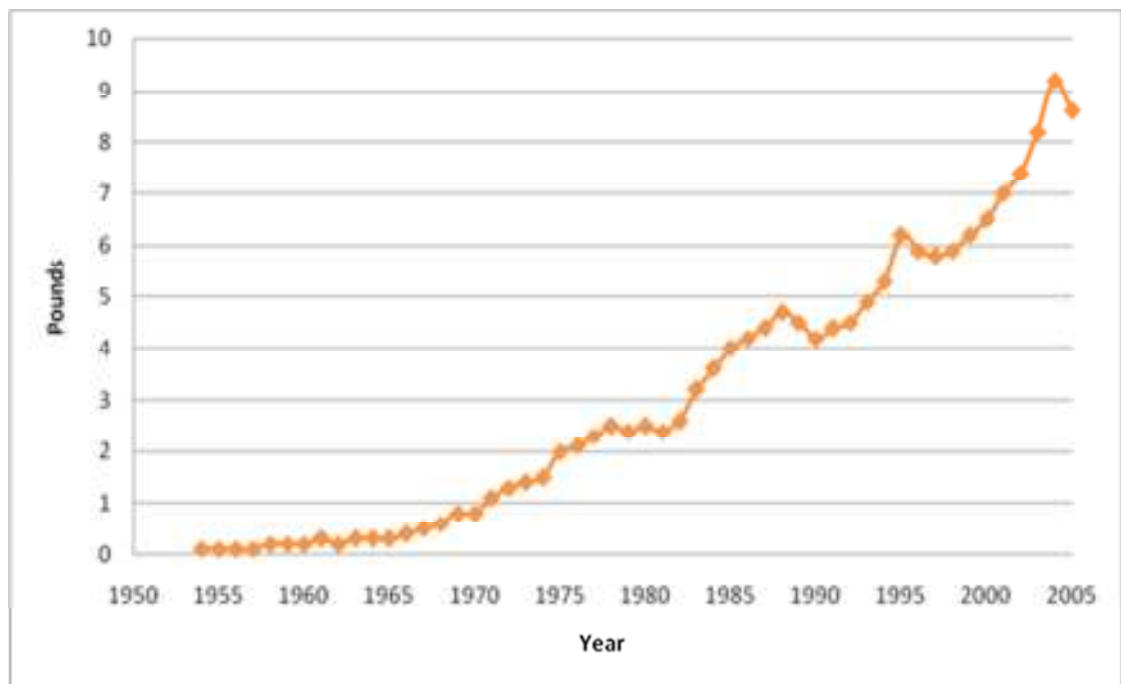


Figure 2.2: Per capita yoghurt consumption in United State from year 1954 to 2005 (Source: Economic Research Service, USDA)

Yoghurt is produced by the growth of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in heated milk (Kosikowski & Mistry, 1997) incubated at the optimum conditions of 40 to 45°C for 2 to 3 hours (Tamime & Robinson, 2007). During this time, the starter culture metabolizes lactose in the milk, producing the lactic acid which reduces the pH of milk to pH within 4.6 to 4.2 (Tamime & Robinson, 2007). This is the major determinant in producing the characteristics of yoghurt in terms of the flavor and texture. Damim, Alcântara, Nunes, & Oliveira (2009) described the texture of yoghurt resulting from the curdling of milk that occurs when casein becomes unstable and coagulates to form a firm gel. This gel is composed of strands of casein micelles and whey entrapped within this matrix. This matrix consists of, 1) the disulfide bonding between k-casein and denatured whey protein and 2) casein aggregation when the pH decreases to the isoelectric point of casein. Lactic acid also plays a major role in the preservation of the product by creating a pH that limits the growth of many microorganisms, including pathogens (Walstra, 1999). The shelf-life of yoghurt is about 20 days under refrigeration. (O'Neil et al., 1979).

Yoghurt can be categorized due to its physical, chemical or flavour properties. Physically, yoghurt may be a set yoghurt with firm gel, a stirred yoghurt with smooth gel in which the gel has been broken or as drinking yoghurt with a viscous liquid (Spreer, 1998;

Tamime & Robinson, 2007). Chemically, yoghurt may be a full, low or non-fat product. Flavour may be described as plain or natural or with fruit and other flavourings (Tamime & Robinson, 2007). Commercially, yoghurt processing involves the standardisation of milk, homogenization, heat treatment, inoculation of starter culture, fermentation, cooling and packaging. To adapt the MIT system to yoghurt processing, yoghurt fermentation could be carried out during distribution. Since the current fermentation time is very short, less than 12 h, an extended fermentation would be required in order to expand the yoghurt distribution and shelf-life. There are a number of challenges in preparing an MIT yoghurt, in particular controlling the growth of contaminants and ensuring the final product is acceptable in terms of the physical and flavour characteristics. The steps in yoghurt manufacture, including milk standardization, heat treatment, starter culture composition and inoculum level, and fermentation temperature could be altered to extend the fermentation time. These factors will affect the acidification and gelation processes (Peng, Serra, Horne, & Lucey, 2009).

2.6 Factors affecting yoghurt fermentation

2.6.1 Milk standardization

The main and most crucial ingredient in yoghurt processing is milk. Milk composition is described in terms of milk fat and milk solids not fat (MSNF) which consists of protein, lactose and minerals. Standardization of fat and MSNF content in milk is essential in yoghurt manufacture as this influences the quality and consistency of the end product. The fat content of yoghurt varies, from as low as 0.1 to 10 g per 100 g depending on the type of yoghurt; full, medium or low-fat yoghurt (Tamime & Robinson, 2007). The percentage of MSNF (mainly lactose, protein and mineral matter) in milk for yoghurt manufacture depends on the legal standards of the country in which the product will be sold or the physical properties or flavour of the end product.

The composition of cow's milk is given in Table 2.2. The major component in milk is water. Next is lactose, the major carbohydrate of milk. Lactose is essential in yoghurt production by providing the nutrition or energy source for the yoghurt starter bacteria. Fat imparts richness or smoothness to dairy products and directly provides an excellent mouthfeel. Protein plays an important role in the formation of the coagulum, influencing the consistency or viscosity of yoghurt (Tamime & Robinson, 2007). The level of protein is proportional to the viscosity of yoghurt. The major proteins of milk are caseins and whey

proteins. Caseins are insolubilized protein and begin to precipitate when the pH of milk is reduced to pH 4.6. The soluble portion at pH 4.6 is known as whey proteins consisting of albumins and globulins (Chandan & O'Rell, 2006).

The total solids content of the milk base influences the yoghurt firmness (Penna, Converti, & Oliveira, 2006; Tamime & Deeth, 1980; Nor-Khaizura et. al., 2012). The milk base protein content (Tamime, Kalab, & Davies, 1984; Trachoo & Mistry, 1998) and protein type (Cho, Lucey, & Singh, 1999; Penna, et al., 2006; Sodini, Remeuf, Haddad & Corrieu, 2004; Tamime, et al., 1984) are important factors in determining yoghurt texture.

Table 2.2: Composition of cow’s milk

	Water	Fat	Protein	Lactose	Ash
Average (%)	86.6	4.1	3.6	5.0	0.7
Range, average (%)	84.5-87.7	3.4-5.1	3.3-3.9	4.9-5.0	0.68-0.74

Source: Adapted from Swaisgood (1996)

The use of a reconstituted yoghurt milk base prepared from dried dairy ingredients is an alternative to standardised fresh milk. Skim milk powder (SMP) is widely used to prepare a yoghurt milk base (Isleten & Karagul-Yuceer, 2006). These authors also mentioned that the sensory properties of reconstituted milk ideally should be similar to fresh skim milk. The use of SMP is preferable to whole milk for the manufacture of fermented milks due to problems with oxidized flavours (McKenna, 1997; McKenna & Anema, 1993). The milk powder should be free from any inhibitory agents and have good microbiological and physical quality. Some specific requirements for SMP include a whey protein nitrogen index of 4.5-5.9; cysteine number, 38-48; thiol number, 7.5-9.4 and heat number, 80-83 (Wilcek, 1990). In the preparation of reconstituted skim milk from SMP, the hydration time is crucial in order to achieve the proper re-equilibration of the minerals, which requires around 3 h (Anema & Li, 2003). The normal practice is to rehydrate the powder to about 12 g per 100 g solid non-fats (SNF) (Tamime & Robinson, 2007). The Codex Standards (FAO/WHO, 2003) state that fermented milk products including yoghurt, must contain a minimum of 2.7% milk protein (% w/w) and less than 15% milk fat (% w/w).

To ensure the characteristics in yoghurt, stabilizers are often added into the yoghurt milk base. Stabilizers can improve the body and texture, viscosity or consistency, appearance and mouthfeel. Yoghurt coagulum is often subject to mechanical treatment during manufacture, for example stirring the coagulum in the fermentation tank for stirred yoghurt production, mixing to incorporate the fruit or flavours into the coagulum and subsequent post-fermentation treatment of the coagulum (e.g. pasteurization, UHT) (Tamime & Robinson, 2007). Stabilizers can avoid defects during stirring. Other functions of stabilizers incorporated into the yoghurt mix listed by Chandan and O'Rell (2006) are as follows: minimise whey separation and bind free water, maintain gel structure after pumping, mixing and cooling, and increase shelf-life of the product. Ingredients that are usually added as yoghurt stabilizers are starch, gelatin, guar gum, locust bean gum, carrageenan, pectin and xanthan gum. The addition of stabilizers is not suitable for plain yoghurt as they may affect the product aroma and flavour (Tamime & Robinson, 2007) and may affect the consumer perception of yoghurt (Amatayakul, et al., 2006).

Yoghurt texture can be improved by increasing the milk total solids by three methods 1) concentrating the milk base through evaporation, 2) reverse osmosis (RO) and 3) fortification with dried dairy ingredient such as skim milk powder (SMP), skim milk concentrate (SMC) or buttermilk powder (BMP) (Sodini, et al., 2004).

Many studies have been carried out to enhance the texture of yoghurt by fortification with dried dairy protein such as skim milk powder (Damin, et al., 2009; Guzmán-González, et al., 1999), buttermilk powder (Trachoo & Mistry, 1998), whey protein concentrates (Damin, et al., 2009; Guzmán-González, et al., 1999; Lucey, Munro, & Singh, 1999; Patocka, Cervenkova, Narine, & Jelen, 2006; Remeuf, Mohammed, Sodini, & Tissier, 2003), whey protein isolates (Isleten & Karagul-Yuceer, 2006; Patocka, et al., 2006), milk protein concentrate (Guzmán-González, et al., 1999), sodium caseinate (Damin, et al., 2009; Isleten & Karagul-Yuceer, 2006) and other milk-protein based ingredients (Lankes, Ozer, & Robinson, 1998; Rohm & Schmid, 1993). These ingredients have gained acceptance as a feasible way to increase total solids in low-fat and non-fat yoghurt (Tamime & Robinson, 2007). Sodini et al. (2004) also mentioned that the effect of milk base protein enrichment could be influenced by the heat treatment of the milk base.

2.6.2 Fortification with dried dairy ingredient

Increasing the total solids content in low-fat and non-fat yoghurt will prevent poor firmness and surface whey separation (Lucey, 2002). Skim milk powder (SMP) is dried non-fat milk and is the most commonly used fortification ingredient to increase the total solid content of the yoghurt milk base. Yoghurt fortified with SMP was observed to have a dense matrix, composed of short micellar chains and small micellar clusters (Tamime, et al., 1984). Buttermilk powder (BMP) is the by-product of sweet cream butter manufacture. BMP can act as an emulsifier due to the high content of phospholipids (Tamime & Robinson, 2007). Yoghurt manufactured from a milk base fortified with BMP has been reported as acceptable (Trachoo & Mistry, 1998).

Whey protein concentrates (WPC) or isolates (WPI) are the by-products from cheese manufacture and often added to a yoghurt milk base (Penna, Baruffaldi, & Oliveira, 1997). The addition of WPC to the yoghurt milk base can reduce syneresis, increase yoghurt viscosity (Kailasapathy & Supriadi, 1996) and water holding capacity (Remeuf, et al., 2003), yet, the undesirable flavor of WPC can limit its application in food (Damodaran, 1996).

Milk protein concentrate (MPC) is a concentrated milk product containing 40-90% of milk protein and sodium caseinate (NaCN) consisting mainly of casein. Both are produced by initially separating whole milk into cream and skim milk. For the MPC, the skim milk is concentrated using ultrafiltration then the product is spray dried. NaCN is produced from casein that has been precipitated from milk using rennet enzyme. This casein is washed and the purified casein protein is treated with sodium hydroxide to produce a soluble casein compound, NaCN. The addition of MPC (Soukoulis, Panagiotidis, Koureli & Tzia, 2007) and NaCN (Isleten & Karagul-Yuceer, 2006) to the milk base improve yoghurt texture and reduce syneresis in set yoghurt. Sodium caseinate has a high protein content with emulsification and water binding properties that contribute to the texture of yoghurt (Isleten & Karagul-Yuceer, 2006).

Yoghurt fermentation time is influenced by the protein components of yoghurt milk base (Puvanenthiran, Williams, & Augustin, 2002). The addition of WPC and NaCN does reduce the fermentation time (Damin, et al., 2009; Lucey, Teo, Munro, & Singh, 1997), yet the opposite effect has been observed for SMP (Damin, et al., 2009). The latter is similar to the finding obtained of Isleten and Karagul-Yuceer (2006), where the addition of dried dairy ingredients including whey isolate, SMP and NaCN did not affect the fermentation time,

although these components greatly influenced the yoghurt texture. This may be explained the buffering effect of the increased solids content in yoghurt milk, meaning more acid development by the starter cultures was necessary to achieve the casein isoelectric point (Lee & Lucey, 2010).

The influence of fortification material on the fermentation time may also depend on the starter cultures used (Isleten & Karagul-Yuceer, 2006). Using a probiotic as single starter culture (*L. acidophilus* LA5 or *L. rhamnosus* LR35) and fortification of milk bases with SMP, MPC and casein hydrolysate increased the fermentation rate and increased the texture of the yoghurt. This was less pronounced in yoghurt prepared with mixed culture starters (probiotic with *S. thermophilus*) (Sodini, Lucas, Oliveira, Remeuf, & Corrieu, 2002). For the single culture, the addition of dried dairy ingredient really influenced the fermentation time with the shortest time produced with milk fortified with casein hydrolysate (Oliveira, Sodini, Remeuf, & Corrieu, 2001; Sodini, et al., 2002).

For yoghurt texture, yoghurt fortified with NaCN is reported to have a stronger gel than the unfortified control and WPI-fortified yoghurts (Isleten & Karagul-Yuceer, 2006). Yoghurt enriched with NaCN produced a coarse texture when assessed visually using a spoon (Tamime, et al., 1984). This was possibly due to large casein particles and a robust micellar chain. They found the yoghurt firmness made from a milk base fortified with NaCN was 30% higher than that from a milk base fortified with SMP, although the former had lower total solid content, 12.8% rather than 16%. The yoghurt rheology was also influenced by the fortification of the milk base with dried dairy ingredients in yoghurt prepared using probiotic cultures (Sodini, et al., 2002). They found the highest texture in yoghurt manufactured with added MPC and lowest in yoghurt with added casein hydrolysate. Peng et al. (2009) mentioned that the yoghurt texture based on physicochemical properties related to the nature and type of protein interactions are not well understood. Possible interactions in the yoghurt are hydrophobic and electrostatic interactions, hydrogen bonding, steric repulsion and dissolution of CCP, which collectively influence the yoghurt physical and rheological properties (Peng, et al., 2009). Dissolution or solubilization of CCP could weakened casein-casein interaction and may contribute to soft gel (Peng et al., 2009). Generally, the casein-based powders are more effective than whey protein products in producing a firmer yoghurt (Bhullar, Uddin, & Shah, 2002; Dave & Shah, 1998).

The milk base used may stimulate probiotic growth, providing some advantage in manufacturing yoghurts containing probiotics. For example, casein hydrolysate stimulates the growth of *L. acidophilus* LA5 (Sodini, et al., 2002). Different casein hydrolysates may also have different influences on the growth of starter or probiotic cultures, and this is believed to be due to variations in the amino acid and peptide composition. Two casein hydrolysates (CH1 contain 73.2 of total nitrogen and CH2, 74.6) used to fortify a yoghurt milk base produced different results, with CH2 producing higher growth of starter culture and shorter fermentation time (Sodini, et al., 2002). This could be due to slightly higher total nitrogen content in CH2. The opposite finding was obtained by several researchers (Isleten & Karagul-Yuceer, 2006; Soukoulis, et al., 2007), with no major effect of milk base fortification on the starter culture growth when using the yoghurt cultures *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. In general, the starter and milk base interactions appear to have a major influence on yoghurt manufacture.

2.6.3 Heat treatment

Heat treatment is one of the crucial stages in yoghurt manufacture. The major purpose of heating is to eliminate all the pathogenic and spoilage microorganisms. In addition, the destruction of competitive microorganisms provides a favourable condition for yoghurt bacteria to grow (Chandan & O'Rell, 2006). In the industry, the yoghurt mix is usually heated at 90°C with a minimum holding time of 30 min (N. Kusumaningrum, personal communication, May 10, 2009). For a high temperature-short time (HTST) pasteurization, the equivalent temperature and time combination is 73°C for 15 s, while ultra-high temperature (UHT) treatment uses temperatures more than 90°C and as high as 148°C for 2 s (Chandan & Shahani, 1993). Treatment at 90-95°C with a holding time of 5-10 min has also been found to be satisfactory (Labropoulos, Palmer, & Lopez, 1981; Mottar, Bassier, Joniau, & Baert, 1989; Parnell-Clunies, Kakuda, & Deman, 1986; Schmidt, Vargas, Smith, & Jezeski, 1985). In yoghurt manufacture it is important that 70-95% of the whey protein is denatured to enhance water absorption. This ensures yoghurt with a smooth consistency and high viscosity (Chandan & O'Rell, 2006). The heat treatment of the yoghurt mix is normally achieved using industrial heat exchangers.

Heating milk is also needed for changes in the physicochemical properties of the milk constituents which are relevant in yoghurt making (Tamime & Robinson, 2007). β -

lactoglobulin, is the main whey protein that is denatured during heating (Lee & Lucey, 2010). This shifts the yoghurt gelation point towards higher pH values (Lucey, Tamehana, Singh, & Munro, 1998), producing a higher isoelectric point at pH 5.3. Denaturation of β -lactoglobulin up to 60% influences the yoghurt texture. Further denaturation, between 60 to 90% of β -lactoglobulin, has less effect on the yoghurt texture. Therefore, the heat treatment of milk base contributes to the fermentation time (Labropoulos, Palmer, & Lopez, 1984; Parnell-Clunies, et al., 1986; Shaker, Jumah, & Abu-Jdayil, 2000) and firmness of yoghurt (Augustin, Cheng, & Clarke, 1999; Dannenberg & Kessler, 1988).

The use of UHT as a heat treatment for the yoghurt milk base is not common. Yet, the sterilization effect of UHT is vital to prevent the growth of contaminating bacteria during the longer fermentation necessary for the production of MIT yoghurt. UHT can destroy all microorganisms including spores, inactivate some enzymes and affect the chemical changes, colour and flavor of milks (Fox & McSweeney, 1998), producing an astringency flavor (Harwalkar et al., 1989). UHT of milk is a continuous heating process at 135 to 150°C for 2-8 sec (Krasaekoopt, Bhandari, & Deeth, 2003) and can be direct or indirect. Most studies use indirect UHT processes. This is due to a better texture and viscosity of yoghurt produced using the indirect compared with the direct method (Mottar, et al., 1989).

There are several advantages in using UHT for yoghurt manufacture (1) better process control and sanitation, (2) energy and time savings, (3) high microbial quality, (4) longer shelf-life for the product (Labropoulos, et al., 1981; Schmidt, et al., 1985) and (5) stimulation of the growth and activity of yoghurt cultures (Smith, Schmidt, & Adams, 1982). The quality of yoghurt made from UHT milk compared with conventionally heated milk has been extensively reviewed by Krasaekoopt et al. (2003) (Table 2.3).

Briefly, yoghurt made from UHT milk has (a) lower viscosity and gel strength, (b) less syneresis, (c) a similar flavour to product manufactured from a pasteurized milk base, (d) minor differences in the microstructure (e) different texture that might be due to different denaturation effects of UHT heating and conventional heating on the whey protein, (f) improved texture when fortified with SMP, (e) enhanced pH reduction (De Brabandere & De Baerdemaeker, 1999).

Table 2.3: Comparison on the quality of yoghurt produced by UHT and pasteurized milk.

Evaluation	Yoghurt produced from UHT milk Vs pasteurized milk (conventional)	References
Texture (Firmness and/or Apparent viscosity)	<p>Yoghurt produced using UHT milk has a weaker gel, lower viscosity and less shear time compared with conventional processes.</p> <p>Yoghurt made from UHT milk fortified with 16, 18 and 20% of low heat skim milk powder has delayed gelation with lower viscosity. 20% of the total solids fortified in UHT milk have a similar viscosity to 16% total solids in conventional processes.</p>	<p>(Labropoulos, et al., 1981; Mottar, et al., 1989; Parnell-Clunies, et al., 1986)</p> <p>(Krasaekoopt, Kew, Bhandari, & Deeth, 2002; Krasaekoopt, Bhandari & Deeth, 2004)</p>
Microstructure	<p>The microstructure studied by SEM and TEM shows a minor difference between yoghurts produced by UHT milk and conventional processing.</p> <p>In conventional yoghurt, micelles tend to fuse and form a dense network that may result in firm gel texture and high viscosity. Compared with UHT yoghurt, the low gel strength and viscosity and loose microstructure could be due to the filamentous appendages that disrupt the fusion of casein particles by forming floccules by particle to particle attachment in UHT yoghurt.</p>	<p>(Parnell-Clunies, Kakuda, Deman, & Cazzola, 1988; Parnell-Clunies, Kakuda, & Smith, 1987)</p> <p>(Krasaekoopt, et al., 2003)</p>
Syneresis	<p>UHT yoghurt was observed to have less syneresis compared with conventionally processed yogurt.</p> <p>This could be due to the increase in water holding capacity (WHC) by denaturation, whereas increased exposure of charged groups and increased surface area enhances protein-water interactions.</p>	<p>(Savello & Dargan, 1997; Schmidt, et al., 1985)</p> <p>(Kinsella, 1984; Parnell-Clunies, et al., 1986)</p>
Denaturation of whey protein	<p>UHT processing was reported to produce less denatured whey protein compared to conventional process.</p> <p>UHT and conventionally heated milk are observed to have similar levels of denaturation of whey protein</p>	<p>(Labropoulos, et al., 1981; Krasaekoopt, Bhandari & Deeth, 2004)</p> <p>(Dargan & Savello, 1990; Mottar, et al., 1989)</p>

2.6.4 Starter culture composition

The commercial process of yoghurt manufacture uses a defined mixture of lactic acid bacteria. The combination of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (STLB) is normally used as the starter culture. These starter cultures are thermophilic bacteria with an optimum growth at a temperature of 37 to 45°C, homofermentative and some strains can produce exopolysaccharide (EPS) (Tamime & Robinson, 2007). The rationale for selecting the combination of starter cultures is to achieve the desired flavour and texture characteristics. The culture is added to the milk base either by direct inoculation using concentrated, frozen or freeze-dried cultures or indirect inoculation using a pre-cultured inoculum at levels from 1 to 5% (Sodini, et al., 2004).

The Codex standard defines yoghurt as a milk product obtained by the fermentation of milk, or products obtained from milk, by the action of suitable microorganisms resulting in a reduction of pH, with or without coagulation (isoelectric precipitation) (FAO/WHO, 2003). The suitable microorganisms for yoghurt, according to Codex, are as follows; symbiotic cultures of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Alternative yoghurt cultures mentioned in the Codex standard include a mixture of *S. thermophilus* and any *Lactobacillus* species. Moreover, other microorganisms than those constituting the specific starter culture(s) specified above may be added (FAO/WHO, 2003).

Many types of lactobacilli and bifidobacteria have been used. These bacteria may be added as a probiotic or adjunct culture with the standard bacteria for yoghurt manufacture. The selection of the starter cultures can also affect the growth of probiotics, depending on proto-cooperation, inhibition or competition (Dave & Shah, 1997; Saxelin et al., 1999). Probiotic bacteria (*Lactobacillus* spp. e.g. *L. acidophilus*, *L. casei*, *Bifidobacterium* spp. and *Enterococcus* spp.) are usually added for producing a health promoting yoghurt. Probiotic bacteria have a beneficial effect on intestinal function and promote good health (Sanders, 1999). Some probiotic bacteria are claimed to aid lactose digestion (Vesa et al., 1996), prevent traveler's diarrhea (Oksanen et al., 1990) and enhance the immune activity (Meydani & Ha, 2000). Certain levels of probiotic bacteria are required for these functions. For instance, the occurrence of traveler's diarrhea can be reduced with 10^9 cfu day⁻¹ of strain *L. acidophilus* GG (Oksanen, et al., 1990). Therefore, it is important to maintain a high number of probiotic bacteria in yoghurt after manufacture in order for them to function as probiotics.

For yoghurt fermentation time, the yoghurt starter culture of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* produce a very short fermentation time, 2 to 3 h at 40 to 45°C (Tamime & Robinson, 2007). Yet, most probiotic bacteria grow slowly in milk and the rate of acid production is usually too slow to support adequate fermentation in yoghurt (Shah, 2000). Several other researchers have also reported that probiotic bacteria produce poor acidification in milk when compared to yoghurt starter cultures (Almeida, Tamime, & Oliveira, 2008; Marshall & Tamime, 1997; Oliveira, et al., 2001; Saxelin, et al., 1999; Sodini, et al., 2002). This could be due to a lack of proteolytic activity in probiotic bacteria (Klaver, Kingma, & Weerkamp, 1993; Lucas, et al., 2004).

Starter culture composition has a great effect on fermentation time. When the mixed starter culture (*S. thermophilus* (ST) with probiotic, *L. acidophilus* (STLA) or *L. rhamnosus* (STLR)) were used, the fermentation time decreased two to three times compared to using a single culture of probiotic bacteria, *L. acidophilus* (LA) or *L. rhamnosus* (LR) (Dave & Shah, 1997; Oliveira, et al., 2001; Sodini, et al., 2002). Sodini et al. (2002) observed that a mixed culture of STLA produced a fermentation time of approximately 4 to 8 h compared with LA by itself which had a fermentation time of 8 to 13 h. In another study, Damin, Minowa, Alcântara, & Oliveira (2008) found the shortest fermentation time to reach pH 4.5 was obtained with milk fermented by *S. thermophilus* with *L. bulgaricus* (5.4 h), and that the fermentation time was longer time when *S. thermophilus* was co-cultured with *Bifidobacterium lactis* (8.3 h) and *L. acidophilus* (9.3 h); the differences between them were significant ($p < 0.05$).

Probiotics tend to grow slowly in yoghurt milk base, do not compete well with many starter strains and the probiotics show poor stability during storage. This may be due to competition between lactobacilli, slowing the growth of probiotic lactobacilli (Sodini, et al., 2002). Mixed cultures of *S. thermophilus* and a probiotic such as *L. acidophilus* showed that the former predominates under all culture conditions used (Dave & Shah, 1997; Oliveira, et al., 2001; Vinderola, et al., 2000). Probiotic growth was better when a single culture was used instead of a mixed culture (Sodini et al., 2002). However, Dave and Shah (1997) observed no difference in single or mixed culture for *L. acidophilus* growth.

The starter culture can influence yoghurt firmness (Hassan, Frank, Schmidt & Shalabi, 1996; Hess, Roberts, & Ziegler, 1997; Rohm & Kovac, 1994) depending whether or not the culture strain is an exopolysaccharide (EPS) producer. This is due to the EPS which has a large

molecular mass, interacting with casein or physically preventing casein micelles from coming into close contact, therefore, restricting the increase of yoghurt firmness (Sodini, et al., 2004). Some probiotic cultures also influence the rheological parameters, with higher values found in yoghurt with the single culture more than with mixed culture (Sodini, et al., 2002). This contradicts Oliveira et al. (2001), where they observed the culture composition did not affect the yoghurt texture.

2.6.5 Inoculum level

The inoculum level of starter culture may influence the acidification process, and consequently the fermentation time (Nor-Khaizura et al., 2012) and yoghurt gelation (Lee & Lucey, 2004; Peng, et al., 2009). Lowering the inoculum level decreases the acidification rate (Kristo, Biliaderis, & Tzanetakis, 2003; Sebastiani, Gelsomino, & Walser, 1998). This also affects the rheology of yoghurt, which decreases under longer fermentation (Kristo, et al., 2003). Higher inoculum levels increase the rheology of yoghurt (Lee & Lucey, 2004). The permeability, pore size and whey separation of the yoghurt gel is increased with a long fermentation time due to a lower inoculum (Lee & Lucey, 2004). However, according to Sodini et al. (2004), the inoculum level has a small effect. Ronnegard and Dejmeck (1993) found not much effect on the yoghurt viscosity when the inoculum level varied between 1 to 5%.

2.6.6 Fermentation temperature

The fermentation temperature affects the yoghurt fermentation time and texture. Fermentation temperatures higher (43.5 and 45°C) than the optimal (42°C) for standard commercial yoghurt cultures, were reported not to affect pH development compared with lowering the temperature (40.5 and 39°C) where the pH drop slowed (De Brabandere & De Baerdemaeker, 1999). Lowering the fermentation temperature causes a systematic decrease in the time required to reach the final pH of 4.5, which can be explained by a decrease in the metabolic activity of the bacteria (Haque, Richardson, & Morris, 2001). Mortazavian et al. (2006) observed that fermentation at 37°C required approximately 6.17 h, compared with 40 and 44°C requiring 5.26 and 4.39 h, respectively. At even lower fermentation temperatures (e.g. ~30°C), the fermentation time can be extended up to 12 h and good quality of yoghurt is produced (Lucey & Singh, 1998). Using probiotic bacteria, the fermentation temperature has a

similar influence on the pH reduction in milk. Østlie, Treimo, and Narvhus (2005) reported after 48 h of fermentation, depending on probiotic strains (*L. aciophilus* LA5, *L. acidophilus* 1748, *L. reuteri* SD2112, *L. johnsonii* LA1 and *Bifidobacterium animalis* BB12), pH decreased from 6.7 to 4.1-5.1 at a fermentation temperature of 30°C, to 3.8-4.7 at 37°C and 3.8-4.5 at 45°C. Further lowering the fermentation temperature to 25°C using the combination of STLA extended the fermentation time to 168 h, but the yoghurt texture was defective (Nor-Khaizura et. al., 2012).

At the typical fermentation temperature for yoghurt, 42°C or higher, yoghurt has a fast gelation time. This causes the yoghurt gel network to be more prone to rearrangements and these changes may lead to greater whey separation (Lucey, 2001; Mellema, Walstra, van Opheusden, & van Vliet, 2002). The yoghurt microstructure shows that gels fermented at 42°C have less branches, coarser, thinner strands and larger pores compared to gels fermented at 30°C (Lucey & Singh, 1998). Yoghurt incubated at a lower temperature (e.g. <40°C) has a slightly longer gelation time, and the product is normally firmer, more viscous, less prone to syneresis and with less lumpy or grainy defects on stirring the coagulum during cooling (Lee & Lucey, 2003; Lucey, 2002). A few studies observed that stirred yoghurt viscosity was higher at lower incubation temperatures (<40°C) compared with higher temperatures (>40°C) (Lee & Lucey, 2006; Martin, et al., 1999; Sodini, et al., 2004). The micrograph structure of yoghurt fermented at lower temperatures showed a highly cross-linked and branch protein network and small pores (Lee & Lucey, 2003, 2004). However, when yoghurt is fermented at lower temperatures (e.g. 25°C) using the probiotic bacteria as co-culture (STLA), the texture was defective compared to yoghurt fermented with the standard co-culture (STLB) (Nor-Khaizura et. al., 2012).

2.7 Possible mechanism of yoghurt gelation during the long fermentation

During long fermentation, the acidification rate becomes slower. This condition directly increases the yoghurt gelation or coagulation. Lucey and Singh (1998) describe the increased coagulation as a two-step phenomenon. Aggregation of heated milk base is expected to begin at higher pH, at about pH 5.3 (isoelectric pH of β -lactoglobulin) and continue to pH 4.6 (isoelectric pH of casein). During the long fermentation time, the elapsed time between these two pH levels is also long. At the first step of coagulation, the number of bonds created is higher due to the time needed to reach to pH 4.6. Therefore, at the second step of

coagulation, rearrangement (further aggregation of strands and clusters) occur in gel network, causing whey separation and the formation of larger pores (Peng, et al., 2009). This was exhibited in the microstructure of yoghurt made with a long fermentation time, where large strands and fewer apparent interconnections in the strands were seen compared to the fine structure and more branches in yoghurt prepared over a short fermentation time (Peng, et al., 2009).

2.8 Tool to assist in preparing an MIT product

Predictive microbiology or modelling can be used to assist in monitoring and predicting the fermentation as well as designing the best conditions for fermentation to fit with the requirements of an MIT food. Predictive microbiology describes microbial responses to different environmental conditions, which enable an objective evaluation of the effect of processing, distribution and storage operations on the microbiological safety and quality of foods (McMeekin, Olley, Ratkowsky, & Ross, 2002). Predictive microbiology is cost effective compared to the traditional microbiological testing to determine shelf-life and safety. Whiting and Buchanan (1993) classified predictive food microbiology according to three levels - primary, secondary and tertiary models. Primary models describe the change in the bacterial number with time under particular environmental and cultural conditions. The response can be measured directly by total viable count (TVC), toxin formation, substrate level or metabolic products and indirectly by absorbance, optical density or impedance. This generates information on the generation time, lag phase duration, exponential growth rate and maximum population density (Whiting, 1995; Whiting & Buchanan, 1993, 1994). Secondary models describe the response of one or more parameters of the primary model (e.g. generation time) in accordance with one or more changes in cultural or environmental conditions (e.g. pH, water activity, relative humidity, temperature). Tertiary models are the application of one or more primary and secondary models, incorporated into a user-friendly computer package.

The important aspects of practical model development are the range of characteristics investigated (growth, death, survival, toxin formation etc). Variables consist of temperature, water activity, pH, nitrate concentration, gaseous atmosphere, organic acid or other preservative concentrations (Ross, Dalgaard, & Tienungoon, 2000). Reproducible responses

are important for developing predictive microbiology in order to be able to predict future behaviour (McMeekin, et al., 2002).

There are several examples of predictive microbiology research conducted in dairy manufacture. Roupas (2008) reported that statistical modelling accurately predicted curd pH and moisture during cheese making. The author added that mathematical models that can predict the cell growth and lactic acid production would be very useful in determining the quality of cheese. The modelling provided improved control in gelation during the cooling of rennet casein gels, and the structure and quality of dairy products such as processed cheese (Zhong & Daubert, 2004).

In yoghurt processing, Soukoulis et al. (2007) and De Brabandere and De Baerdemaeker (1999) proposed the use of predictive modelling as a monitoring system during yoghurt fermentation. Due to the complexity of the fermentation and the many factors involved in yoghurt coagulation, the mechanisms involved remain poorly understood (Peng, et al., 2009). Prediction of fermentation is difficult, so it is a common practice to control it empirically (Soukoulis, et al., 2007). In industry, pH measurement is used to control yoghurt manufacture, as acidification is the parameter for monitoring fermentation (De Brabandere and De Baerdemaeker, 1999).

The fermentation based on pH reduction could be illustrated with a three phase process 1) lag phase (slow pH reduction), 2) logarithm phase (rapid pH reduction) and 3) slowdown of acidification rate (Soukoulis et al., 2007). This three phase process forms a sigmoidal fermentation curve. The curve is dependent on many parameters (De Brabandere and De Baerdemaeker, 1999) such as the yoghurt milk base, fortification ingredients, heat treatment, starter culture composition and fermentation temperature (Soukoulis et al., 2007). The modified Gompertz model was shown to be excellent to describe the pH reduction (Soukoulis, 2007; De Brabandere and De Baerdemaeker, 1999) and viscosity development (Soukoulis et al., 2007) during yoghurt fermentation. This supports continuously monitoring pH during yoghurt fermentation as a useful tool for checking product quality and for predictive or corrective purposes (Soukoulis et al., 2007). Other than modelling, other monitoring systems suggested include a combination of near infrared (NIR) and the electronic nose (Cimander, Carlsson, & Mandenius, 2002; Navrátil, Cimander & Mandenius, 2004).

2.9 Potential and challenges for yoghurt as an MIT product

Based on the information on the manufacture of yoghurt presented in this review, it should be possible to prepare MIT yoghurt. Yoghurt is an ideal product to investigate the development of MIT fermented foods as the fermentation period and shelf-life for the standard product are relatively short. This means that results from experiments are produced in a relatively short time frame, compared for the ripening of cheese, for example. In addition, using the MIT concept, it is possible to extend the shelf-life and distribution of this relatively short shelf-life product. In order to prepare an MIT product, the fermentation needs to be extended. This may present challenges in terms of the product texture, flavour and possible contamination. Possible ways to overcome these problems are fortification and UHT treatment of the yoghurt base. In order to design and predict the fermentation of yoghurt under different conditions, predictive microbiology or modelling is an appropriate tool.

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CHAPTER THREE : MATERIALS AND METHODS

3.1 Materials

3.1.1 Cultures

The lactic acid starter bacteria used were *Streptococcus thermophilus* STM5 (ST) and *Lactobacillus acidophilus* LA5 (LA) obtained as freeze-dried cultures, *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) and *Lactobacillus casei* (LC) obtained frozen. These starter cultures were supplied by the Fonterra Research Centre (Palmerston North, New Zealand). Each organism was cultured twice in skim milk medium (10% w/v skim milk powder in distilled water, sterilized at 110°C for 10 min) at 37 °C for 18 h each time before use (Figure 3.1). This procedure produced a consistent concentration of starter cultures of about 10^8 cfu mL⁻¹ in each bacterium; confirmed by plate counting.

3.2 Methods

3.2.1 Preparation of reconstituted skim milk

Skim milk powder (SMP) was obtained from Fonterra (Fonterra Co-operative Group, New Zealand). The typical composition (% w/w) of this SMP was as follows: lactose 54.1%, protein 33.4%, minerals 7.9%, moisture 3.8% and fat 0.8%. Yoghurt milk base was prepared by reconstituting SMP in distilled water to 12% (w/v) (unless stated otherwise). The SMP was dispersed by agitation using a magnetic stirring unit (Heidolph MR 3001) for 3 h at 25°C (Lee & Lucey, 2004) in a plastic beaker covered with aluminium foil. This reconstitution method is a gentle process and does not significantly damage the casein micelles compared with high shear and/or homogenization processes (Bock, Milliken, & Schmidt, 2008). The reconstituted skim milk was stored at 4-6°C overnight before use.

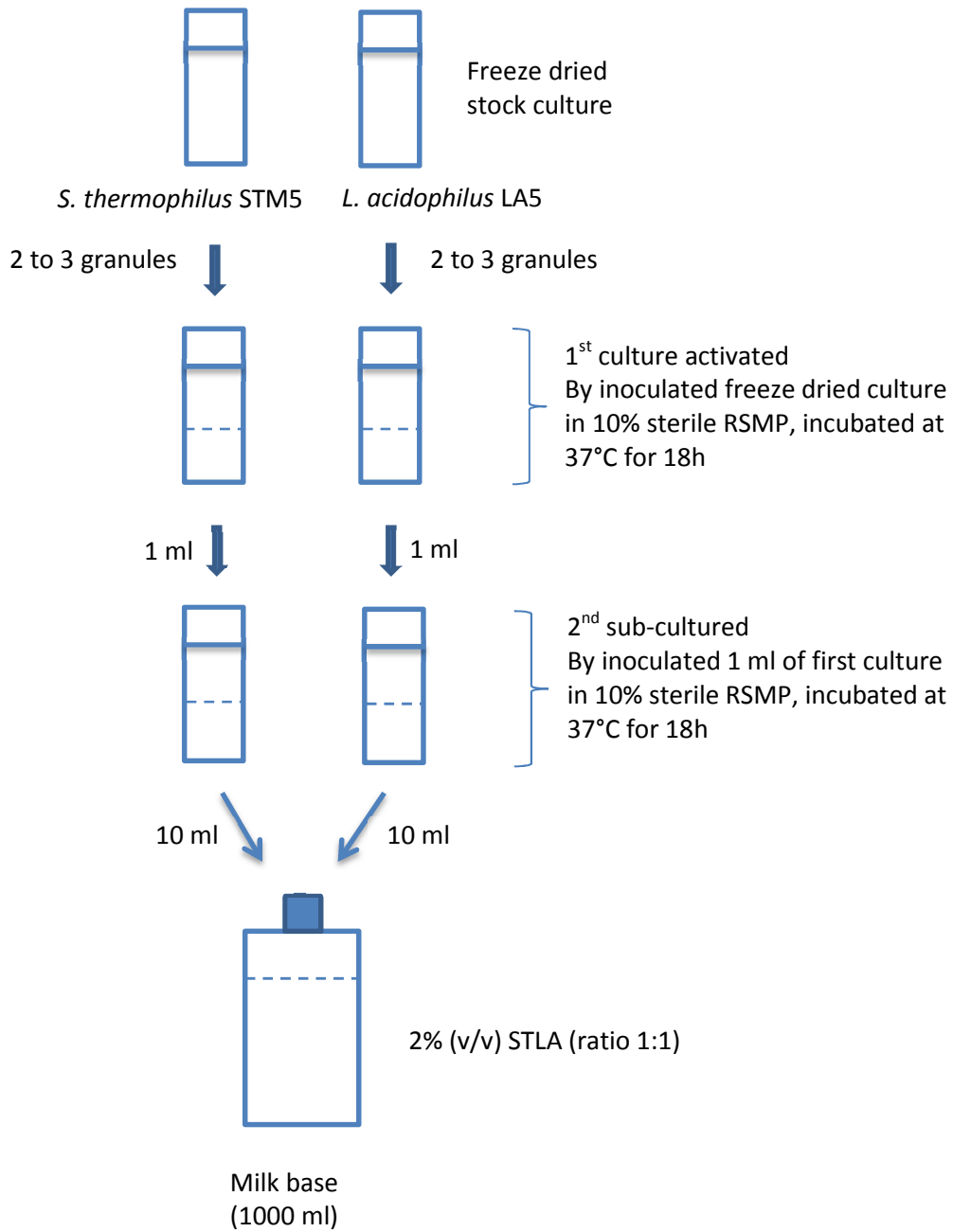


Figure 3.1: Diagram of preparing starter culture for MIT set yoghurt; example illustrated is combination of *S. thermophilus* STM5 and *L. acidophilus* LA5 (STLA) at ratio 1:1.

3.2.2 Heat treatment of milk base

The reconstituted skim milk was taken from refrigerator and transferred to the Institute of Food, Nutrition and Human Health (IFNHH) Pilot Plant for ultra-high-temperature (UHT) processing using an indirect UHT plant (Alfa Laval, Lund, Sweden). After each use, plant was cleaned in place using Sodium hydroxide. Prior to an experimental run, plant was pre-sterilized by circulating water at 120°C outlet temperature for 1h and by exposing UV light in the milk collection area. The holding temperature was set at 138°C and the flow rate at 0.833 L min⁻¹ to obtain a holding time of 6 s. These UHT conditions, 138°C for 6 s, are based on a previous study by Schmidt et al. (1985). The UHT milk was collected by hand aseptically into sterile Schott Duran bottles at 6-10°C in the laminar flow cabinet. After that, the UHT milk was stored at 4°C for 24 h before use.



Figure 3.2: Indirect UHT plant (Alfa Laval) use for UHT treatment for yoghurt milk base

3.2.3 Processing of yoghurt

Yoghurt processing was carried out in the Massey University IFNHH Product Development Lab. The milk was brought out from the refrigerator and allowed to come to the fermentation temperature ($\sim 25^{\circ}\text{C}$) in an incubator. The 12% (w/v) UHT skim milk was then inoculated with 2.0 or 0.2% (v/v) (unless stated otherwise) of a combined *Streptococcus thermophilus* STM5 and *Lactobacillus acidophilus* LA5 (STLA) starter culture (unless stated otherwise). The milk was shaken manually for 60 s and left in the fermentation bottle for 10 min after inoculation before it was poured into a sterile plastic container (55 mm height; 40 mm diameter) (LabServ, Auckland, New Zealand). The containers were filled to a headspace of approximately 4.0 cm (sample volume ≈ 50 mL). The containers were then sealed with screw lids and placed in an incubator (Sanyo incubator) set at 25°C (unless otherwise stated) for fermentation.



Figure 3.3: Yoghurt in the incubator

3.2.4 Measurement of pH

Changes in pH during fermentation were monitored using a glass electrode pH meter (Orion model 250 A/610). The pH meter was calibrated using standard buffer solutions (MERCK) at pH 4.0 and 7.0. The probe was rinsed with distilled water before being placed in a yoghurt container under aseptic conditions. The probe was sanitized with 70% ethanol at the beginning and at the end of pH measurement.

3.2.5 Microbiological analysis

Samples (1 g) were aseptically removed from the containers and diluted by mixing with 9 mL of 0.1% peptone water. Further serial 10-fold dilutions were made as required. MI7 agar (MERCK) was used to enumerate viable *S. thermophilus*, MRS agar (MERCK) for viable *L. acidophilus*, and acidified MRS media (MERCK), after pH adjustment to 5.4, for viable *L. bulgaricus* (ISO, 2003). The standard spread plate method was employed to determine viable cell counts of the starter organisms. Inoculated plates were incubated for 72 h at 37°C aerobically for *S. thermophilus* and anaerobically for *L. bulgaricus* and *L. acidophilus*. The results were recorded as log (colony-forming unit) g⁻¹ (log cfu g⁻¹) yoghurt.

3.2.6 Determination of titrable acidity

The titratable acidity was also measured for monitoring the production lactic acid during the fermentation (Fox & McSweeney, 1998). The sample was brought to a temperature between 20 and 25°C. It was then mixed carefully by means of a spatula, using a rotary motion which passed from the lower layers to the surface layers of the sample. Approximately 10 g of sample was weighed into a 50 mL beaker and approximately 10 mL of distilled water was added and mixed in. The pH meter probe was inserted into the suspension until properly immersed. The contents of the beaker, whilst being stirred (manually), were titrated with 0.1N sodium hydroxide solution (BDH Ltd, Poole, England) to pH 8.30±0.01. The volume (in mL) of sodium hydroxide used was recorded. The titrable acidity (w), expressed as millimoles (100 g)⁻¹, was calculated using the following equation:

$$w = v \times 0.9/m$$

where v is the volume (mL) of NaOH used in the titration; m is the mass (g) of the test sample; and 0.9 is the conversion factor for lactic acid (ISO, 1997).

3.2.7 Measurement of firmness

Undisturbed yoghurt samples in plastic containers (55 mm height; 40 mm diameter) (LabServ, Auckland, New Zealand) were evaluated for firmness using a TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, Surrey, U.K.) with a load cell of 5 kg. The sample was back extruded with a flat acetal plate (30 mm diameter) moving downwards at 1 mm s^{-1} . Force readings were automatically recorded at the rate of 400 points per second (pps). Tests were performed at $4\text{-}6^\circ\text{C}$ (Pereira et al., 2003). Firmness was defined as the average of the force readings (in newtons, N) recorded between the fifth and fifteenth seconds inclusive (Figure 3.4). The chosen depth had to be greater than 75% of the depth of the sample, so as to avoid the disc coming into contact with the base of the container during testing, which could produce an erroneous result. Calibration of height was conducted every time a sample measurement was carried out. Measurements were performed on samples at $4\text{-}6^\circ\text{C}$.

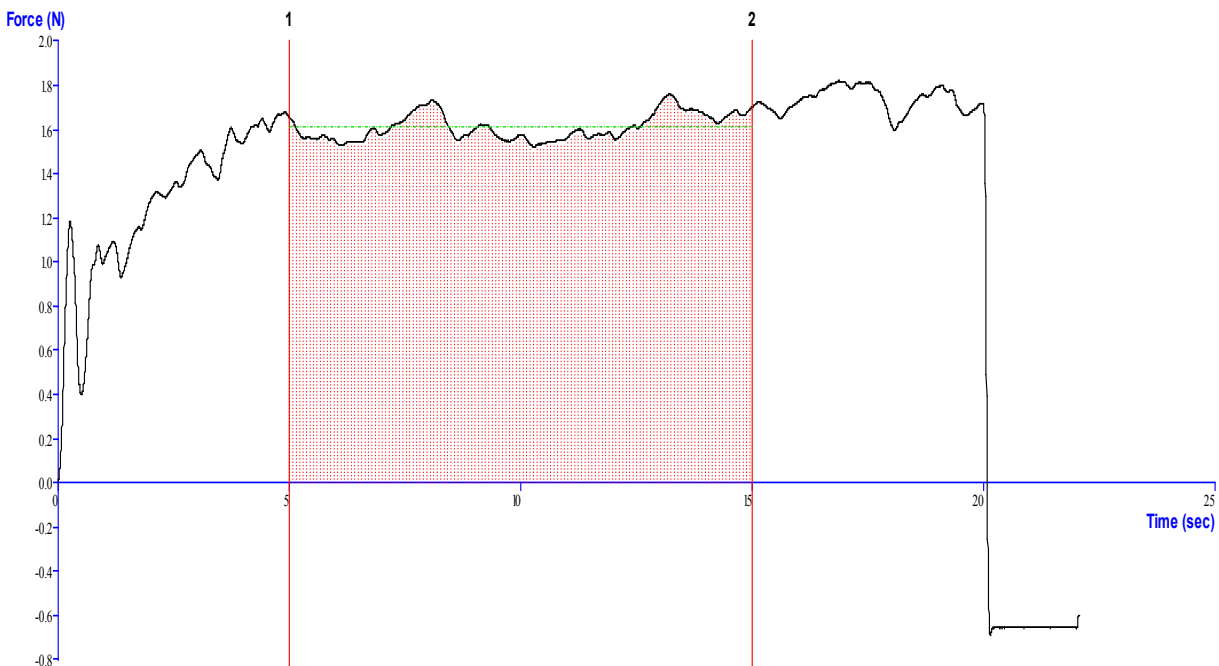


Figure 3.4: Texture profile during firmness measurement of MIT set yoghurt by back extrusion using TA.XT2 Texture Analyzer.

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CHAPTER FOUR : DESIGNING PROCESSING AND FERMENTATION CONDITIONS OF SET YOGHURT AS A MADE-IN-TRANSIT (MIT) PRODUCT

4.1 Abstract

Extending yoghurt fermentation time could facilitate yoghurt distribution by allowing the fermentation to occur during transportation in a process known as made-in-transit. Multiple factors have been suggested to influence yoghurt fermentation time. The objective of this chapter was to determine the starter culture type, inoculum size and fermentation temperature for extending yoghurt fermentation to 168 h. For screening purposes, the growth profiles of selected starter cultures, combinations of *Streptococcus thermophilus* STM5 with *Lactobacillus delbrueckii* subsp. *bulgaricus*, with *Lactobacillus acidophilus* LA5 and with *Lactobacillus casei* in a ratio of 1:1 at 0.2 and 2.0% (v/v) inocula at 20, 25, 35 and 40°C were determined using impedance measurement (BacTrac™ 4300). A 2³ factorial experiment was used to compare two starter combinations (*Streptococcus thermophilus* STM5 with *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* STM5 with *Lactobacillus acidophilus* LA5), two inoculum sizes (2.0 and 0.2% (v/v)) and two fermentation temperatures (25 or 35°C) in yoghurt processing. Fermentation was monitored over 168 h (7 days) using pH, titratable acidity, starter culture count and firmness. The impedance screening trial showed that all three combinations of starter culture could grow at between 20 to 40°C with the combinations of *Streptococcus thermophilus* STM5 with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *S. thermophilus* with *Lactobacillus acidophilus* LA5 found to have slower growth at lower temperature, most appropriate for the extended incubation time required. The 2³ factorial experiments revealed that the combination of *Streptococcus thermophilus* STM5 with *Lactobacillus acidophilus* LA5 at 0.2% (v/v) fermented at 25°C was the most appropriate for extending yoghurt fermentation to 168 h. These conditions produced yoghurt with a final pH of 4.72 and yoghurt produced under these conditions failed to gel.

4.2 Introduction

Yoghurt fermentation time is critical for producing an MIT product. The fermentation time and the product distribution time must be about the same. Various yoghurt fermentation times have been reported including 22 h (Tamime & Robinson, 2007), 6 to 10 h (Torre, Tamime, & Muir, 2003), 3 to 4 h (Tamime, 2006) and 3.5 to 5 h (Ozer & Kirmaci, 2010). In one

yoghurt manufacture in New Zealand the fermentation time is between 6 to 12 hours (N. Kusumaningrum, personal communication, May 10, 2009). In general the yoghurt fermentation time is 2.5 to 3.0 h at 40 to 45°C and 16 h at ca. 30°C (Tamime & Robinson, 2007). The variable fermentation times are due to type of starter culture, fermentation temperature and the composition and heat treatment of the milk base (Labropoulos et al., 1984; Hassan et al., 2003; Tamime & Robinson, 2007; Soukoulis et al., 2007). The primary objective of the present study was to investigate the effect of type of starter culture, inoculum size and fermentation temperature on the fermentation time. The fermentation time desired for MIT yoghurt was 168 h, on the basis of the time needed to distribute yoghurt from New Zealand to markets in Australasia and the Pacific.

4.3 Materials and Methods

4.3.1 Microbial growth measurement using impedance analysis (BacTrac™ 4300)

This analysis was carried out to determine the growth profile of selected lactic acid bacteria at various incubation temperatures. BacTrac cells were sterilized by filling with 10 ml distilled water and autoclaving at 121°C for 15 min. The sterile water in the cells was then aseptically replaced with growth medium consisting of 10 ml 12% (w/v) reconstituted skim milk powder that had been sterilized by ultra-high temperature process (138°C 6 s).

The combinations of starter cultures in a ratio of 1:1 used in this study were: *Streptococcus thermophilus* STM5 with *Lactobacillus delbrueckii subspp bulgaricus* (STLB), *Streptococcus thermophilus* STM5 with *Lactobacillus acidophilus* LA5 (STLA), and *Streptococcus thermophilus* STM5 with *Lactobacillus casei* (STLC). Starter cultures were then inoculated into the growth medium at 0.2% and 2.0% (v/v) inoculum. Two inoculum sizes, 2.0 and 0.2% (v/v) were prepared. For 2.0% (v/v) inoculum size, 0.2 mL of starter culture combination (ratio of 1:1) was added and 0.02 mL was added for 0.2% (v/v). After inoculation the BacTrac cells were gently shaken manually by hand, and then placed in the BacTrac incubator. The time interval between inoculation of the starter cultures and placing the BacTrac cells in the incubator was under 15 minutes.

Impedance was measured using the BacTrac™ 4300 micro-organism growth analyser (SyLab, Purkersdorf-Vienna, Austria). The BacTrac instrument was pre-set with the “device”

and “parameter” settings. In the device setting, the incubation temperature and the measurement interval of 10 min were selected. Incubation temperatures were 20, 25, 35 and 40°C. In the “parameter setting”, identification information for each of the BacTrac cells used and incubation total time (168 h) was inserted. During the incubation time, M-value (in percentage) was measured and recorded by the BacTrac data system. The M-value is the electrical impedance changes in the growth medium resulting from microbial metabolic processes (Flint and Brooks, 2001).

The growth curves obtained (by single measurement) from BacTrac impedance measurements provided preliminary information on the possibility to delay the starter culture growth at their minimum temperature and lower inoculum size. Accordingly, the fermentation process could be delayed. In the next experiment, the suitable starter culture, inoculum size and fermentation temperature was selected for producing the set yoghurt as an MIT product.

4.3.2 2³ factorial experiment design

A 2³ factorial experiment design generated by MINITAB 15 (Minitab Inc., State College, PA, USA) was used to study factors affecting the acidification rate and texture of set yoghurts (Table 4.1). Eight experiments, carried out in random order, were defined according to 1) starter culture inoculum size: 0.2% and 2.0% (v/v); 2) starter culture type (in a ratio of 1:1): *Streptococcus thermophilus* STM5 with *Lactobacillus delbrueckii* subsp. *bulgaricus* (STLB) and *Streptococcus thermophilus* STM5 with *Lactobacillus acidophilus* LA5 (STLA) and 3) fermentation temperature: 25 and 35°C. Products were analyzed for pH, titratable acidity and starter culture count every 2 h for the first 12 h and then every 12 h for 144 h. For firmness, it was measured on the first 12 h and 24 h and then every 24 h for 144 h.

CHAPTER FOUR: DESIGNING PROCESSING AND FERMENTATION CONDITIONS OF SET YOGHURT AS A MADE-IN-TRANSIT (MIT) PRODUCT

Table 4.1: 2³ factorial experiment designs for investigating the effect of starter culture, inoculum size and fermentation temperature on producing made-in-transit (MIT) set yoghurt.

Run	Experimental variables		
	Starter culture	Inoculum size (% (v/v))	Fermentation temperature (°C)
1	STLB	0.2	25
2	STLB	0.2	35
3	STLB	2.0	25
4	STLB	2.0	35
5	STLA	0.2	25
6	STLA	0.2	35
7	STLA	2.0	25
8	STLA	2.0	35

4.3.3 Cultures

See section 3.1.1 (Chapter 3).

4.3.4 Preparation of reconstituted skim milk

See section 3.2.1 (Chapter 3).

4.3.5 Heat treatment of milk base

See section 3.2.2 (Chapter 3).

4.3.6 Processing of yoghurt

See section 3.2.3 (Chapter 3).

4.3.7 Measurement of pH

See section 3.2.4 (Chapter 3).

4.3.8 Microbiological analysis

See section 3.2.5 (Chapter 3).

4.3.9 Determination of titratable acidity

See section 3.2.6 (Chapter 3).

4.3.10 Measurement of firmness

See section 3.2.7 (Chapter 3).

4.3.11 Statistical Analyses

All samples were prepared and fermented in duplicate. Two-way analysis of variance was performed using MINITAB to determine the effects of starter culture, inoculum size and fermentation temperature on the pH, titrable acidity, starter culture concentration and texture of set yoghurt during and at the end of the fermentation time. Mean value of measurements at the end of fermentation time were compared using Tukey's multiple comparison tests.

4.4 Results & Discussion

4.4.1 Growth profiles of starter cultures at various incubation temperatures and inoculum sizes as measured by impedance (BacTrac™4300)

The growth profiles of combinations of *Streptococcus thermophilus* STM5 with *Lactobacillus delbrueckii* subsp. *bulgaricus* (STLB) or with *Lactobacillus acidophilus* LA5 (STLA) or with *Lactobacillus casei* (STLC) were observed at various incubation temperatures (20, 25, 35 and 40°C) and inoculum sizes (0.2 and 2.0% (v/v)).

All combinations of starter culture produced a long lag effect, which is a low initial growth rate (Figure 4.1) when incubated at 20°C, owing to this temperature being lower than the optimum for starter culture growth (40 to 42°C). Among the three combinations of starter culture, STLC showed faster growth than did STLB and STLA. The minimum growth temperature for *Streptococcus thermophilus* is 20°C, for *Lactobacillus casei* is between 15 and 20°C, for *Lactobacillus acidophilus* is 20 to 22°C and for *Lactobacillus delbrueckii* subsp. *bulgaricus* is 22°C (Cogan, 1996; Holt et al., 1994). The lag effect for 2.0 and 0.2% STLC were approximately 10 and 20 h, respectively. A longer lag effect of approximately 40 h was observed for STLB and STLA starter combinations. STLB and STLA have quite similar growth patterns. At higher inoculum size (2.0% (v/v)), the maximum growth indicated by the impedance M-value (% M) was higher than for the lower inoculum size (0.2% (v/v)) for STLB and STLA throughout 168h of incubation time. Yet for STLC the lower inoculum size showed lower M-value for the first 60 h and then the M-value observed similar between 2.0% and 0.2% (v/v).

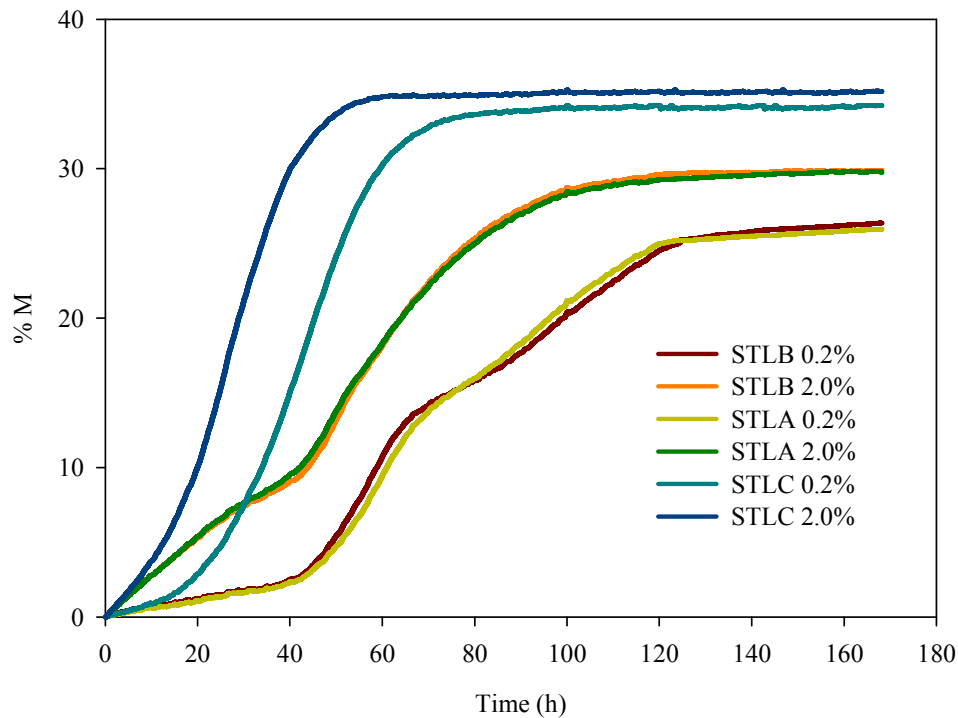


Figure 4.1: Growth profiles of STLB, STLA and STLC at 20°C fermentation temperature and inoculum sizes of 0.2 and 2.0% (v/v) (ratio of 1:1).

Growth profiles for STLB, STLA and STLC at 25°C with inoculum sizes of 0.2 and 2.0% (v/v) (Figure 4.2) showed faster growth and shorter lag effect than for incubation at 20°C. This trend also observed at 35°C (Figure 4.3) and 40°C (Figure 4.4) fermentation temperatures, with still faster growth, where the exponential growth start at earlier times as fermentation temperature increased. These results were expected because these last two incubation temperatures are within the optimum growth temperature range for the three starter cultures combinations (Cogan, 1996; Holt et al., 1994; Radke-Mitchell & Sandine, 1985).

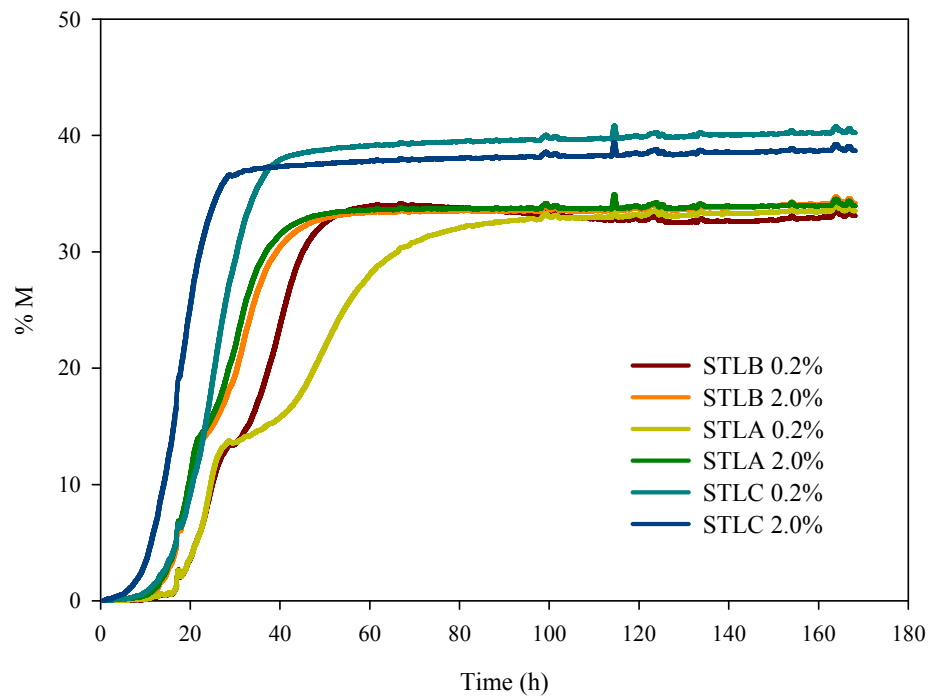


Figure 4.2: Growth profiles of STLB, STLA and STLC at 25°C fermentation temperature and inoculum sizes of 0.2 and 2.0% (v/v) (ratio of 1:1)

There are no other reports on extending yoghurt fermentation time to 168 h (7 days). However, it is known that lowering the incubation temperature will slow the growth of lactic acid bacteria (Tamime & Robinson; 2007, Jay, 2007; Lee & Lucey, 2004).

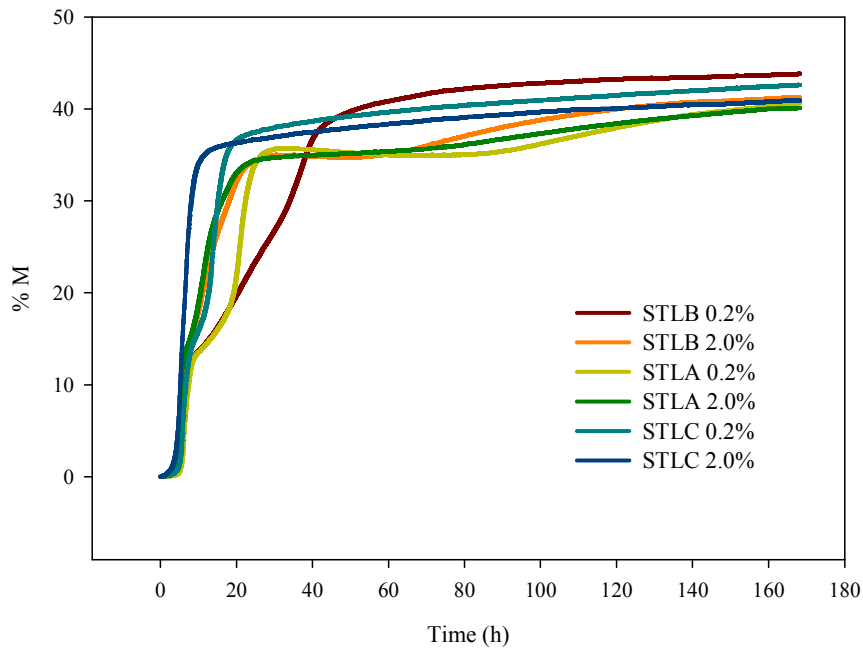


Figure 4.3: Growth profiles of STLB, STLA and STLC at 35°C and inoculum sizes of 0.2 and 2.0% (v/v) (ratio of 1:1).

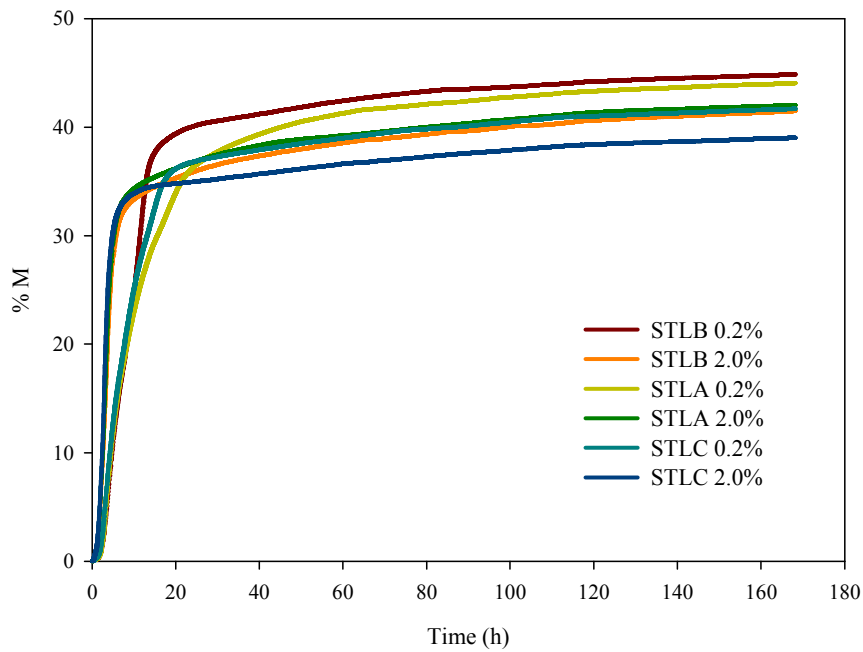


Figure 4.4: Growth profiles of STLB, STLA and STLC at 40°C and inoculum sizes of 0.2 and 2.0% (v/v) (ratio of 1:1).

At 40°C incubation temperature, the effect of inoculum size on slowing the starter culture growth was observed only on the first 10 h. Afterward, the M value (% M) for inoculum size of 0.2% (v/v) of all starter culture combinations reveal a steep increase even having a higher M value than starter culture with inoculum size of 2.0% (v/v). This condition is probably due to the low initial starter culture concentration that utilize small amount of nutrient. Yet during the exponential stage, the starter culture starts to grow rapidly with more available nutrient.

The range of incubation temperatures chosen for these experiments was based on the minimum and maximum growth temperatures of lactic acid bacteria that could be considered for use as MIT starter cultures (Cogan, 1996; Holt et al., 1994). Even though starter culture optimum temperature is 37 to 42°C, the starter cultures were found to be able to grow at lower incubation temperatures. At lower incubation temperatures, a longer lag effect was produced by the starter cultures compared with incubation at higher temperatures. The longer lag in the growth curve is attributed to the temperatures being less than optimal for the starter cultures to grow (Cohan, 1996; Holt et al., 1994). Tamime (1977) also observed a longer fermentation time resulting from reducing or increasing the fermentation temperature away from the optimal temperature.

The inoculum size is also known to influence starter culture growth (Tamime & Robinson, 2007). The normal inoculum size for starter cultures used in the manufacture of yoghurt is 2.0% (at a species ratio of 1:1, if two types of starter culture are used). In order to slow starter culture growth and to give a longer fermentation time, the inoculum size could be reduced.

The effect of starter culture, inoculum size and fermentation temperature on acidification was obtained by measuring the final pH of the BacTrac samples. The acceptable pH for set yoghurt is 4.2 to 4.6 (Tamime & Robinson, 2007). For given starter culture and inoculum size, the final pH of the samples incubated at the lower temperatures was found to be higher than at higher temperatures (Table 4.2). This reflects the slower rate and extent of fermentation at the lower temperatures. The final pHs of the samples incubated at 35 and 40°C was in the range 3.27 to 3.83. This range is lower than the acceptable pH range for set yoghurt. The final pHs of samples incubated at 20 and 25°C lay in the range 3.39 to 5.59, with most being higher than the acceptable pH for set yoghurt. The closest to an acceptable pH

CHAPTER FOUR: DESIGNING PROCESSING AND FERMENTATION CONDITIONS OF SET YOGHURT AS A MADE-IN-TRANSIT (MIT) PRODUCT

(4.6 to 4.2) was obtained using STLA with a 0.2% (v/v) inoculum size incubated at 25°C with pH 4.85. This combination of conditions was selected for further trials to develop set yoghurt as an MIT product.

Table 4.2: Final pH of the BacTrac samples after 168h of incubation.

Starter culture composition	Inoculum percentage (% (v/v))	Incubation temperature (°C)	pH
STLB	0.2	20	5.59
STLB	0.2	25	3.97
STLB	0.2	35	3.83
STLB	0.2	40	3.40
STLB	2.0	20	5.16
STLB	2.0	25	3.51
STLB	2.0	35	3.42
STLB	2.0	40	3.32
STLA	0.2	20	5.43
STLA	0.2	25	4.85
STLA	0.2	35	3.80
STLA	0.2	40	3.58
STLA	2.0	20	5.15
STLA	2.0	25	4.13
STLA	2.0	35	3.69
STLA	2.0	40	3.59
STLC	0.2	20	4.10
STLC	0.2	25	3.79
STLC	0.2	35	3.46
STLC	0.2	40	3.42
STLC	2.0	20	3.93
STLC	2.0	25	3.72
STLC	2.0	35	3.27
STLC	2.0	40	3.39

4.4.2 Effect of starter culture, inoculum size and fermentation temperature on pH, titratable acidity, starter culture growth and firmness of MIT set yoghurt

The ranges of conditions that had the potential to extend fermentation time to enable the manufacture of a made-in-transit type product were starter cultures STLB and STLA, total inoculum sizes 0.2 and 2.0% (v/v) and fermentation temperatures 25 and 35°C. As mentioned previously, the combination STLA/0.2% inoculum size/fermentation temperature 25°C was particularly promising. STLB is the standard starter culture for yoghurt processing. Therefore it was chosen for comparison with STLA. The usual inoculum size used in yoghurt processing is 2.0% (v/v), and this was chosen for comparison with 0.2% (v/v). For fermentation temperature, 35°C was chosen for comparison with 25°C instead of with the standard yoghurt fermentation temperature (40 to 45°C); this was because at the standard fermentation temperature, it is not possible to extend fermentation time at all.

Starter culture, inoculum size and fermentation temperature significantly influenced ($p < 0.05$) the pH reduction in yoghurt milk base. The fastest pH reduction occurred with 2.0% (v/v) STLB fermented at 35°C (Figure 4.5). This result was expected as these conditions were closest to those used in typical yoghurt fermentation; 2.0% (v/v) STLB fermented at 40 to 45°C (Tamime & Robinson, 2007). The pH reduction in set yoghurt with STLB was slower with a reduced inoculum size (0.2% (v/v)) and lower fermentation temperature (25°C). The biggest influence was probably the less than optimal fermentation temperature for STLB (Tamime & Robinson, 2007; Lee & Lucey, 2004). The final pH with STLB was below the acceptable pH for set yoghurt (pH 3.28 to 3.89) (Figure 4.7).

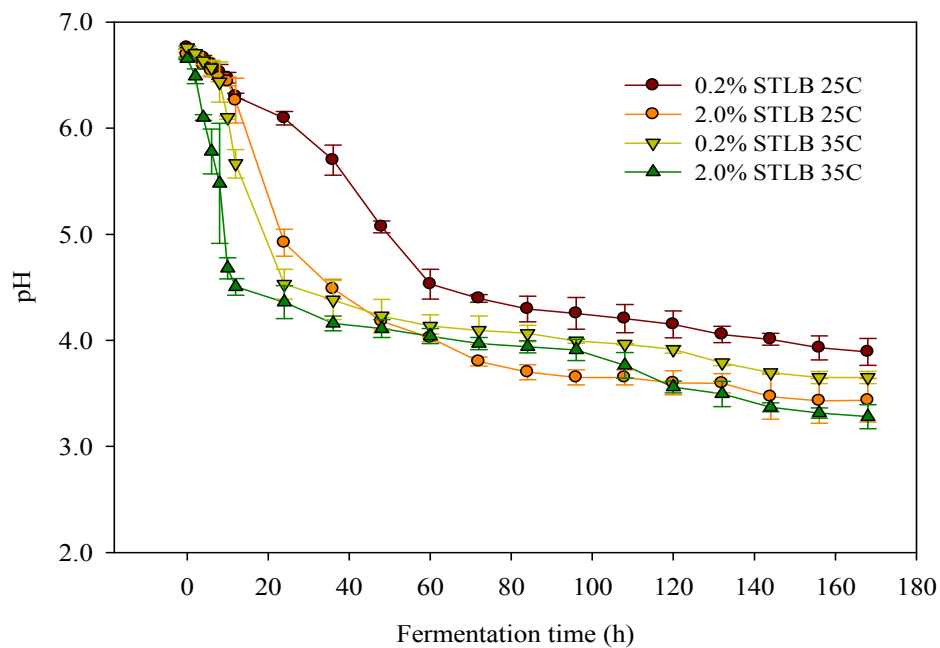


Figure 4.5: pH profile of MIT set yoghurt during fermentation using *Streptococcus thermophiles* STM5 with *Lactobacillus delbrueckii* subsp *bulgaricus* (STLB), inoculum size 0.2 and 2.0% (v/v) and fermentation temperature 25 and 35°C (n=2). Internal bar = \pm (2 x sample standard deviation).

The pH profile with 0.2% (v/v) STLA at 25°C (Figure 4.6) showed an extended fermentation. The pH steadily decreased during 168h of fermentation. The final pH was 4.77, which is above the isoelectric point of casein (pH 4.6). Figure 4.7 shows that other fermentation conditions produced yoghurt below pH 4.00, which is within the range 3.28 to 3.94. Even with a non-standard starter culture, a higher inoculum size and fermentation temperature will accelerate the fermentation, producing yoghurt with a lower final pH. Yoghurt with a pH lower than the acceptable range of 4.20 to 4.60 is too sour (Tamime & Robinson, 2007).

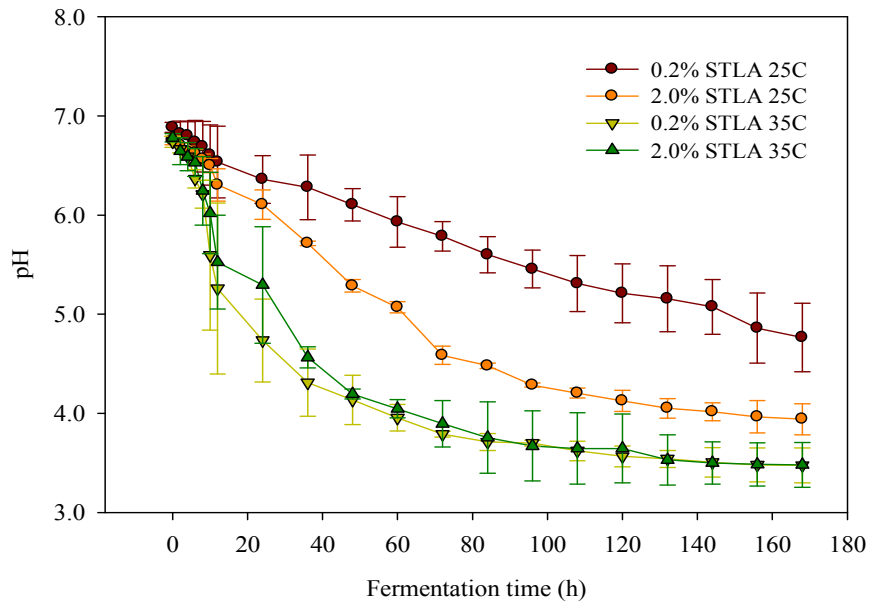


Figure 4.6: pH profiles of MIT set yoghurt during fermentation using *Streptococcus thermophiles* STM5 with *Lactobacillus acidophilus* LA5 (STLA), inoculum size 0.2 and 2.0% (v/v) and fermentation temperature 25 and 35°C (n=2). Internal bar = \pm (2 x sample standard deviation).

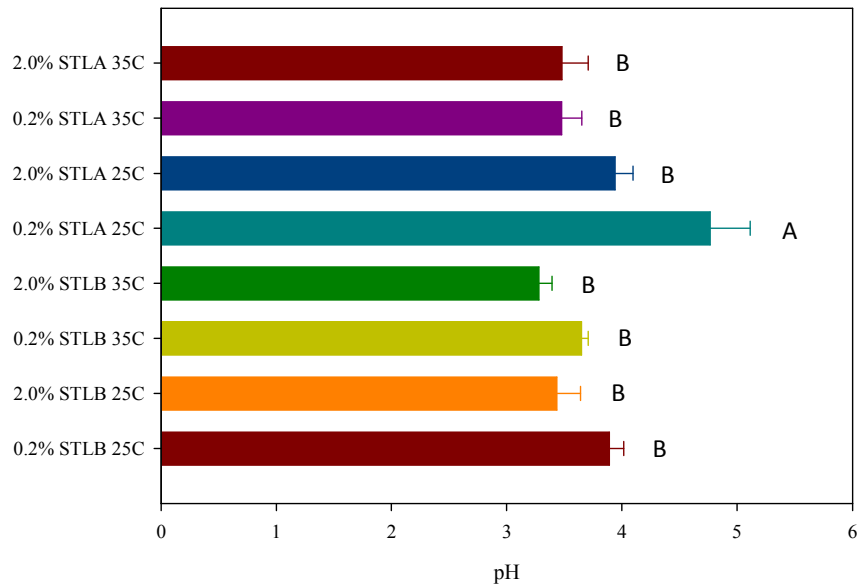


Figure 4.7: Final pH of MIT set yoghurt at the end of fermentation (168 h) using STLB and STLA at inoculum size 0.2 and 2.0% (v/v) and fermentation temperature 25 and 35°C (n=2). Means with different letters are significantly different ($p < 0.05$). Internal bar = \pm (2 x sample standard deviation).

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The final titratable acidity (TA), expressed as lactic acid, of set yoghurt fermented made using STLB, was 1.076 to 1.215 mmol (100 g)⁻¹ (Figure 4.8 and Figure 4.10). The TA profiles reflected the pH profiles, with yoghurt fermented using 2.0% (v/v) STLB at 35°C showing the fastest production of lactic acid. The TAs of yoghurts fermented using STLA were in the range 0.872 to 1.188 mmol (100 g)⁻¹ (Figure 4.9 and Figure 4.10). Incubation at 25°C and 0.2% (v/v) inoculum resulted in slower acidification, producing yoghurt with a higher pH and lower TA at the end of the fermentation (168h). The IDF (1991, 1992) have suggested a minimum of 0.7g lactic acid per 100g of retail yoghurt. Therefore, even though the yoghurt produced using STLA produced a lower titratable acidity than when using STLB, the amount produced was still sufficient (Figure 4.10).

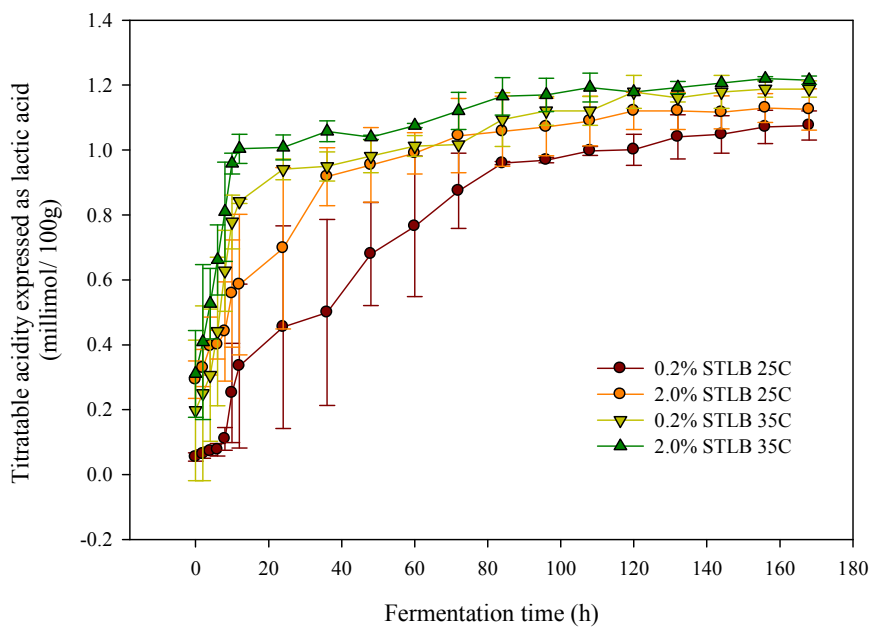


Figure 4.8: Titratable acidity expressed as lactic acid of MIT set yoghurt during fermentation using *Streptococcus thermophilus* STM5 with *Lactobacillus delbrueckii* subsp *bulgaricus* (STLB), inoculum size 0.2 and 2.0% (v/v) and fermentation temperature 25 and 35°C (n=2). Internal bar = ± (2 x sample standard deviation).

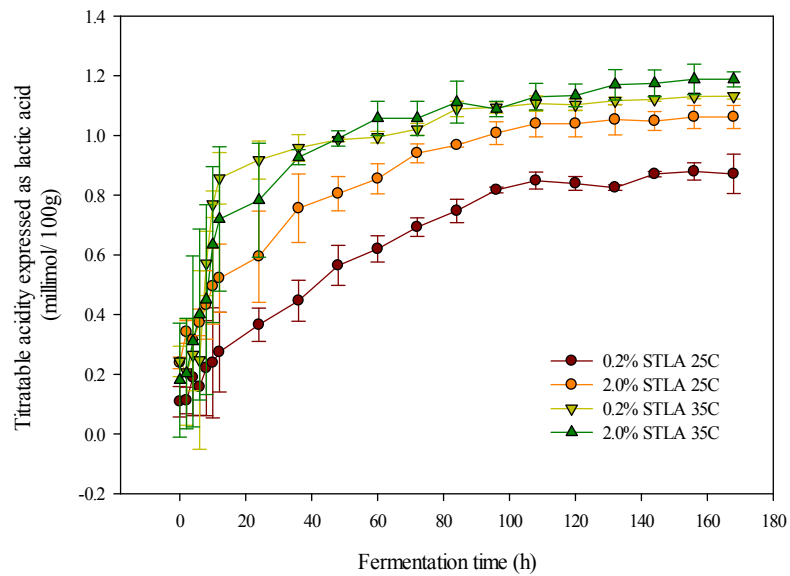


Figure 4.9: Titratable acidity expressed as lactic acid of MIT set yoghurt during fermentation using *Streptococcus thermophiles* STM5 with *Lactobacillus acidophilus* LA5 (STLA), inoculum size 0.2 and 2.0% (v/v) and fermentation temperature 25 and 35°C (n=2). Internal bar = \pm (2 x sample standard deviation).

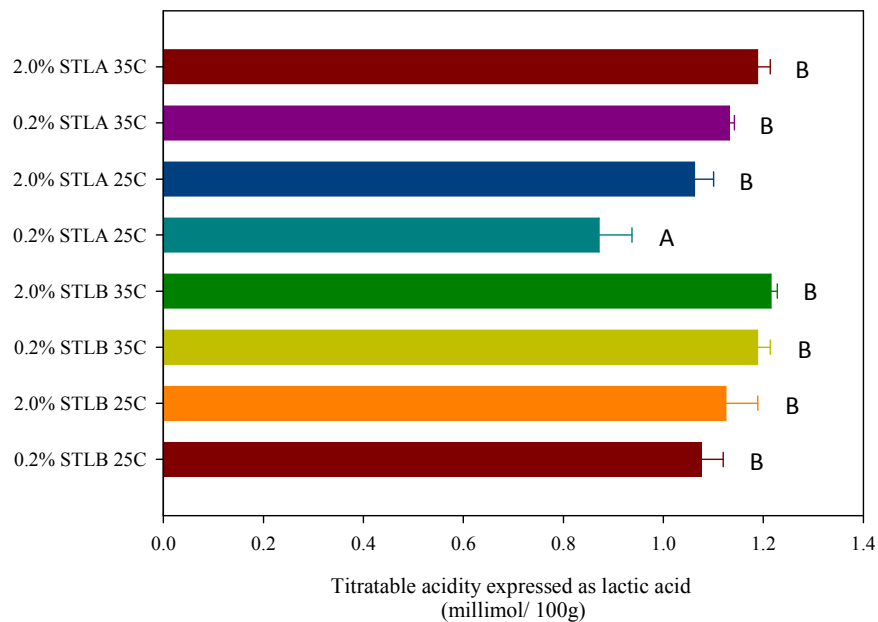


Figure 4.10: Final titratable acidity of MIT set yoghurt at the end of fermentation (168 h) using STLB and STLA at inoculum size 0.2 and 2.0% (v/v) and fermentation temperature 25

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and 35°C (n=2). Means with different letters are significantly different ($p < 0.05$). Internal bar = \pm (2 x sample standard deviation).

Starter culture growth during fermentation to produce MIT set yoghurt was determined using plate counts. There were no significant differences ($p < 0.05$) in the growth of *S. thermophilus* STM5 in combination with *L. bulgaricus* and growth in combination with *L. acidophilus* LA5 (Figure 4.11 and Figure 4.12). The final *S. thermophilus* STM5 count in combination with *L. bulgaricus* was in the range of 7.25 to 7.46 log (cfu g⁻¹) (Figure 4.11a). *L. bulgaricus* grew at both 25 and 35°C and inoculum sizes of both 0.2 and 2.0% (v/v). The final count of *L. bulgaricus* was in the range 6.15 to 7.01 log (cfu g⁻¹) (Figure 4.11b). *S. thermophilus* STM5 and *L. bulgaricus* grew more slowly at lower fermentation temperatures and inoculum sizes than at higher.

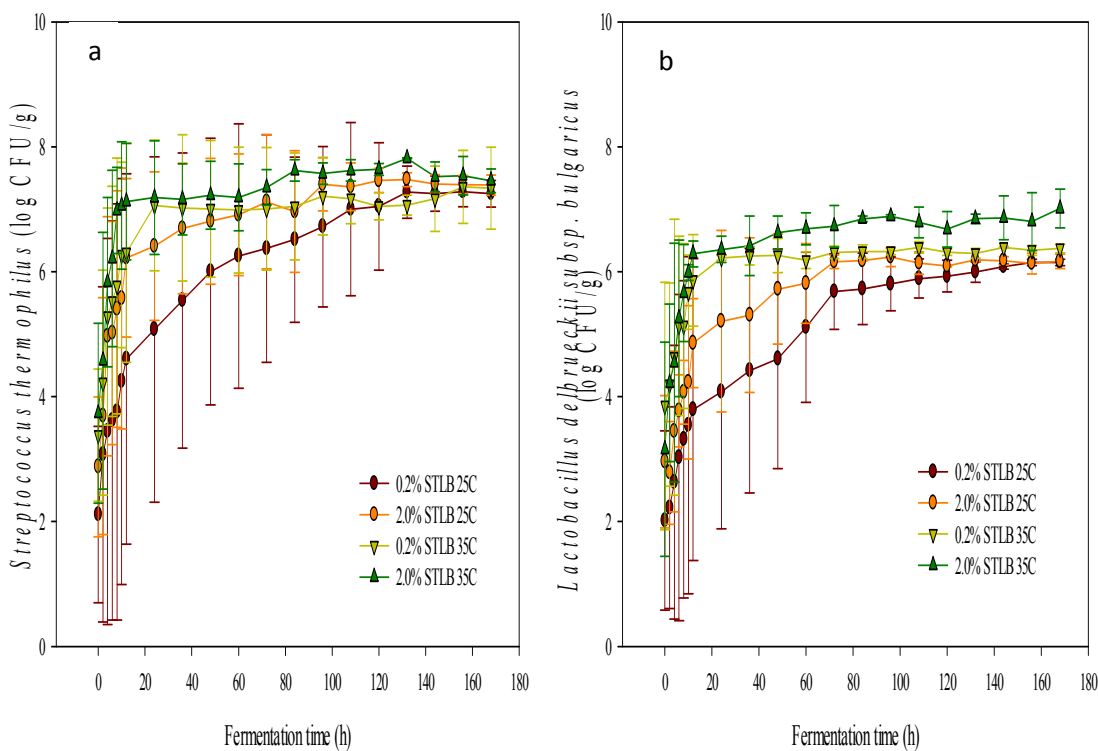


Figure 4.11: Growth of (a) *Streptococcus thermophilus* STM5 and (b) *Lactobacillus delbrueckii ssp bulgaricus* (STLB) in MIT set yoghurt during fermentation, inoculum size 0.2 and 2.0% (v/v) and fermentation temperature 25 and 35°C (n=2). Internal bar = \pm (2 x sample standard deviation).

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Fermentations using *S. thermophilus* STM5 in combination with *L. acidophilus* LA5 produced counts of 7.07 to 7.40 log cfu g⁻¹ (Figure 4.12a). Higher counts of *S. thermophilus* (8.0 to 9.0 log cfu g⁻¹) were reported by Oliveira et al. (2001) using STLA as the starter culture fermented at 42°C. They mentioned that, this could be due to the mechanism of nutritional competition. *Lactobacillus acidophilus* LA5 grew in combination with *S. thermophilus* STM5 (Figure 4.12b) with the final counts at the end of fermentation in the range 6.56 to 7.25 log cfu g⁻¹. Oliveira et al. (2001) observed the concentration of *L. acidophilus* in fermented milk after 24 hours of storage at 4°C is in the ranged of 7.68 to 8.64 log cfu mL⁻¹ when using a milk base supplemented with whey, casein and milk protein.

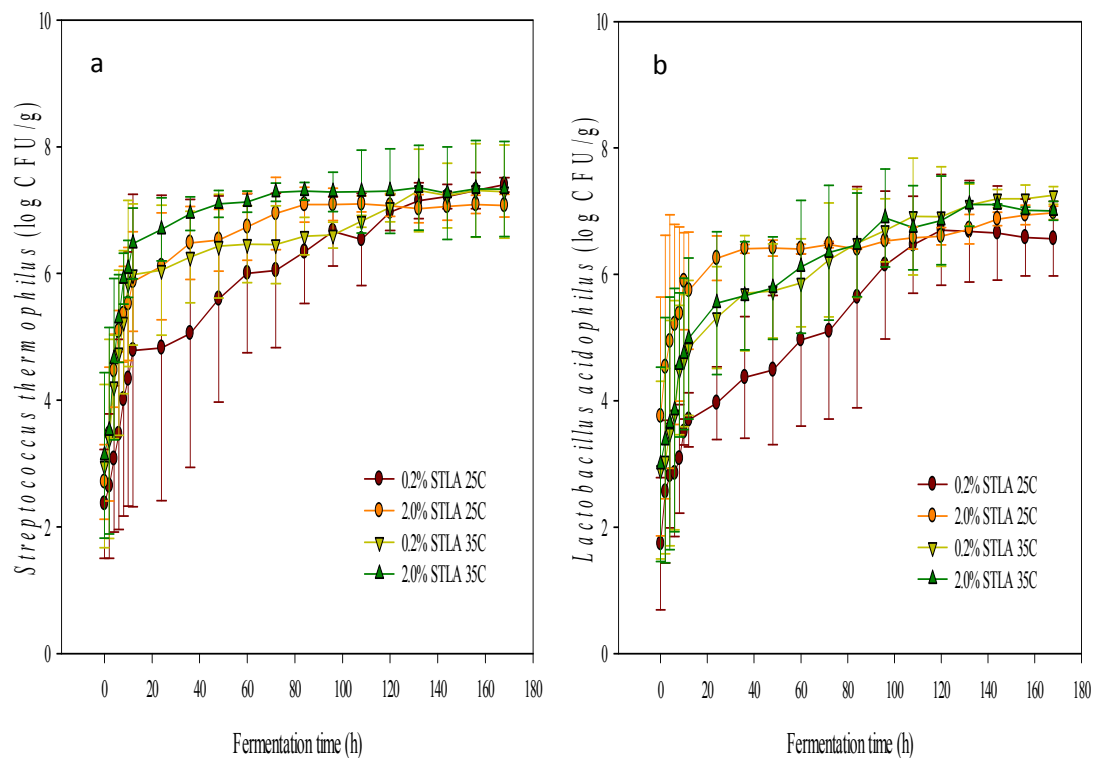


Figure 4.12: Growth of (a) *Streptococcus thermophilus* STM5 and (b) *Lactobacillus acidophilus* LA5 (STLA) in MIT set yoghurt during fermentation, inoculum size 0.2 and 2.0% (v/v), fermentation temperature 25 and 35°C (n=2). Internal bar = ± (2 x sample standard deviation).

In both starter culture combinations (STLB and STLA), the starter culture growth profile was affected by the fermentation temperature and inoculum size, especially during the first 120 h. After 120 h, the starter culture counts showed little change up to the end of fermentation (168h) for all conditions. The higher fermentation temperature (35°C) caused faster growth than the lower temperature (25°C) during the exponential stage, as it was close to the optimum temperature of the starter culture. A similar effect was caused by inoculum size.

A crucial characteristic of set yoghurt is firmness. However, no minimum or maximum standard was found for yoghurt firmness. Variations in the fermentation conditions affected the firmness of MIT set yoghurt. Yoghurt produced with STLB was found to have firmness in the range 0.778 to 1.746 N (Figure 4.13 and Figure 4.15). The lowest firmness (0.778 N) occurred with the lower fermentation temperature and lower inoculum size (25°C and 0.2% (v/v)). Firmness was measured with the same equipment as that used in other published studies (TA-XT2 Texture Analyzer) where the following firmness results were recorded: 0.17-1.57 N by cylindrical plunger of 12.7 mm (Fizman et al., 1999), 1.3-2.2 N by back extrusion cell of 35 mm (Tudorica et al., 2002), 0.47- 0.85 N by plunger of 43 mm (Diaz-Jimenez et al., 2004) and 0.2-0.8 N by probe of 12.7 mm (Amatayakul et al., 2006). The variation of yoghurt firmness is affected by milk composition, starter culture and supplementary materials. Yoghurt made in this study was observed to have firmness values within the ranges reported in previous studies except for condition of 0.2% (v/v) STLA fermented at 25°C.

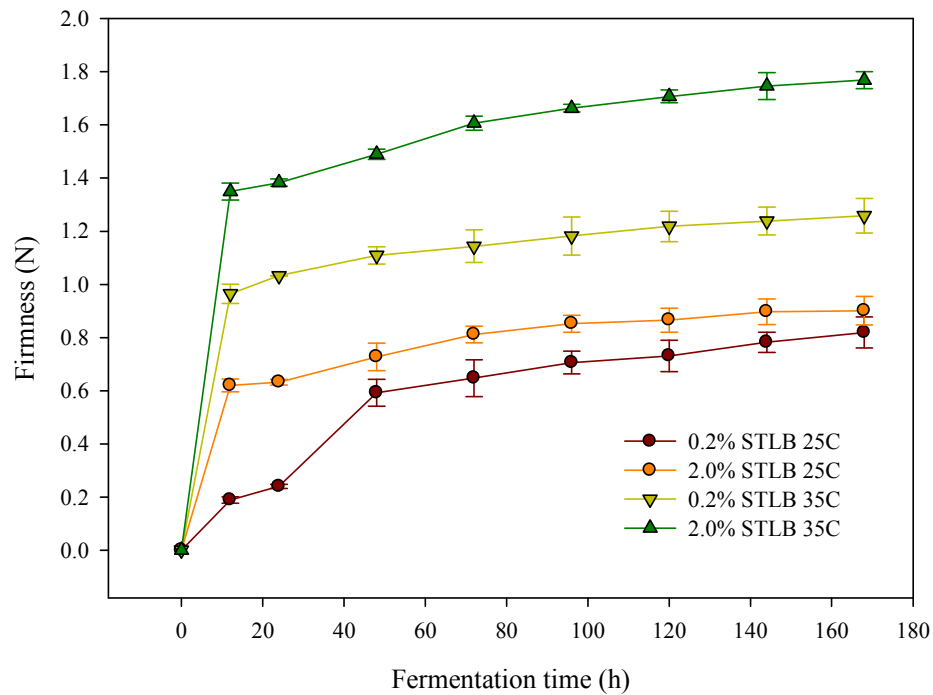


Figure 4.13: Firmness of MIT set yoghurt during fermentation using *Streptococcus thermophilus* STM5 with *Lactobacillus delbrueckii* subsp *bulgaricus* (STLB), inoculum size 0.2 and 2.0%, fermentation temperature 25 and 35°C (n=2). Internal bar = \pm (2 x sample standard deviation).

The firmness of MIT set yoghurt produced by STLA was 0 to 1.415 N (Figure 4.14 and Figure 4.15). Yoghurt produced using STLA at 0.2% (v/v) inoculum size and 25°C had not gelled by the end of fermentation. The reason for this is possibly the fact that the pH of the yoghurt failed to fall as far as the isoelectric pH of casein by the end of fermentation (Figure 4.7). Other conditions used to produce MIT set yoghurt using STLA resulted in gelation.

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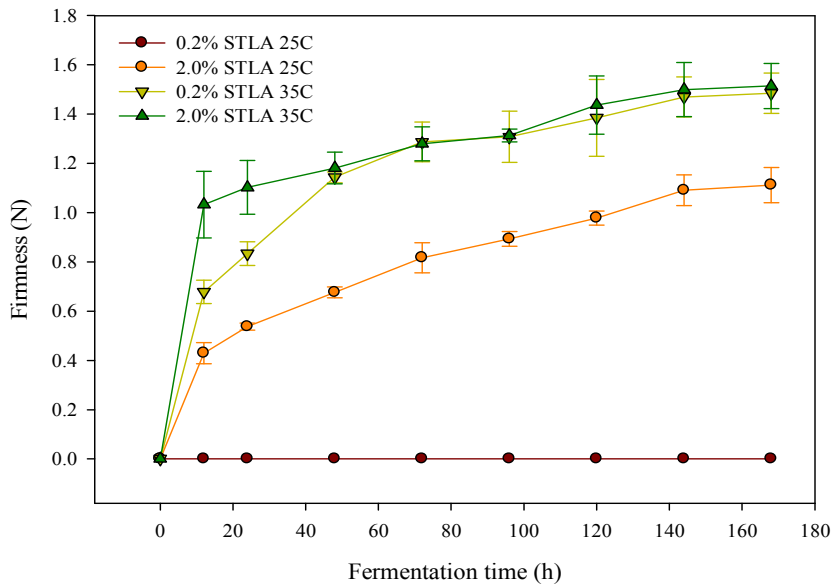


Figure 4.14: Firmness of MIT set yoghurt during fermentation using *Streptococcus thermophilus* STM5 with *Lactobacillus acidophilus* LA5 (STLA), inoculum size 0.2 and 2.0%, fermentation temperature 25 and 35°C. Internal bar = \pm (2 x sample standard deviation).

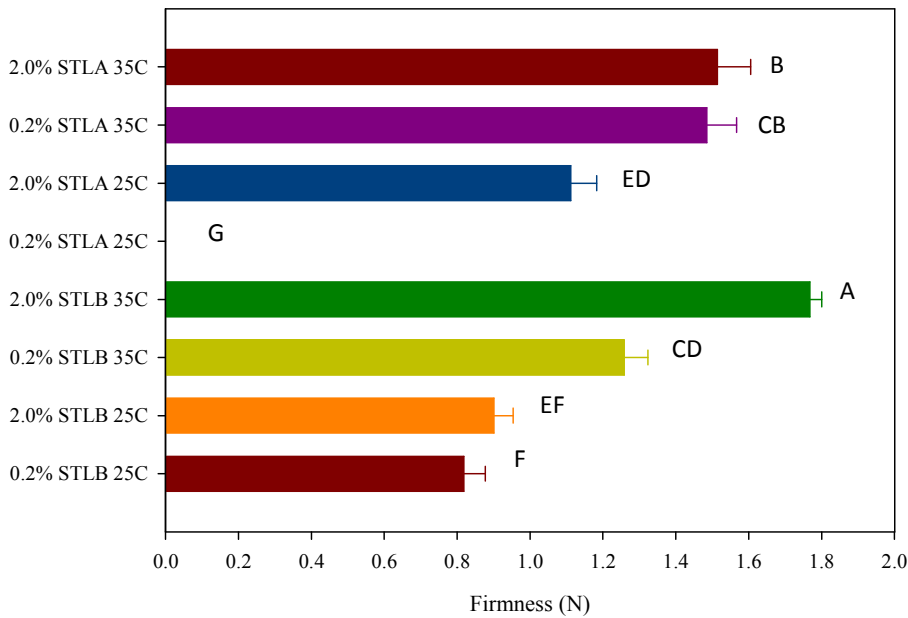


Figure 4.15: Final firmness of MIT set yoghurt at the end of fermentation (168 h) using STLB and STLA at inoculum size 0.2 and 2.0% and fermentation temperature 25 and 35°C (n=2). Means with different letters are significantly different ($p < 0.05$). Internal bar = \pm (2 x sample standard deviation).

The lower firmness of set yoghurt could be related with heat treatment of yoghurt milk base. Speer (1998) reported that heat treatment of yoghurt milk base could influence the yoghurt consistency. There are two possible reasons that this occurs. Firstly, the heating process was observed to cause a denaturation of whey protein (especially β -lactoglobulin). The denatured whey protein becomes associated with casein micelles (Mottar et al., 1989) and at the same time it can perform as a bridging material with other denatured whey protein (Lucey & Singh, 1998). This consequently increased the number and strength of bonds between protein particles (Lucey & Singh, 1998). Compared to pasteurization, UHT processes have been reported to cause lower percentage of β -lactoglobulin denaturation; is about 60 to 90% for UHT, while pasteurization could cause 90 to 100% (Kessler, 2002). Thus, heat treated milk base with a higher percentage of whey protein denaturation would be expected to produce a firmer yoghurt gel (Schmidth et al. 1985).

Secondly, Labropoulos et al. (1981) suggested the firmness of yoghurt is mainly due to subtle differences in protein structure caused by different heat processes rather than directly due to degree of whey protein denaturation. This is because the yoghurt firmness was observed not to have increased with time of heating at high temperature, even though the WPD was increased (Labropoulos et al., 1981; Parnell-Clunies et al., 1986).

4.5 Conclusion

Starter culture composition, inoculum size and fermentation temperature affected yoghurt fermentation time. Fermentation conditions suitable for MIT set yoghurt comprised *Streptococcus thermophilus* STM5 with *Lactobacillus acidophilus* LA5 (STLA), an inoculum size of 0.2% (v/v) inoculum and a fermentation temperature of 25°C. These enabled the fermentation to be extended to 168 h. At the end of fermentation, the pH of the yoghurt was 4.77. However, no yoghurt gel developed. The next part of the study focused on improving the texture of MIT set yoghurt.

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CHAPTER FIVE : EFFECT OF INCREASING THE CONCENTRATION OF RECONSTITUTED SKIM MILK AS THE MILK BASE OF SET YOGHURT AS A MADE-IN-TRANSIT (MIT) PRODUCT

5.1 Abstract

The fermentation time, yoghurt acidity expressed as lactic acid, starter culture growth and firmness of made-in-transit set yoghurts produced using different concentrations of reconstituted skim milk powder from 12 to 20% (w/v) as the milk bases were investigated. All milk base formulations were ultra-high temperature sterilized at 138°C for 6 s. The results revealed that increasing the skim milk powder concentration increased the firmness of made-in-transit set yoghurt. At 20% skim milk powder, yoghurt firmness was 1.266 N. Fermentation of made-in-transit set culture yoghurt with 20% skim milk powder was observed to be faster than with the other conditions. The fermentation time for a medium containing from 14 to 20% skim milk powder could be extended to 168h, reaching a final pH of 4.50 to 4.37, and at the same time improving the texture of the made-in-transit set yoghurt. The skim milk powder concentration had no influence on the total viable counts of starter bacteria in the yoghurt. The texture of made-in-transit set yoghurt may be improved by increasing the concentration of skim milk powder.

5.2 Introduction

Although fermentation time can be extended to 168 h by inoculating with *Streptococcus thermophilus* STM5 and *Lactobacillus acidophilus* LA5 and fermenting at 25°C, the body and texture of the MIT set yoghurt produced are poor. It is very important for set yoghurt to have a firm enough body to be spooned and the texture should be fine and smooth (O'Neil *et al.*, 1979). Increasing total solids can improve yoghurt texture as measured by sensory and instrument analyses (Amatayakul *et al.*, 2006). Tamime and Robinson (2007) found that yoghurt texture improved as milk base total solids increased from 12 to 20g /100g. Therefore, the objective of this study was to investigate the effects of increasing the concentration of skim milk powder in the yoghurt milk base on the texture, and fermentation time extension, of MIT set yoghurt.

5.3 Materials and Methods

5.3.1 Experimental Design

An experiment was carried out to study the effect of skim milk powder (SMP) concentration in yoghurt milk base (comprising SMP and water) on the acidification rate and texture of set yoghurts during fermentation. SMP concentration was set at 12, 14, 16, 18 and 20% (w/v). The ferment was analyzed every 2 h for the first 12 h, and then every 12 h for 144 h for pH and titratable acidity; every 2 h for the first 12 h, and then every 24 h for 144 h for starter culture and on the first 12 h, and then every 24 h for 144 h for firmness.

5.3.2 Cultures

See section 3.1.1 (Chapter 3).

5.3.3 Preparation of reconstituted skim milk

See section 3.2.1 (Chapter 3).

5.3.4 Heat treatment of milk base

See section 3.2.2 (Chapter 3).

5.3.5 Processing of yoghurt

See section 3.2.3 (Chapter 3).

5.3.6 Measurement of pH

See section 3.2.4 (Chapter 3).

5.3.7 Microbiological analysis

See section 3.2.5 (Chapter 3).

5.3.8 Determination of titratable acidity

See section 3.2.6 (Chapter 3).

5.3.9 Measurement of firmness

See section 3.2.7 (Chapter 3).

5.3.10 Statistical Analyses

All experiments were done in duplicate. One-way analysis of variance was performed using MINITAB 15 to determine the effects of increasing SMP concentration on the pH, titratable acidity, starter culture growth and texture of set yoghurt. Means were compared using Tukey's multiple comparison tests.

5.4 Results and discussion

Different SMP concentrations resulted in no significant differences ($p > 0.05$) in the rate of fermentation based on pH for set culture yoghurt over the first 24 h (Figure 5.1). A steep pH reduction was found between 24 and 72 h, especially at 18 and 20% SMP. The reduction in pH was observed to be dependent on the SMP concentration, where higher SMP concentrations caused a more rapid pH reduction than lower SMP concentrations used in the manufacture of MIT set culture yoghurt. Increasing the SMP concentration increased the lactose content of the milk base. The lactose content in the SMP used was 54.1%. The 12 to 20% SMP used therefore provided 6.49 to 10.82% lactose in the yoghurt base. Therefore, the increase in the rate of pH reduction with increasing SMP concentration was expected, as increasing the lactose content provided more substrate for lactic acid formation by the starter bacteria. Yoghurt with between 14 and 20% SMP (inclusive) was observed to have a final pH within the range of pH 4.50 to 4.37. Only set culture yoghurt produced using 12% SMP had a final pH higher than 4.5 (pH 4.63) (Figure 5.2). Previous studies on the effect of milk powder concentration reported the final pH to be in the range pH 4.2 to 4.6 (Soukoulis *et al.*, 2007; Staff, 1998).

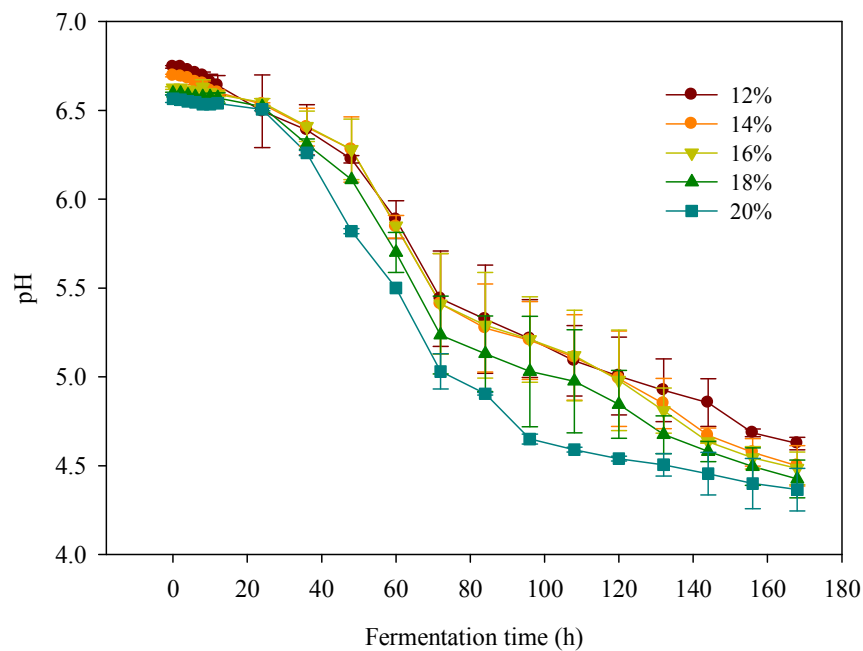


Figure 5.1: pH profiles of MIT set yoghurt at 12, 14, 16, 18 and 20% SMP as the yoghurt milk base. Internal bar = \pm (2 x sample standard deviation).

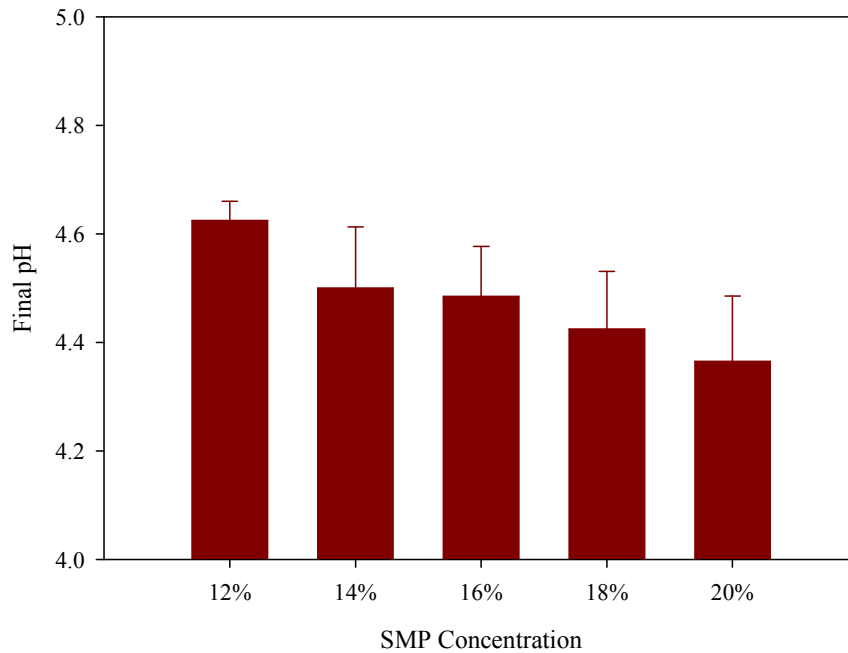


Figure 5.2: pH of MIT set yoghurt produced at 12, 14, 16, 18 and 20% SMP as the yoghurt milk base at the end of the 168 h fermentation time. Internal bar = \pm (2 x sample standard deviation).

The variation in yoghurt acidity expressed as lactic acid with time (Figure 5.3) mirrored the pH profile, the rate of production of lactic acid increasing with increasing SMP concentration. At the three lowest concentrations of SMP, 16, 14 and 12%, there were no significant differences in yoghurt acidity. Walstra and Jenness (1984) reported that increasing the level of solids not fat in yoghurt mix raised the titratable acidity of the milk owing to the buffering action of the additional proteins, phosphates, citrates, lactates and other milk constituents.

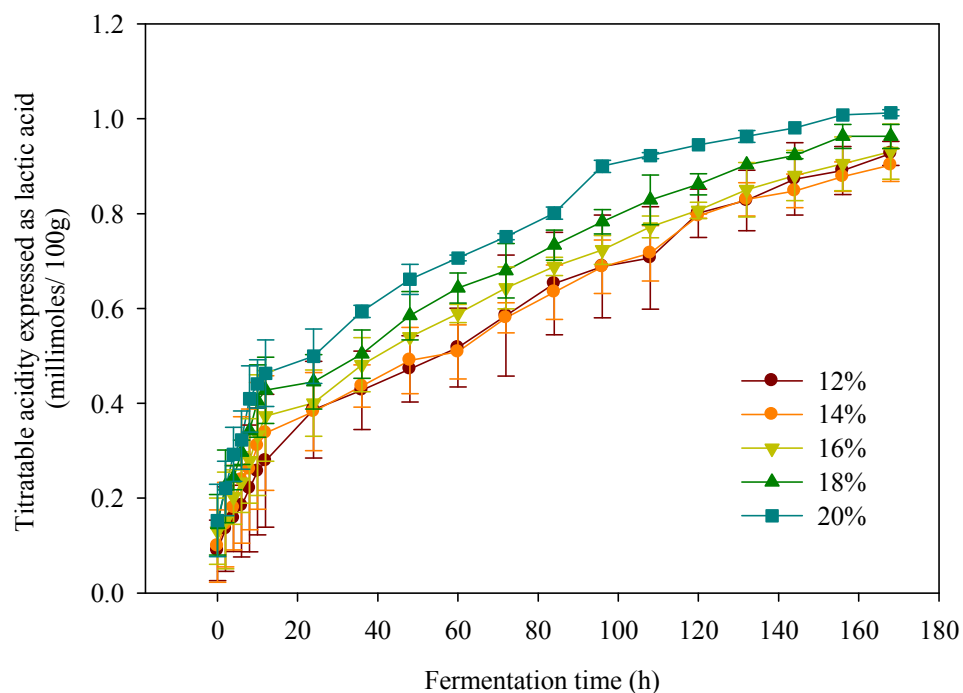


Figure 5.3: Yoghurt acidity expressed as lactic acid in MIT set yoghurt at 12, 14, 16, 18 and 20% SMP as the yoghurt milk base over the 168 h fermentation time. Internal bar = \pm (2 x sample standard deviation).

Table 5.1 and 5.2 show *S. thermophilus* STM5 and *L. acidophilus* LA5 counts during the 168 h fermentation time. Increasing the concentration of SMP did not have any significant effects on the starter culture growth, which agrees with Krasaekoopt, *et al.* (2004). However, Al-Dabbagh & Allan (1989) observed that different levels of total solids can influence the generation times and cell counts of yoghurt starter culture organisms; optimum conditions were 12 and 14g/ 100g for *Lactobacillus delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, respectively. In the present work on MIT yoghurt, at the end of the fermentation time, the *S. thermophilus* STM5 count was in the range 7.77 to 8.06 log cfu g⁻¹ and *L. acidophilus* LA5 in the range 7.83 to 8.17 log cfu g⁻¹. The final starter culture count met the minimum required total count of starter bacteria (1.0 x 10⁷ cfu g⁻¹), and the probiotic species count exceeded the 1.0 x 10⁶ cfu g⁻¹ specified in the Codex Standard (FAO/WHO, 2003).

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Table 5.1: Viable cell counts of *Streptococcus thermophilus* STM5 (log cfu g⁻¹) in MIT set yoghurt produced at 12, 14, 16, 18 and 20% SMP as the yoghurt milk base during incubation at 25°C.

Time (h)	SMP Concentration				
	12%	14%	16%	18%	20%
0	4.98 ^a	4.98	4.98	4.98	4.98
2	5.17	5.07	5.12	5.10	5.11
4	5.25	5.19	5.22	5.20	5.21
6	5.73	5.44	5.59	5.51	5.55
8	6.30	6.05	6.18	6.11	6.14
10	6.47	6.68	6.58	6.63	6.60
12	6.66	6.77	6.72	6.74	6.73
24	7.01	6.89	6.95	6.92	6.93
48	7.06	7.15	7.11	7.13	7.12
72	7.41	7.53	7.47	7.50	7.48
96	7.65	7.66	7.66	7.66	7.66
120	8.21	7.87	8.04	7.96	8.00
144	8.26	8.10	8.18	8.14	8.16
168	7.77	8.06	7.92	7.99	7.95

^a At a given time of growth (i.e. within a row) there were no significant differences in viable cell count

Table 5.2: Viable cell counts of *Lactobacillus acidophilus* LA5 (log cfu g⁻¹) in MIT set yoghurt produced at 12, 14, 16, 18 and 20% SMP as the yoghurt milk base during incubation at 25°C.

Time (h)	SMP Concentration				
	12%	14%	16%	18%	20%
0	4.20 ^a	4.22	4.30	4.28	4.44
2	4.24	4.30	4.32	4.50	4.65
4	4.53	4.52	4.52	4.68	4.75
6	4.83	4.82	4.80	4.95	4.99
8	4.75	4.85	4.98	5.14	5.28
10	5.20	5.12	5.20	5.44	5.52
12	5.46	5.56	5.64	5.87	5.86
24	5.82	5.96	6.09	6.07	6.28
48	6.55	6.83	6.60	6.74	6.74
72	7.62	7.31	7.30	7.11	7.15
96	7.84	7.73	7.43	7.42	7.47
120	8.05	7.83	7.94	7.55	7.67
144	8.27	8.10	8.02	7.88	7.72
168	8.17	8.04	7.93	7.99	7.83

^a At a given time of growth (i.e. within a row) there were no significant differences in viable cell count

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Increasing SMP concentration influenced the development of firmness (Figure 5.4) in set yoghurt. The texture of set yoghurt was improved by increasing the SMP concentration. The higher concentrations of SMP (18 and 20%) were found to produce set culture yoghurt with markedly higher firmness. The improvement in yoghurt firmness with increasing SMP concentration in this work is in line with Amatayakul *et al.* (2006) and Krasaekoopt *et al.* (2004). Krasaekoopt *et al.* (2004) reported that 20% total solids produced higher firmness than 16% total solids. Increasing the yoghurt milk base solids concentration increases the level of milk protein. This in turn increases the density of the gel network and reduces the pore size of the yoghurt microstructure (Krasaekoopt *et al.*, 2004). Gastaldi *et al.* (1997) reported that the firmness of yoghurt increased with increasing total solids owing to the consequent increase in water binding capacity. They also mentioned that yoghurt firmness was dependent on total solids because a higher total solids results in a higher concentration of casein particles, which could enhance the interaction between these particles (Gastaldi *et al.*, 1997).

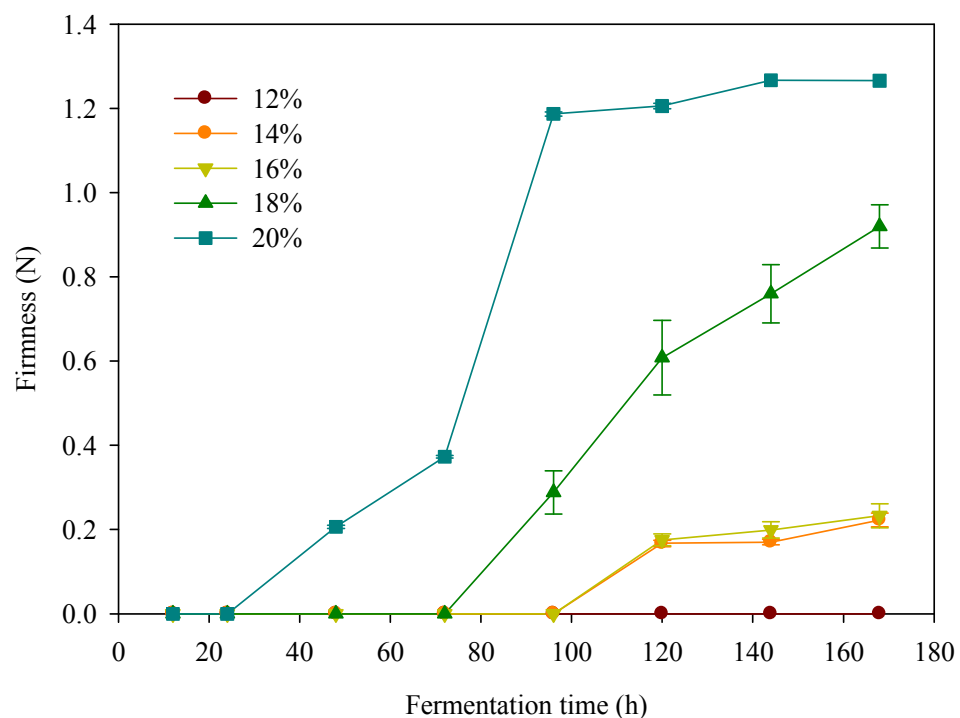


Figure 5.4: Firmness of MIT set yoghurt at 12, 14, 16, 18 and 20% SMP as the yoghurt milk base over the 168 h fermentation time. Internal bar = \pm (2 x sample standard deviation).

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The final characteristics of MIT set yoghurt produced at 12, 14, 16, 18 and 20% SMP are summarized in Table 5.3. Both texture and the length of fermentation are crucial to the success of MIT yoghurt. The fermentation time for normal yoghurt is between 4 to 12 h based on pH. This time variation is dependent on the specific processing methods used and the characteristics of the yoghurt to be produced. In the present work, the fermentation time was set at 168h, which resulted in an acceptable final pH in the range 4.2 to 4.6. Increasing the SMP concentration was observed to increase the firmness of yoghurt. A final pH of 4.5 was used as a guideline in this study as it is the maximum final pH for yoghurt stipulated in the Australia New Zealand Food Standards Code for fermented milk products (FSANZ, 1996).

Table 5.3: Final characteristics of MIT set yoghurt produced at 12, 14, 16, 18 and 20% SMP as the yoghurt milk base.

SMP concentration (%)	Final pH	Acidity expressed as lactic acid	Firmness (N)	Fermentation time (h) ^a
12	4.63 ± 0.04	0.93 ± 0.03	0.00 ± 0.00	>168
14	4.50 ± 0.11	0.90 ± 0.03	0.223 ± 0.02	168
16	4.49 ± 0.09	0.93 ± 0.06	0.223 ± 0.03	168
18	4.43 ± 0.11	0.96 ± 0.03	0.920 ± 0.05	156
20	4.37 ± 0.12	1.01 ± 0.01	1.266 ± 0.01	144

^a Fermentation time is measured when the pH measurement reaches pH 4.5

5.5 Conclusion

Increasing the concentration of the skim milk powder (12 to 20% (w/v)) used as a yoghurt milk base can improve the firmness of set yoghurt. Starter culture growth based on viable cell counts was not influenced by the SMP concentration. The final pH levels of set culture yoghurt produced with 14 to 20% SMP were within the acceptable pH range for set yoghurt for a fermentation time of 168h. Yoghurt base composition and fermentation conditions that result in an acceptable set yoghurt have been established, yet, the use of higher total solid up to 18 and 20% SMP is maybe uneconomical. Further options for improving yoghurt texture were investigated and are described in the next chapter (Chapter 6).

5.6 References

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CHAPTER SIX : THE EFFECT OF MILK BASE FORTIFICATION AND HEAT TREATMENT ON SET YOGHURT AS A MADE-IN-TRANSIT (MIT) PRODUCT

6.1 Abstract

The fermentation time, yoghurt acidity expressed as lactic acid, starter culture growth and firmness of made-in-transit set yoghurt produced from skim milk powder fortified with dried protein-containing dairy ingredients using two methods of heat processing were studied. Skim milk powder as the milk base was fortified with five dried dairy ingredients: skim milk powder, butter milk powder, whey protein concentrate, milk protein concentrate and sodium caseinate. The premixed milk base was reconstituted in distilled water followed by two methods of heat treatment; 1) ultra-high temperature (UHT) sterilization, and 2) a combination of high temperature long time (HTLT) pasteurization and UHT sterilization. Each milk base mixture was inoculated with *Streptococcus thermophilus* STM5 and *Lactobacillus acidophilus* LA5 (0.2% (v/v) total inocula, see section 6.3.2), and incubated at 25°C for 168 h. The effect of fortification was found more significant ($p < 0.05$) than the effect of heat treatment in improving the texture of MIT set yoghurt. Among all combinations, set yoghurt fortified with NaCN and UHT sterilization was observed to produce the highest firmness with final pH 4.12.

6.2 Introduction

The extended yoghurt fermentation (0.2% (v/v) of STLA fermented at 25°C) produced a poor texture of set yoghurt at the end of 168h of fermentation (Chapter 4). In the previous chapter (Chapter 5), the effect of increasing the concentration of skim milk powder in the milk base from 12 to 20% (w/v) was reported. The firmness of the yoghurt improved to 0.223 to 1.266 N when produced using 14 to 20% (w/v) of SMP, respectively. The highest SMP concentration resulted in the highest firmness. However, the use of 20% SMP as a yoghurt milk base is uneconomical.

Other methods of protein fortification and heat treatment (Damin et al., 2009) have been reported to enhance the firmness of yoghurt. Several dried dairy ingredients have been used. These have included skim milk powder (Damin et al., 2009; Guzman-Gonzalez et al., 1999; Moddler and Kalab, 1983), buttermilk powder (Trachoo and Mistry, 1998), whey protein concentrate (Damin et al., 2009; Guzman-Gonzalez et al., 1999), milk protein concentrate

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(Guzman-Gonzalez et al., 1999) and sodium caseinate (Damin et al., 2009; Isleten & Karagul-Yuceer, 2006).

Fortification with such ingredients increases the protein level, which is the principal factor influencing yoghurt texture (Prentice, 1992). Prentice also mentioned that the fortification with casein containing ingredient could contribute to the development of chains and aggregates of casein micelles. The ingredient type and its proportion in the dry matter also affect the final yoghurt texture (Penna et al., 2006; Puvanenthiran et al., 2002; Sodini et al., 2004).

Yoghurt texture is also influenced by heat treatment of the milk base. During heat treatment, whey protein is denatured and some of it becomes associated with casein micelles (Mottar et al., 1989). It also acts as a bridging material between such micelles by whey protein-whey protein interaction (Lucey & Singh, 1998). This increases the number and strength of bonds between protein particles (Lucey & Singh, 1998). Ultra high temperature (UHT) sterilization has been reported to cause a lower percentage of whey protein (β -lactoglobulin) denaturation (60-90%) than does pasteurization (90 to 100%) (Kessler, 2002). This is in accord with the findings of Krasaekoopt et al. (2004) and Schmidt et al. (1985), who reported that UHT treatment of the milk base produced yoghurt gels weaker than those made from pasteurized milk bases. The use of a UHT milk base is, however, unavoidable for made-in-transit set yoghurt as the base must to be sterile to avoid the growth of spoilage organisms during the long fermentation. Therefore, it was considered that a combination of high temperature long time (HTLT) and UHT might improve the texture of made-in-transit set yoghurt.

The objective of the work reported in this chapter was to determine if the texture of set yoghurt for made-in transit product could be improved by fortification of the milk base by testing five different dried dairy ingredients (skim milk powder (SMP), buttermilk powder (BMP), whey protein concentrate (WPC), milk protein concentrate (MPC) and sodium caseinate (NaCN)) in combination with a single heat treatment (UHT sterilization) or a double heat treatment (HTLT followed by UHT sterilization).

6.3 Materials and Methods

6.3.1 Experimental design

A factorial experiment was carried out to study the influence of fortifying material and heat treatment on acidification and starter culture growth in, and the texture of, set culture yoghurt as a made-in-transit product. Twelve experiments were defined according to 1) fortifying material: control (without fortification), SMP, BMP, WPC, MPC, NaCN; and 2) heat treatment: single (UHT) and double (HTLT plus UHT) (Table 6.1). pH was measured at 2 h intervals for the first 12 h and then at 12 h intervals for remaining 144 h of fermentation. Samples for starter culture enumeration were taken at 2 h intervals for the first 12 h and then at 24 h intervals for remaining 144 h. Firmness was measured at the end of the first 12 h and then at 24 h intervals for remaining 144 h. The entire experimental process was performed in duplicate.

Table 6.1: Experimental design for investigating the effect of fortification material and heat treatment on the acidification and texture of set yoghurt for a made-in-transit product

Run	Fortification materials	Heat treatment
1	Control	Single
2	SMP	Single
3	BMP	Single
4	WPC	Single
5	MPC	Single
6	NaCN	Single
7	Control	Double
8	SMP	Double
9	BMP	Double
10	WPC	Double
11	MPC	Double
12	NaCN	Double

6.3.2 Cultures

See Section 3.1.1 (Chapter 3). This procedure produced a consistent concentration of starter culture: $8.54 \pm 0.50 \log \text{ cfu mL}^{-1}$ (log colony forming units mL^{-1}) for *S. thermophilus* STM5 and $8.23 \pm 0.29 \log \text{ cfu mL}^{-1}$ for *L. acidophilus* LA5, confirmed by plate counting.

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6.3.3 Preparation and fortification of the yoghurt milk base

SMP was obtained from Fonterra (Fonterra Co-operative Group, New Zealand). The typical composition of SMP is as follows: lactose 54.1%, protein 33.4%, minerals 7.9%, moisture 3.8% and fat 0.8%. Experimental yoghurt milk bases were prepared using skim milk powder (4.0% protein) alone as a control and skim milk powder (4.0% protein) fortified with SMP, BMP, WPC, MPC or NaCN to bring the final protein content to 5.0% (Table 6.2). The total solids contents of the milk bases were as follows: 12.00 g 100 g⁻¹ in the control, 14.99 g 100 g⁻¹ for SMP, 13.25 g 100 g⁻¹ for WPC, 15.23 g 100 g⁻¹ for BMP, 13.23 g 100 g⁻¹ for MPC and 13.08 g 100 g⁻¹ for NaCN. The dried ingredients were mixed for one minute using a spoon before being reconstituted in distilled water while being agitated using a magnetic stirrer for 3 h at 25°C (Lee & Lucey, 2004). This reconstitution method is a gentle process and does not significantly damage the casein micelles compared with high shear and/or homogenization processes (Bock *et al.* 2008). The milk bases were stored in a refrigerator (4°C) overnight before use (Lee & Lucey, 2004).

Table 6.2: Protein content for fortification of dried dairy ingredients to the milk bases and control milk used for set yoghurt production (g/100g)

Milk base	Control	Fortification (g/ 100g)				
		SMP	BMP	WPC	MPC	NaCN
SMP	4.00	-	-	-	-	-
SMP	4.00	1.00	-	-	-	-
SMP	4.00	-	1.00	-	-	-
SMP	4.00	-	-	1.00	-	-
SMP	4.00	-	-	-	1.00	-
SMP	4.00	-	-	-	-	1.00

SMP = skim milk powder; BMP = buttermilk powder; WPC = whey protein concentrate; MPC = milk protein concentrate; NaCN = sodium caseinate.

6.3.4 Heat treatment of milk base

The yoghurt milk base was heat treated using two methods: 1) a single heat treatment (ultra-high temperature (UHT) sterilization) and 2) a double heat treatment (high-temperature long-time (HTLT) treatment plus UHT sterilization). In UHT sterilization, the milk base was held at 138°C for 6 s (Schmidt *et al.*, 1985). For the double heat treatment, the milk base was HTLT treated (90°C for 30 min) followed by UHT sterilization (138°C for 6 s). HTLT treatment was carried out batch wise using a temperature controlled water bath (Model ED, Julabo

Labortechnik GMBH Germany), and UHT treatment using pilot scale indirect UHT plant (Alfa Laval, Lund, Sweden). For both methods, the heated milk was dispensed into sterile Schott Duran bottles (previously autoclaved at 121°C for 15 min) and stored at 4°C until used for yoghurt making.

6.3.5 Yoghurt processing

See Section 3.2.3 (Chapter Three).

6.3.6 Measurement of pH

See Section 3.2.4 (Chapter Three).

6.3.7 Microbiological analysis

See Section 3.2.5 (Chapter Three).

6.3.8 Determination of titratable acidity

See Section 3.2.6 (Chapter Three).

6.3.9 Measurement of firmness

See Section 3.2.7 (Chapter Three).

6.3.10 Statistical analyses

Analysis of variance was performed using MINITAB 15 (Minitab Inc., State College, PA, USA) to determine the effects of fortification and heat treatment on the pH, titratable acidity and firmness of, and starter culture growth in, MIT set yoghurt. The means were compared using Tukey's multiple range tests.

6.4 Results and Discussion

The effect of fortifying material and heat treatment on pH profiles during culture yoghurt fermentation is shown in Figure 6.1. A somewhat steeper pH reduction over the first 60 h of fermentation was observed in fortified yoghurt bases given a double heat treatment (Figure 6.1(b)) compared with those given a single heat treatment (Figure 6.1(a)). After 60 h, the pH for both heat treatments showed little further reduction, tending to stabilize to close to the value at the end of fermentation, except for yoghurt treated with UHT and fortified with SMP. Each main factor, fortification and heat treatment, significantly influenced ($p < 0.05$) the rate of pH reduction during the 168 h of fermentation (Table 6.3).

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Yoghurt with UHT treatment alone and SMP fortification was observed to experience a more gradual pH reduction than did the other fortified yoghurts, which all showed faster pH reduction compared with the control regardless of the heat treatment applied. In previous studies, yoghurts made from milk bases fortified with MPC or casein hydrolysate (Sodini, Lucas, Oliveira, Remeuf & Corrieu, 2002) or NaCN or WPC (Damin et al., 2009) were shown to exhibit accelerated fermentation.

There was no significant difference ($p > 0.05$) between heat treatments in terms of the final pH of the yoghurt (Figure 6.2). For fortification, on the other hand, a significant difference ($p < 0.05$) was observed between the final pH of the control and the final pHs of yoghurt prepared from fortified milk bases. The final pH of the yoghurt had the following ranking: control > SMP > BMP > MPC > WPC > NaCN for the single heat treatment and control > SMP > BMP \geq WPC > MPC > NaCN for the double heat treatment. All fortified yoghurts except that fortified with SMP had a lower final pH than the acceptable pH range for yoghurt, pH 4.2 to 4.6 (Tamime & Robinson, 2007; Soukoulis et al., 2007). The final pH for yoghurt fortified with BMP was pH 4.20 and 4.17; WPC, pH 4.16 and 4.17; MPC, pH 4.17 and 4.15 and NaCN, pH 4.12 and 4.13 for single and double heat treatments, respectively. A too low pH produces a too sour yoghurt (Tamime & Robinson, 2007).

Titrateable acidity development during fermentation of set yoghurt produced from fortified bases subjected to the two heat treatment methods is shown in Figure 6.3. Yoghurt fortified with SMP, BMP, WPC, MPC and NaCN subjected to the single heat treatment showed a gradually increasing titrateable acidity over the first 24 h. This was followed by a steep increase until 48h after which except in the case of the control, the titrateable acidity started to plateau. The titrateable acidity of the control gradually increased during the 168 h of fermentation. A similar pattern was observed for yoghurts produced from bases that received a double heat treatment. Both factors, fortification and heat treatment, significantly influenced ($p < 0.05$) the titrateable acidity profile of yoghurt during 168 h of fermentation (Table 6.3).

The final titrateable acidity (Figure 6.4) of all yoghurts was above the minimum requirement for yoghurt, which is $0.7 \text{ mmol (100 g)}^{-1}$ (IDF, 1991, 1992). No significant difference ($p < 0.05$) in titrateable acidity was observed between the two heat treatment methods (Table 6.3). Yoghurt fortified with SMP, BMP, WPC, MPC and NaCN produced similar

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final titratable acidities: 0.924 to 0.986 mmol (100 g)⁻¹ for the single heat treatment and 0.945 to 1.013 mmol (100 g)⁻¹ for the double heat treatment.

Streptococcus thermophilus STM5 growth in milk bases subjected to single and double heat treatments is shown in Figure 6.5. A similar trend was observed for both heat treatments. In all experiments, the control base showed the slowest growth. This might have been due to the lower total solids and protein contents of the control base compared with the fortified base. Generally, bacterial growth is influenced by the total solids, protein and other nutrients available (Jay, 2007). Yoghurt fortified with NaCN produced rapid growth of *S. thermophilus* STM5. Both factors, fortification and heat treatment, significantly influenced ($p < 0.05$) the growth profile of *S. thermophilus* STM5 in yoghurt during 168 h of fermentation (Table 6.3).

At the end of fermentation (168h), the *S. thermophilus* STM5 count in all samples was above the minimum requirement, 10⁷ cfu g⁻¹, which is stated in the Codex Standard (FAO/WHO, 2003). The *S. thermophilus* STM5 count in fortified yoghurt was higher than in the control for both heat treatments: 7.97 to 8.13 log cfu g⁻¹ for the single heat treatment, and 7.99 to 8.13 log cfu g⁻¹ for the double heat treatment (Figure 6.6).

The *Lactobacillus acidophilus* LA5 growth that occurred during 168 h of fermentation is shown in Figure 6.7. The *L. acidophilus* LA5 count gradually increased during the first 48 h of fermentation. This was observed in all samples regardless of the heat treatment. Both factors, fortification and heat treatment, significantly influenced ($p < 0.05$) the *L. acidophilus* LA5 growth profile of yoghurt during 168 h fermentation (Table 6.3).

There was no significant difference ($p < 0.05$) between heat treatments in terms of the final count of *L. acidophilus* LA5 count (Figure 6.8). The final count in all control and fortified samples was above the minimum requirement for yoghurt, 10⁶ cfu g⁻¹, which is stated in the Codex Standard (FAO/WHO, 2003). The final *L. acidophilus* LA5 count was 7.71 to 8.04 log cfu g⁻¹ for the single heat treatment, and 7.87 to 8.13 log cfu g⁻¹ for the double heat treatment.

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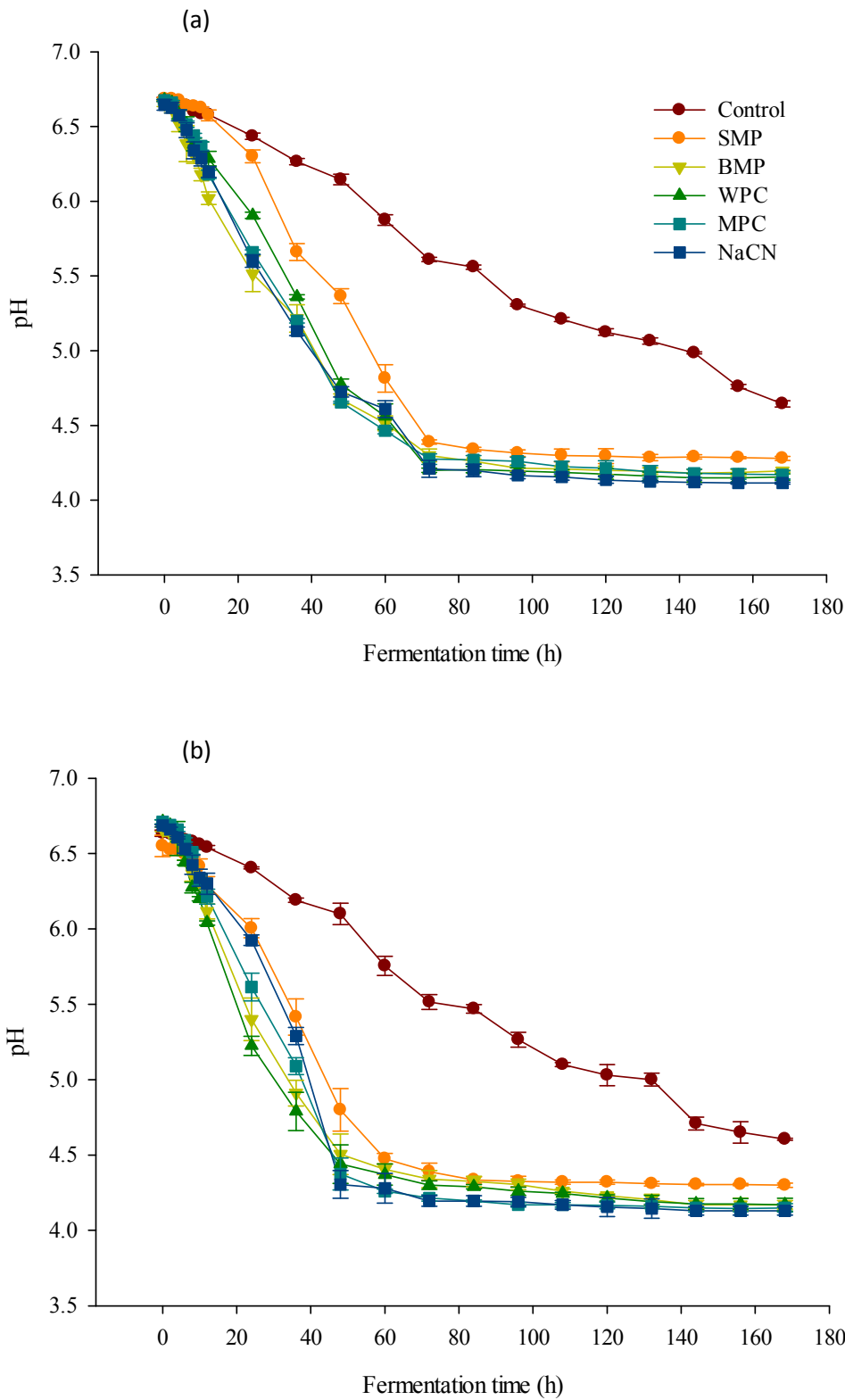


Figure 6.1: pH profile of set yoghurt for MIT product produced using yoghurt milk base fortified with SMP, BMP, WPC, MPC or NaCN and (a) single (UHT sterilization) or (b) double (HTLT plus UHT sterilization) heat treatment. Internal bar = \pm (2 x sample standard deviation).

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Table 6.3: p-values for the effects of fortifying material and heat treatment on pH decline, firmness development, and starter culture growth in yoghurt during and at the end of 168 h of fermentation at 25°C.

Effect	pH	TA	During fermentation			Final values				
			ST ^a	LA ^b	Firmness	pH	TA	ST	LA	Firmness
Fortifying material (FM)	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.002	0.026	0.000
Heat treatment (HT)	0.000	0.000	0.001	0.001	0.880	0.000	0.112	0.654	0.189	0.000
FM x HT	0.000	0.043	0.000	0.000	0.053	0.000	0.998	0.981	0.355	0.000

^a *Streptococcus thermophilus* STM5 concentration ^b *Lactobacillus acidophilus* LA5 concentration

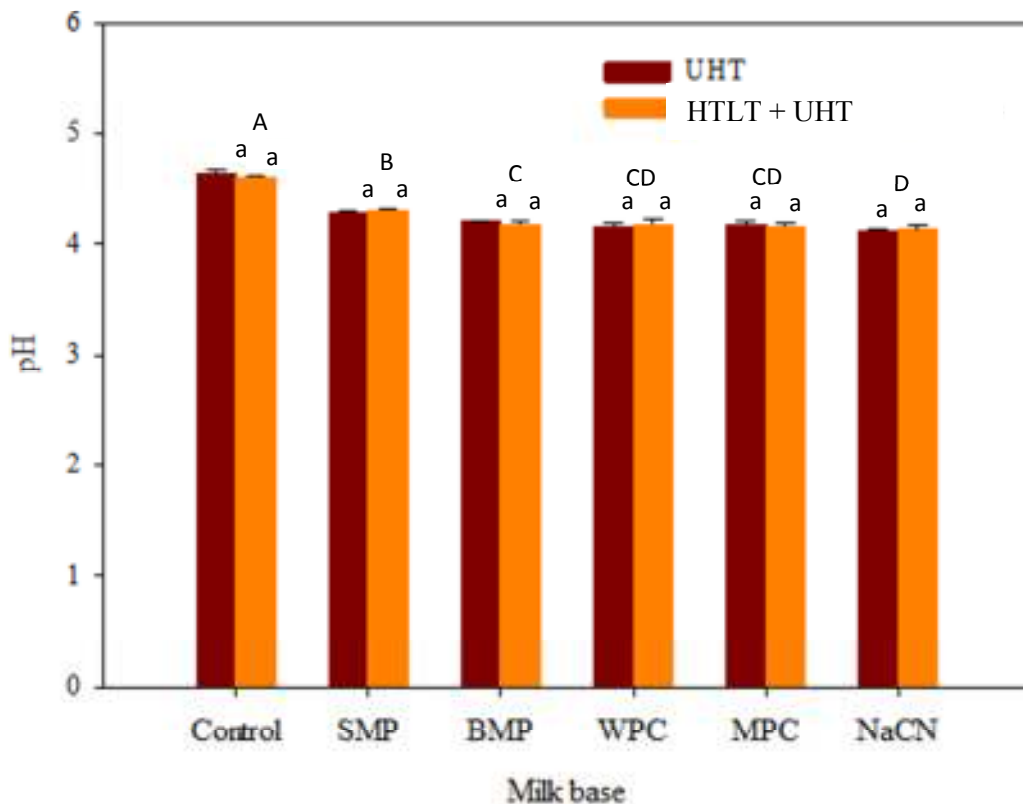


Figure 6.2: Comparison of final pHs of set yoghurt for MIT product produced using yoghurt milk base fortified with SMP, BMP, WPC, MPC or NaCN and single (UHT) and for single (UHT sterilization) and double (HTLT and UHT) heat treatments. Internal bar = ± (2 x sample standard deviation).

^{A, etc} Different letters represent significant differences (p<0.05) between fortifying materials.

^a Different letters would represent significant differences (p<0.05) between heat treatments.

CHAPTER SIX: THE EFFECT OF MILK BASE FORTIFICATION AND HEAT TREATMENT ON SET YOGHURT AS A MADE-IN-TRANSIT (MIT) PRODUCT

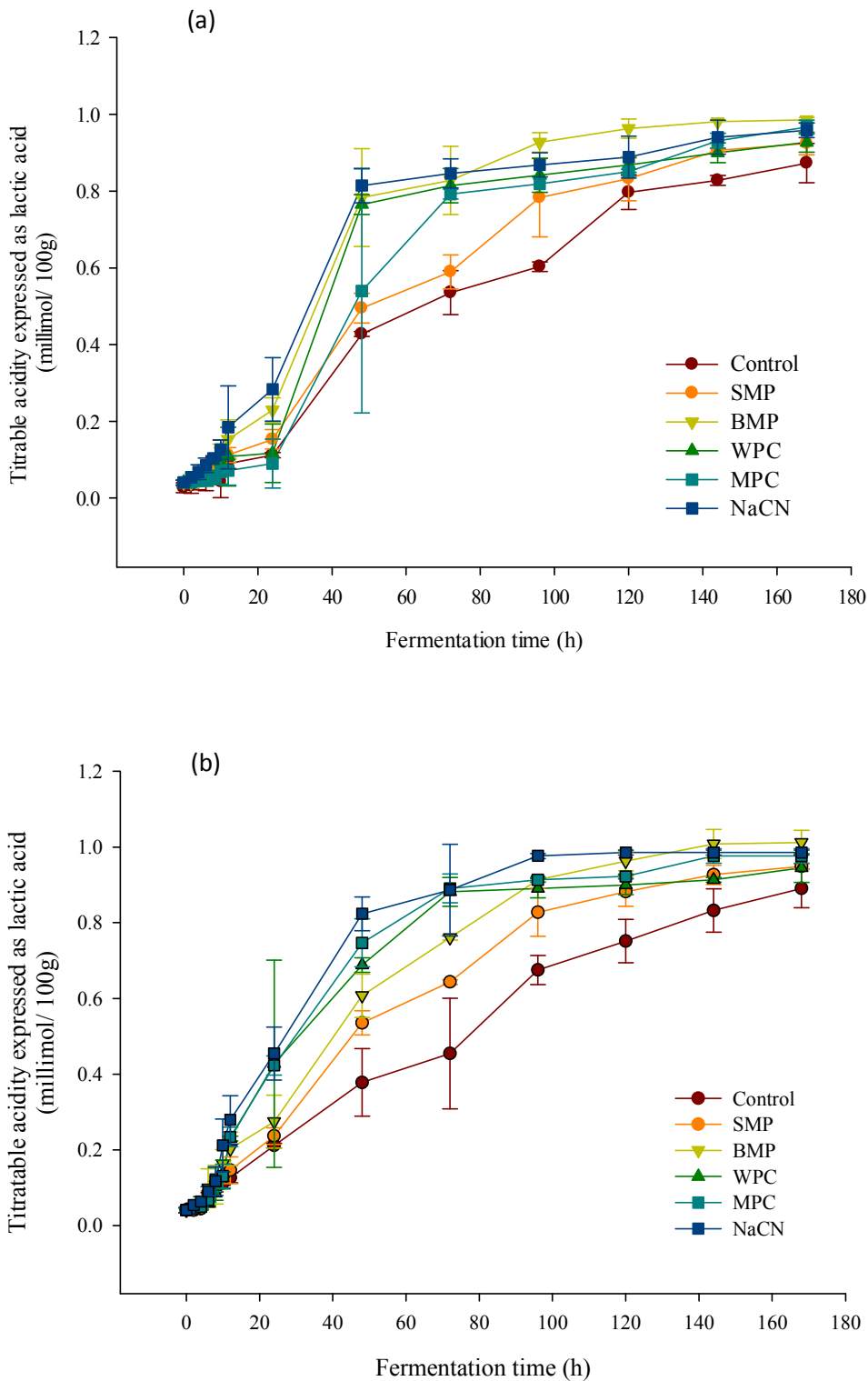


Figure 6.3: Titratable acidity of set yoghurt for MIT product produced using yoghurt milk base fortified with SMP, BMP, WPC, MPC or NaCN and (a) single (UHT sterilization) or (b) double (HTLT plus UHT sterilization) heat treatment. Internal bar = \pm (2 x sample standard deviation).

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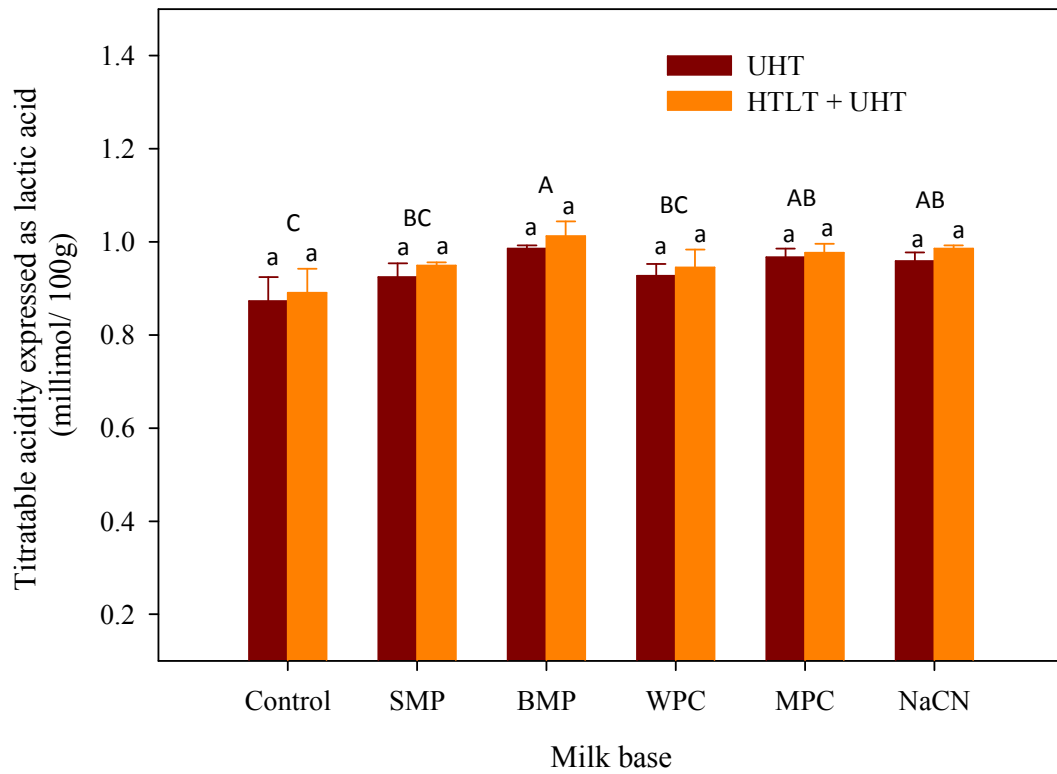


Figure 6.4: Comparison of final titratable acidity for set yoghurt for MIT product produced using yoghurt milk base fortified with SMP, BMP, WPC, MPC or NaCN and single (UHT) and double heat treatment (HTLT and UHT). Internal bar = \pm (2 x sample standard deviation).
A, etc Different letters represent significant differences ($p < 0.05$) between fortifying materials.
^a Different letters would represent significant differences ($p < 0.05$) between heat treatments.

CHAPTER SIX: THE EFFECT OF MILK BASE FORTIFICATION AND HEAT TREATMENT ON SET YOGHURT AS A MADE-IN-TRANSIT (MIT) PRODUCT

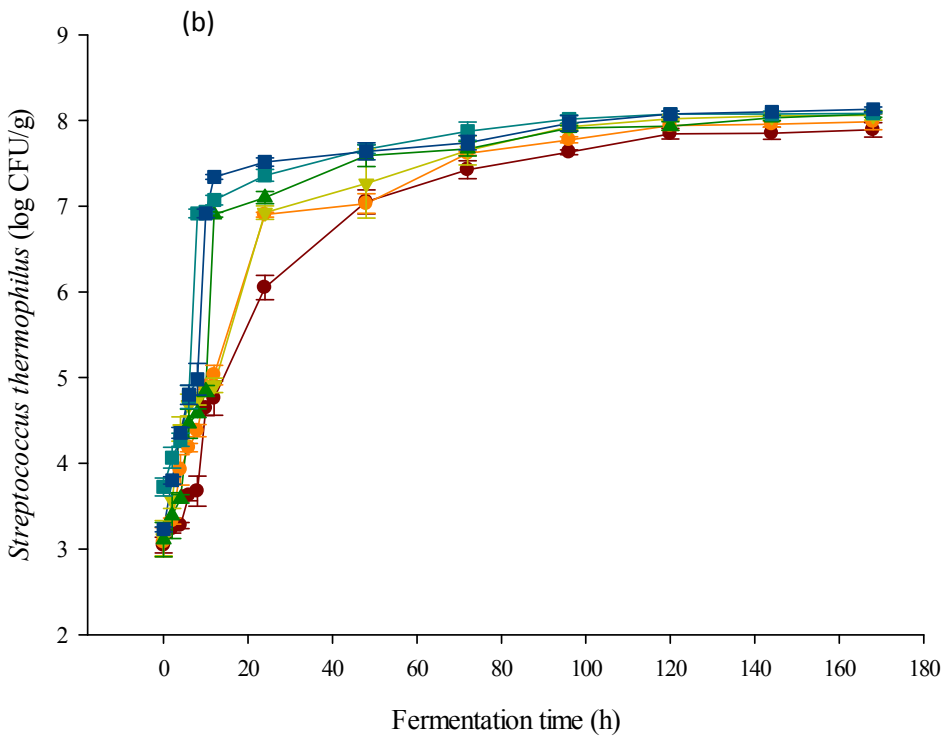
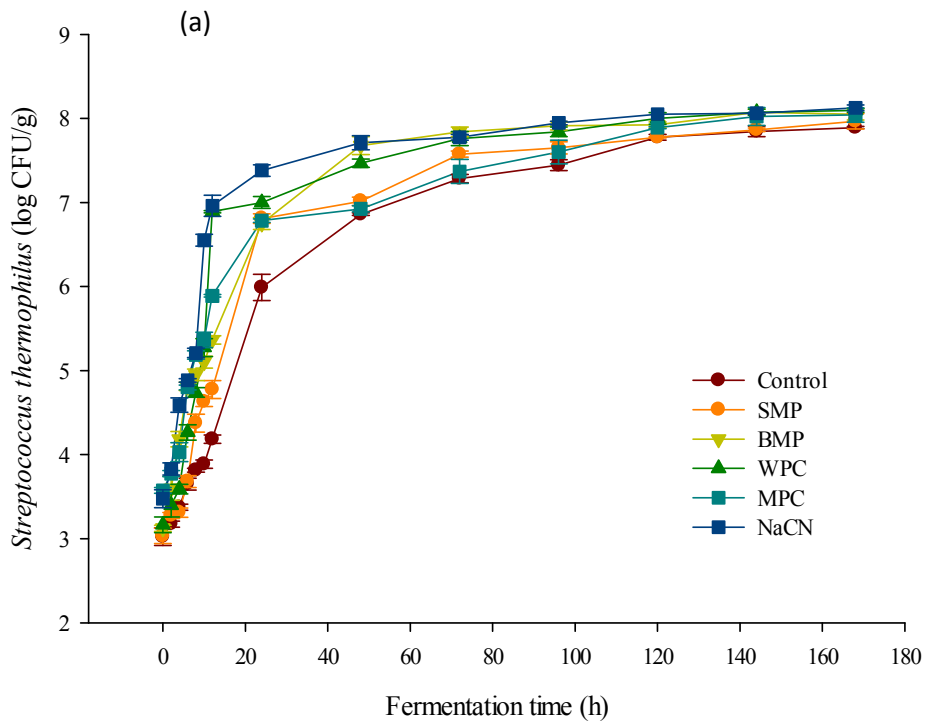


Figure 6.5: *Streptococcus thermophilus* STM5 growth in set yoghurt for MIT product produced using yoghurt milk base fortified with SMP, BMP, WPC, MPC or NaCN and (a) single (UHT sterilization) or (b) double (HTLT plus UHT sterilization) heat treatment. Internal bar = \pm (2 x sample standard deviation).

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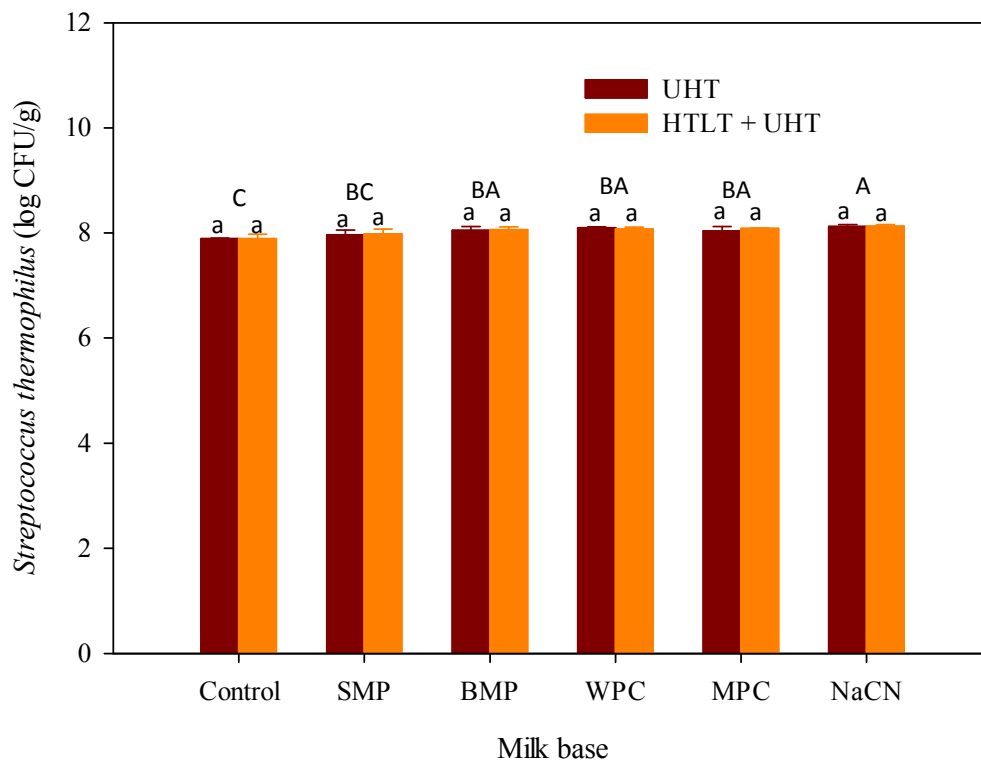


Figure 6.6: Comparison of final count of *Streptococcus thermophilus* STM5 for set yoghurt for MIT product produced using yoghurt milk base fortified with SMP, BMP, WPC, MPC or NaCN and single (UHT) and double heat treatment (HTLT and UHT). Internal bar = \pm (2 x sample standard deviation).

^{A, etc} Different letters represent significant differences ($p < 0.05$) between fortifying materials.

^a Different letters would represent significant differences ($p < 0.05$) between heat treatments.

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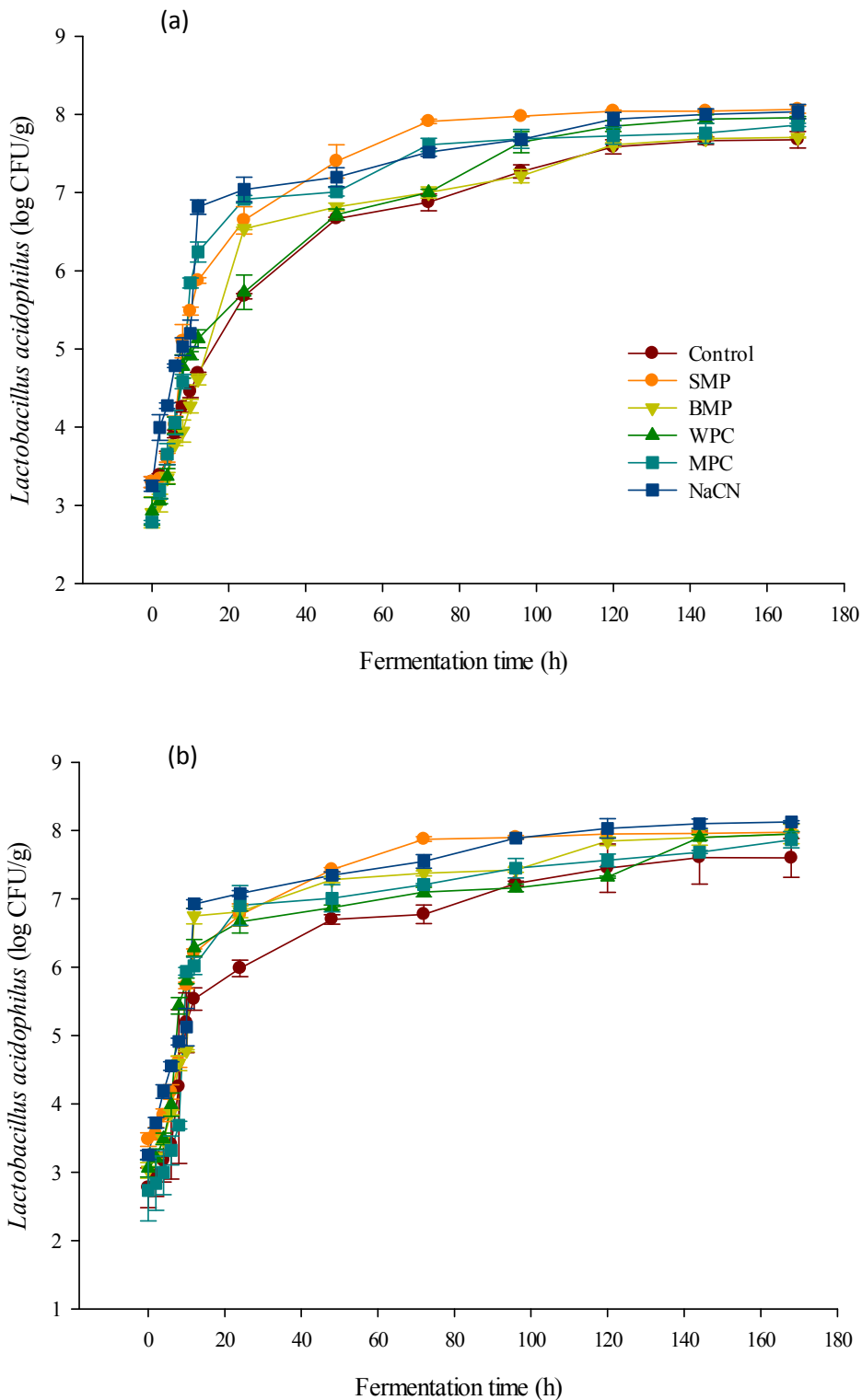


Figure 6.7: *Lactobacillus acidophilus* LA5 count in set yoghurt for MIT product produced using yoghurt milk base fortified with SMP, BMP, WPC, MPC or NaCN and (a) single (UHT sterilization) or (b) double (HTLT plus UHT sterilization) heat treatment. Internal bar = \pm (2 x sample standard deviation).

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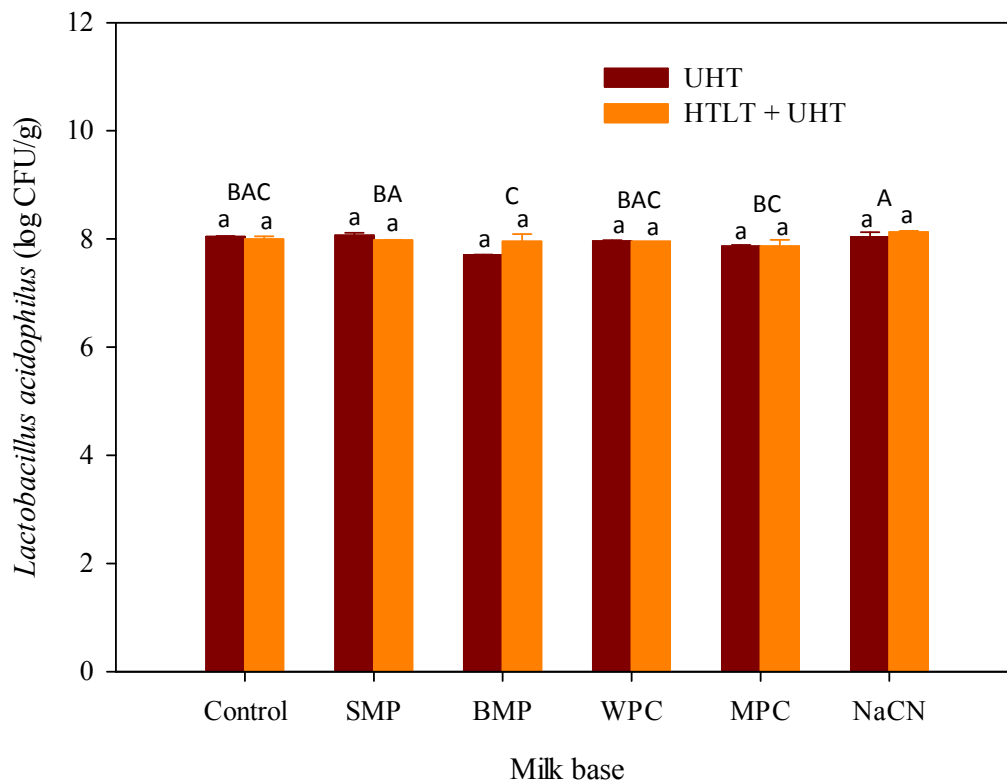


Figure 6.8: Comparison of final count of *Lactobacillus acidophilus* LA5 of set yoghurt for MIT product produced using yoghurt milk base fortified with SMP, BMP, WPC, MPC or NaCN and single (UHT) and double heat treatment (HTLT and UHT). Internal bar = \pm (2 x sample standard deviation).

A, etc Different letters represent significant differences ($p < 0.05$) between fortifying materials.

^a Different letters would represent significant differences ($p < 0.05$) between heat treatments.

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The development of yoghurt firmness during fermentation (Figure 6.9) was significantly influenced by fortifying material ($p < 0.05$), as was the final firmness ($p < 0.05$) (Table 6.3). The lowest rate of firmness development and the lowest final firmness (when firmness did develop) were observed in the control yoghurt. This was probably due to the firmness of yoghurt being highly dependent on the total solids content (Penna, Barrufaldi & Oliveira, 1997), protein content (Tamime, Kalab & Davies, 1984; Trachoo & Mistry, 1998) and type of protein (Tamime et al., 1984; Cho, Lucey & Singh, 1999). In this study the total solids content (see section 6.3.3) and the protein composition varied, while the protein content was 5.0% for all samples except the control (4.0%).

Heat treatment had no effect on firmness development during fermentation (except in the case of the control) but significantly affected the final firmness (Figure 6.10 and Table 6.3). Firmness developed in the control yoghurt only on double heat treatment; apparently, there was no gel development as a result of the single heat treatment. Fortification with SMP gave the smallest improvement in firmness compared with the control, while NaCN gave the biggest, followed by MPC (Figure 6.9). This finding is consistent with Damin et al. (2009) who found that yoghurts fortified with NaCN were firmer than those fortified with SMP and WPC. It is also in line with the findings of Guzman-Gonzalez, Morais and Amigo (2000) who reported that yoghurt containing caseinate was firmer than yoghurts containing other dairy ingredients (skim milk powder and skim milk concentrates). Modler and Kalab (1983) reported that yoghurt fortified with caseinate had a stronger gel than that fortified with MPC and SMP.

There was a significant interaction between fortifying material and heat treatment in terms of their effects on final yoghurt firmness ($p < 0.05$, Table 6.3). The double heat treatment resulted in firmer gels for yoghurts fortified with SMP and WPC, but weaker gels for those fortified with BMP, MPC and NaCN. Remeuf, Mohammed, Sodini and Tissier (2003) stated that the effect of heating (90°C for 5 min) was negligible for NaCN but not for WPC, as heating in that case leads to a high level of protein cross-linking within the gel network, increasing yoghurt firmness and water holding capacity.

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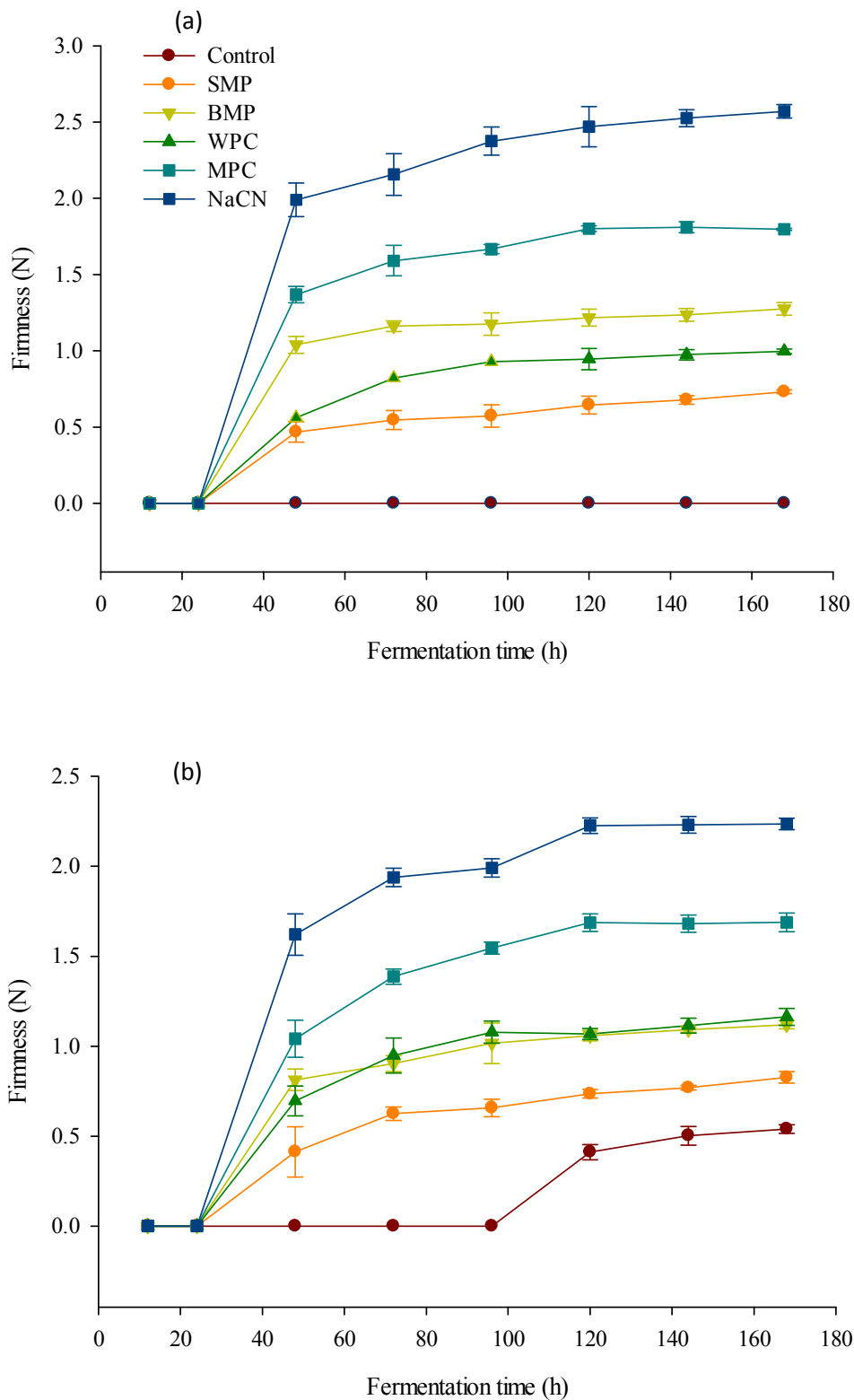


Figure 6.9: Firmness of set yoghurt for MIT product produced using yoghurt milk base fortified with SMP, BMP, WPC, MPC or NaCN and (a) single (UHT sterilization) or (b) double (HTLT plus UHT sterilization) heat treatment. Internal bar = \pm (2 x sample standard deviation).

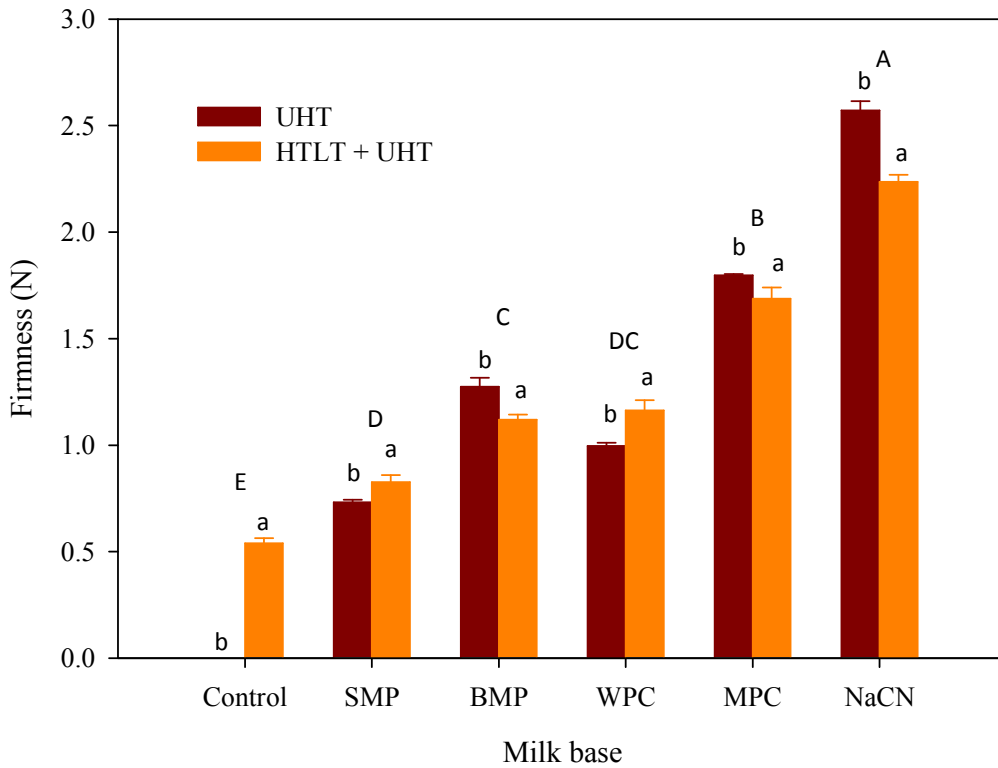


Figure 6.10: Final firmness comparison of set yoghurt for MIT product produced using yoghurt milk base fortified with SMP, BMP, WPC, MPC or NaCN and single (UHT) and double heat treatment (HTLT and UHT). Internal bar = \pm (2x sample standard deviation).

^{A, etc} Different letters represent significant differences ($p < 0.05$) between fortifying materials.

^{a, etc} Different letters represent significant differences ($p < 0.05$) between heat treatments.

6.5 Conclusion

Fortification of a yoghurt milk base with a high protein dairy ingredient (SMP, BMP, WPC, MPC or NaCN) can considerably improve yoghurt texture (measured as an instrumentally determined firmness) for low temperature-long time fermentation conditions (25°C, 168 h). A double heat treatment (a high temperature-long time treatment plus UHT sterilization) was essential for firmness development during fermentation of the unfortified milk base. Such treatment, compared with UHT sterilization alone, improved texture for some fortifying materials (SMP and WPC) but slightly disimproved for others (BMP, MPC and NaCN). Best texture (firmness) yoghurt was obtained with NaCN and MPC fortification followed by UHT sterilization alone, although this resulted in a final yoghurt pH slightly lower than the acceptable pH range (pH 4.2 to 4.6) for yoghurt. In order to produce a made-in-transit set

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yoghurt using a milk base fortified with NaCN or MPC, an optimization to obtain suitable conditions producing acceptable final pH was carried out in the next chapter (Chapter 7).

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CHAPTER SEVEN: THE EFFECT OF MILK BASE FORTIFICATION, FERMENTATION TEMPERATURE, STARTER CULTURE COMPOSITION AND INOCULUM SIZE ON SET YOGHURT AS A MADE-IN-TRANSIT (MIT) PRODUCT

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7.1 Abstract

The effects of milk base fortification, starter culture composition, inoculum size and fermentation temperature on acidification, starter culture growth and firmness of set yoghurt during the long fermentation required for a made-in-transit product were investigated. Two milk products used for fortification, milk protein concentrate and sodium caseinate, were added to a base made with reconstituted skim milk. For the starter culture the two alternatives trialled were 1) a combination of *Streptococcus thermophilus* STM5 and *Lactobacillus acidophilus* LA5 at the ratio of 1:1 (STLA) and 2) a combination of *Streptococcus thermophilus* STM5, *S. thermophilus* (ropy strain) ST10 and *Lactobacillus acidophilus* LA5 in the proportions 0.5:0.5:1 (STSTRLA). These two starter cultures were added to the yoghurt milk base at three inoculum sizes, 0.2% (v/v) (low), 0.002% (v/v) (medium low), and 0.00002% (v/v) (very low). Then, all samples were incubated at the fermentation temperatures 20, 22.5 and 25°C. In general, fortification of the skim milk base with sodium caseinate produced higher final firmness than fortification with milk protein concentrate. Inoculation with *S. thermophilus* STM5 and *L. acidophilus* LA5 produced higher (better) yoghurt firmness. Reducing the inoculum size slowed the acidification rate during 168 h of fermentation. Lowering the fermentation temperature to 22.5 or 20°C resulted in slower acidification rates and incomplete gel development; no gel developed at 20°C and incomplete gelation at 22.5°C.

7.2 Introduction

Yoghurt texture was improved when the yoghurt milk base was fortified with high protein dairy ingredients (Chapter 6). Damin, Alcântara, Nunes and Oliveira (2009) reported that protein fortification is the most important parameter influencing yoghurt texture. Following on from the work reported in Chapter 6, the two best dairy ingredients for fortification, milk protein concentrate (MPC) and sodium caseinate (NaCN), were examined in the current chapter. While improving the texture of the final product, MPC and NaCN were found to increase the acidification rate, resulting in yoghurt with a pH lower than the acceptable range (pH 4.6 to 4.2) at the end of fermentation. Manipulation of the fermentation

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parameters by lowering the fermentation temperature and inoculum size, and altering the starter culture composition were potential means of overcoming this problem (Peng et al., 2009).

In addition to milk base fortification, it was thought that incorporation of a ropy strain starter culture that produces exopolysaccharide (EPS) might also improve yoghurt texture (Sodini, et al., 2004). This would be due to the water binding ability and texture promoting properties of EPS (Broadbent, McMahon, Welker, Oberg, & Moineau, 2003; Duboc & Mollet, 2001). The findings of Jaros et al. (2002) support the use of a ropy strain to improve yoghurt texture. However, contradictory results were reported by several other researchers (Amatayakul, et al., 2006; Hassan & Frank, 1997; Hassan, et al., 1996) who found a lower firmness in yoghurt produced with a ropy strain compared with a control (non-ropy) strain.

The size of the starter inoculum can also affect the acidification process, and the physical and microstructural properties of yoghurt gels (Lee & Lucey, 2004). With a lower inoculum size, slower growth of starter culture and consequently slower acidification are observed (Sebastiani, et al., 1998).

One of the crucial factors in yoghurt fermentation is the fermentation temperature, as this may influence the acidification rate during fermentation (De Brabandere & De Baerdemaeker, 1999). When the fermentation temperature is optimal for the starter culture, in the result is rapid growth of the starter culture and rapid acidification.

The objective of the work reported in this chapter was to determine the optimum fermentation temperature, optimum starter culture composition and optimum inoculum size for producing set yoghurt as an MIT product using a skim milk base fortified with either sodium caseinate (NaCN) or milk protein concentrate (MPC). The response criteria to be used were the pH profile during fermentation, and the final pH, titratable acidity (expressed as lactic acid), starter culture count and texture (firmness) at the end of 168 h of fermentation.

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7.3 Materials and Methods

7.3.1 Experimental design

A factorial experiment with a split plot design (Table 7.1) was carried out to study the influence of various conditions on the acidification rate and texture of yoghurt. Thirty six experiments were defined according to 1) milk base fortification: MPC and NaCN, 2) fermentation temperature: 25, 22.5 and 20°C, 3) starter culture composition: STLA and STSTRLA and 4) inoculum size: 0.2, 0.002 and 0.00002% (v/v) (1:1 for STLA and 0.5:0.5:1 for STSTRLA). Inoculum composition is described below in section 7.3.2. The 36 experiments were divided into 9 blocks of experiments with each fermentation temperature a constant factor in each of three blocks (Table 7.1). The fermentation temperature was considered a hard-to-change factor; as only a single digital incubator was available, experiments with different temperatures were carried out on different days. The levels of the other factors were arranged randomly within the blocks to give all of the possible factor combinations. The blocks of experiment were carried out one at a time. Products were analysed every 2 h for the first 12 h and then every 12 h for remaining 144 h for pH, and at the end of fermentation (168 h) for titratable acidity, starter culture count and firmness.

7.3.2 Cultures

The lactic acid starter bacteria used were *Streptococcus thermophilus* STM5 (ST) and *Lactobacillus acidophilus* LA5 (LA) obtained as freeze-dried cultures, and *S. thermophilus* (ropy strain) ST10 obtained frozen. These starter cultures were supplied by the Fonterra Research Centre (Palmerston North, New Zealand). Each organism was activated twice in skim milk media (10% w/v skim milk powder in distilled water, sterilized at 110°C for 10 min) at 37 °C for 18 h each time before use (Figure 7.1). This procedure produced a consistent concentration of starter cultures of about 10^8 CFU mL⁻¹ in each starter bacteria: 8.55 ± 1.05 log cfu mL⁻¹ (log colony forming units mL⁻¹) for *S. thermophilus* STM5, 8.11 ± 0.30 log cfu mL⁻¹ for *L. acidophilus* LA5 and 8.33 ± 0.25 log cfu mL⁻¹ for *S. thermophilus* (ropy strain) confirmed by plate counting.

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Table 7.1: Split-plot experiment design for investigating the effect of fortification material, fermentation temperature, starter culture composition and inoculum size on the acidification and texture of, and starter culture growth in, set yoghurt as an MIT product.

Experiments	Fermentation temperature (°C)	Inoculum size (%)	Starter culture composition	Fortifying materials
1	25	0.002	STLA	NaCN
		0.00002	STSTRLA	NaCN
		0.002	STSTRLA	MPC
		0.2	STLA	NaCN
2	22.5	0.002	STSTRLA	NaCN
		0.2	STLA	MPC
		0.00002	STSTRLA	MPC
		0.2	STLA	NaCN
3	20	0.002	STSTRLA	MPC
		0.002	STLA	NaCN
		0.00002	STSTRLA	NaCN
		0.2	STLA	MPC
4	25	0.00002	STLA	MPC
		0.2	STSTRLA	MPC
		0.00002	STLA	NaCN
		0.002	STSTRLA	NaCN
5	20	0.2	STSTRLA	MPC
		0.002	STSTRLA	NaCN
		0.2	STLA	NaCN
		0.00002	STLA	MPC
6	20	0.00002	STSTRLA	MPC
		0.002	STLA	MPC
		0.2	STSTRLA	NaCN
		0.00002	STLA	NaCN
7	25	0.2	STSTRLA	NaCN
		0.2	STLA	MPC
		0.00002	STSTRLA	MPC
		0.002	STLA	MPC
8	22.5	0.00002	STLA	NaCN
		0.2	STSTRLA	MPC
		0.002	STLA	MPC
		0.00002	STSTRLA	NaCN
9	22.5	0.2	STSTRLA	NaCN
		0.002	STSTRLA	MPC
		0.00002	STLA	MPC
		0.002	STLA	NaCN

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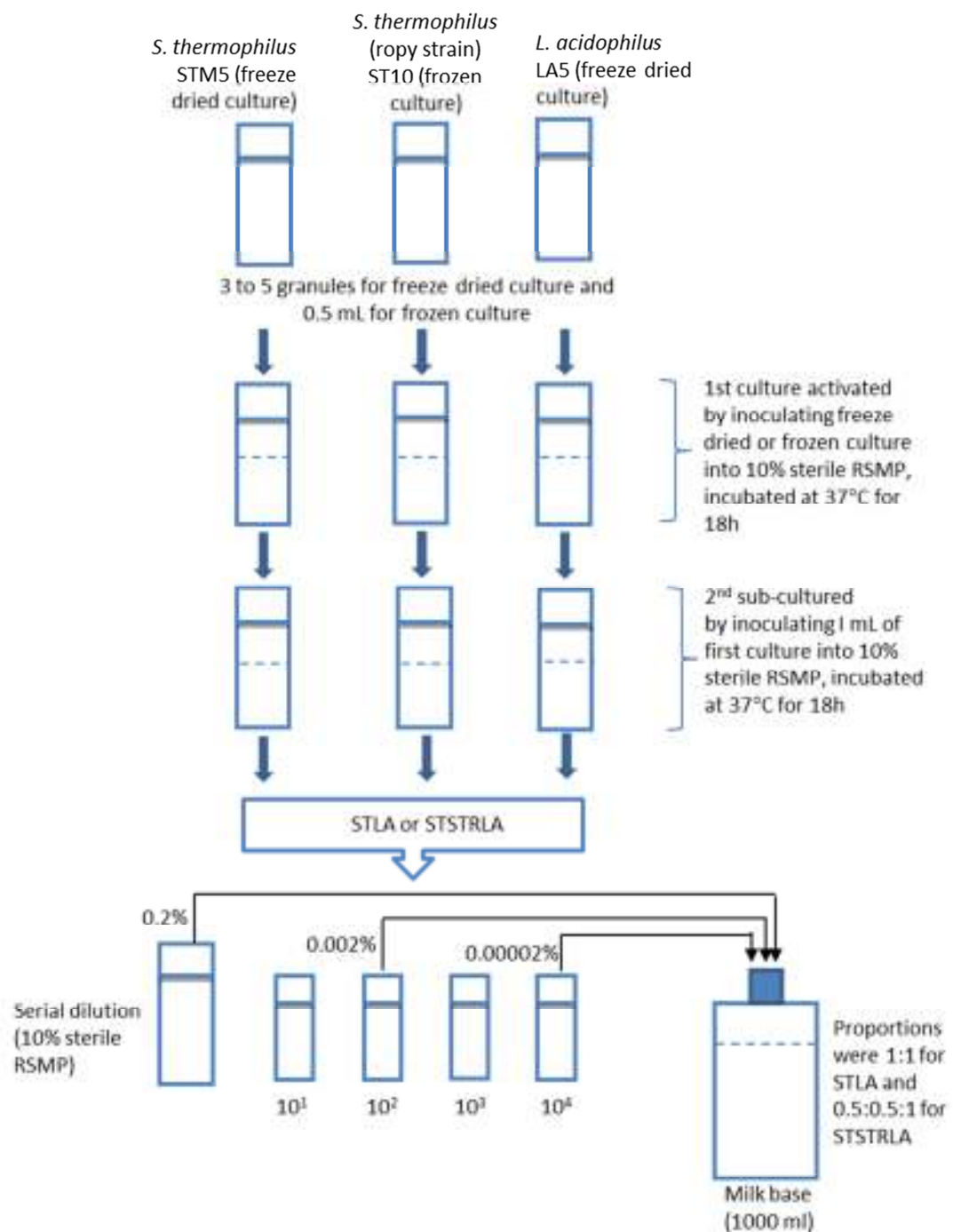


Figure 7.1: Preparation of starter cultures for set yoghurt for a MIT product using 0.2, 0.002 and 0.00002% (v/v) inocula of STLA and STSTRLA.

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7.3.3 Preparation and fortification of the yoghurt milk base

See Section 6.3.3 (Chapter Six).

Experimental yoghurt milk bases were prepared using skim milk powder (4.0% protein) fortified with MPC or NaCN to bring the final protein content to 5.0%.

7.3.4 Heat treatment of milk base

See Section 3.2.2 (Chapter Three).

7.3.5 Yoghurt processing

The milk bases was taken from the refrigerator and allowed to come to the fermentation temperature (~25°C) in the incubator. The base was then inoculated at 0.2, 0.002% or 0.00002% (v/v) with either STLA or STSTRLA. The base was shaken manually for 60 s, allowed to rest for 10 min, and then poured into sterile plastic containers (55 mm height; 40 mm diameter) (LabServ, Auckland, New Zealand). The containers were filled to a headspace of approximately 4.0 cm (sample volume ≈ 50 mL). The containers were then sealed with screw lids and placed into an incubator set at 25, 22.5 or 20°C for fermentation.

7.3.6 Measurement of pH

See Section 3.2.4 (Chapter Three).

7.3.7 Microbiological analysis

See Section 3.2.5 (Chapter Three). *S. thermophilus* ST10 (ropy strain) was enumerated using M17 agar (MERCK).

7.3.8 Determination of titratable acidity

See Section 3.2.6 (Chapter Three).

7.3.9 Measurement of firmness

See Section 3.2.7 (Chapter Three).

7.3.10 Statistical analyses

Analysis of variance was performed using MINITAB 15 (Minitab Inc., State College, PA, USA) to determine the effects of fortifying material, fermentation temperature, starter culture composition and inoculum size on pH during fermentation, and titratable acidity, starter culture growth and the firmness of the final yoghurt on 168 h of fermentation.

7.4 Results and Discussion

The effect of fortifying material, starter culture composition and inoculum size on pH profiles at the fermentation temperatures of 20°C, 22.5°C and 25°C are shown in Figures 7.2-7.4. A significant effect ($p < 0.05$) of fermentation temperature on pH profile was obtained (Table 7.2). The pH profiles of yoghurt fortified with milk protein concentrate (MPC) (Figure 7.2a) and sodium caseinate (NaCN) (Figure 7.2b) at 20°C show a slow and steady pH reduction during 168 h of fermentation. This was expected, as 20°C is the minimum growth temperature for all of the starter bacteria (Cogan, 1996; Holt, et al., 1994); it would be expected that slower growth would result in a lower rate of acidification. The rate of pH fall in yoghurt fortified with MPC and NaCN was generally faster at 22.5°C (Figure 7.3), and faster still at 25°C (Figure 7.4). The better growth of the starter culture at 25°C resulted in more lactic acid production and, in turn, a more rapid pH reduction during the fermentation.

At all fermentation temperatures (20, 22.5 and 25°C), inoculum size significantly affected ($p < 0.05$) pH profile of yoghurt (Table 7.2; Figure 7.5). The very low inoculum concentration (0.00002%) produced the slowest acidification and the highest final pHs, followed by the medium low inoculum size (0.002%) and low inoculum size (0.2%). This result is in agreement with Kristo, Biliaderis, & Tzanetakis (2003), who observed that acidification became slower with decreasing starter culture inoculum size. Starter culture composition (STLA and STSTRLA) also had a significant effect ($p < 0.05$) on the yoghurt pH profile during the 168h of fermentation (Table 7.2); the use of STSTRLA resulted in a more rapid fall in pH.

Fortifying material (MPC, NaCN) did not significantly affect the pH profile, but did have a significant ($p < 0.05$) though small effect on final pH (Table 7.2). This result is consistent with those one obtained in Chapter Six, where no significant difference ($p > 0.05$) in pH profile was found between MPC and NaCN fortification. De Brabandere and De Baerdemaeker (1999) found that dry matter fortification did not affect the pH reduction during yoghurt fermentation.

In brief, the pH profile during fermentation was dependent on the fermentation temperature, starter culture compositions and inoculum size (Table 7.2). Fermentation at 25°C produced a sigmoidal pH profile (Figure 7.4). The pH profile tended to become more linear

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with decreasing temperature. Fermentation temperatures lower than optimal are known to slow pH reduction De Brabandere and De Baerdemaeker (1999).

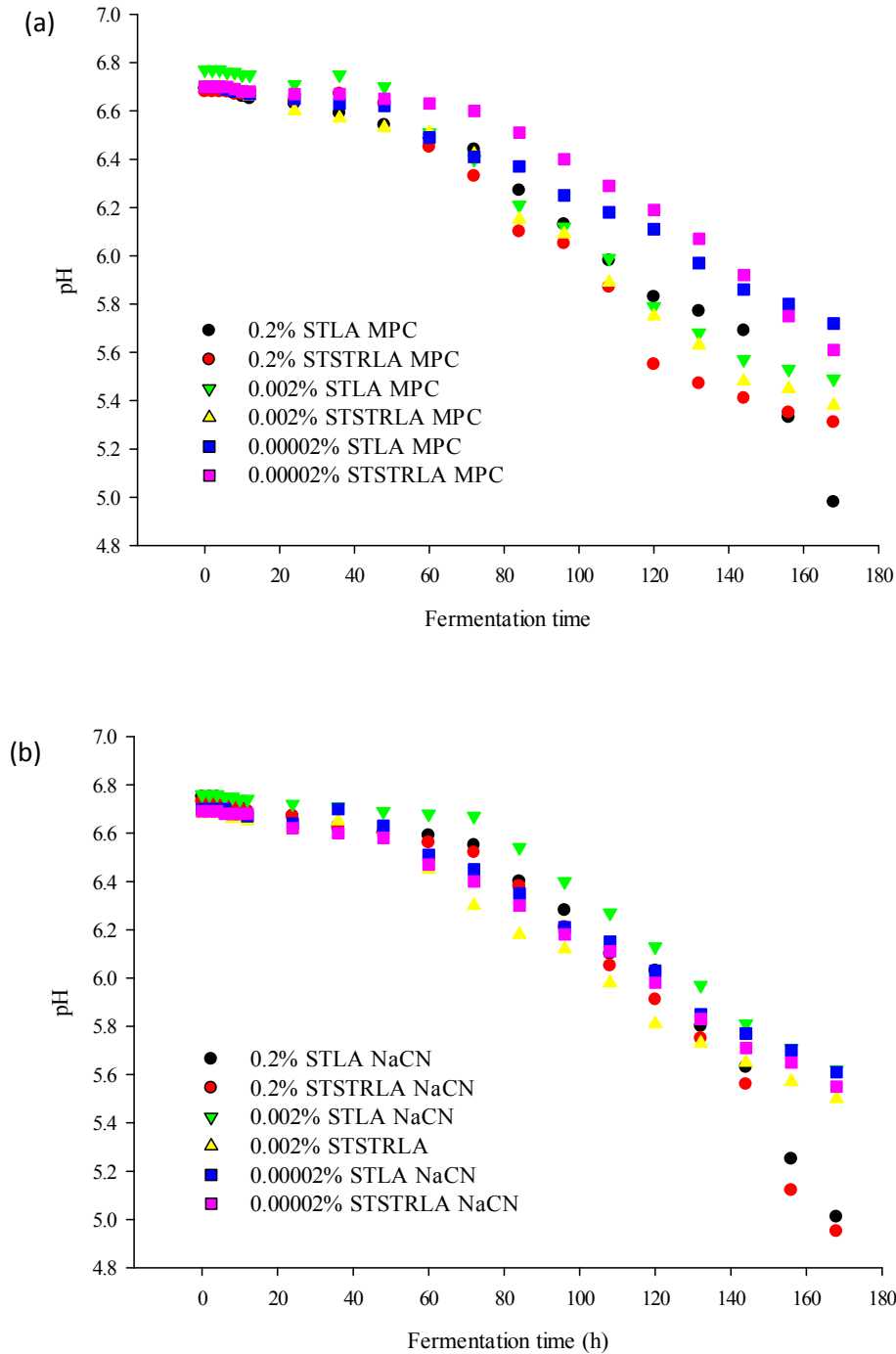


Figure 7.2: pH profiles of set yoghurt fortified with (a) milk protein concentrate (MPC) and (b) sodium caseinate (NaCN) at three different inoculum levels (0.2, 0.002 and 0.00002% (v/v)) of STLA and STSTRLA at a fermentation temperature of 20°C.

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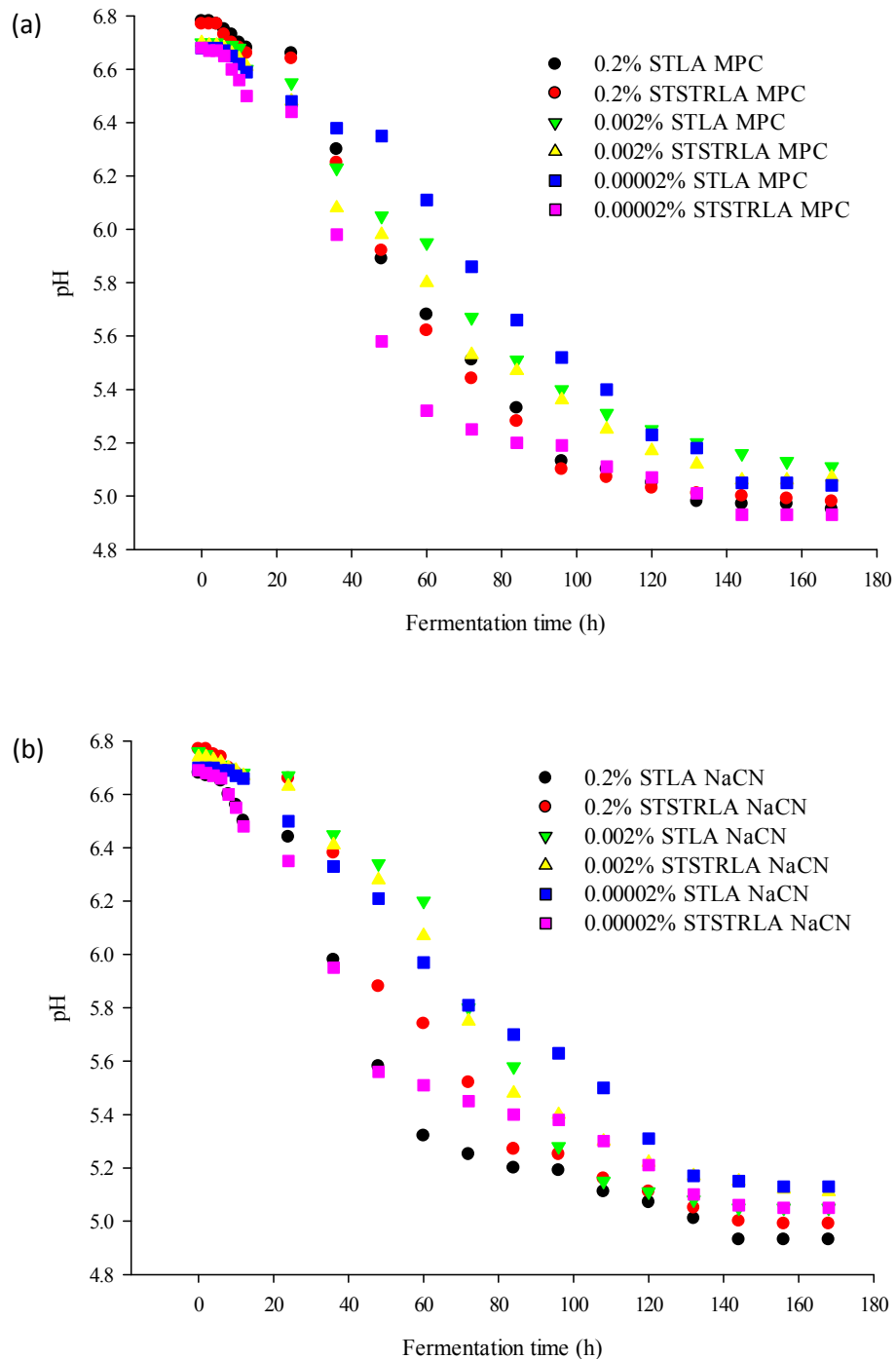


Figure 7.3: pH profiles of set yoghurt fortified with (a) milk protein concentrate (MPC) and (b) sodium caseinate (NaCN) at three different inoculum levels (0.2, 0.002 and 0.00002% (v/v)) of STLA and STSTRLA at a fermentation temperature of 22.5°C.

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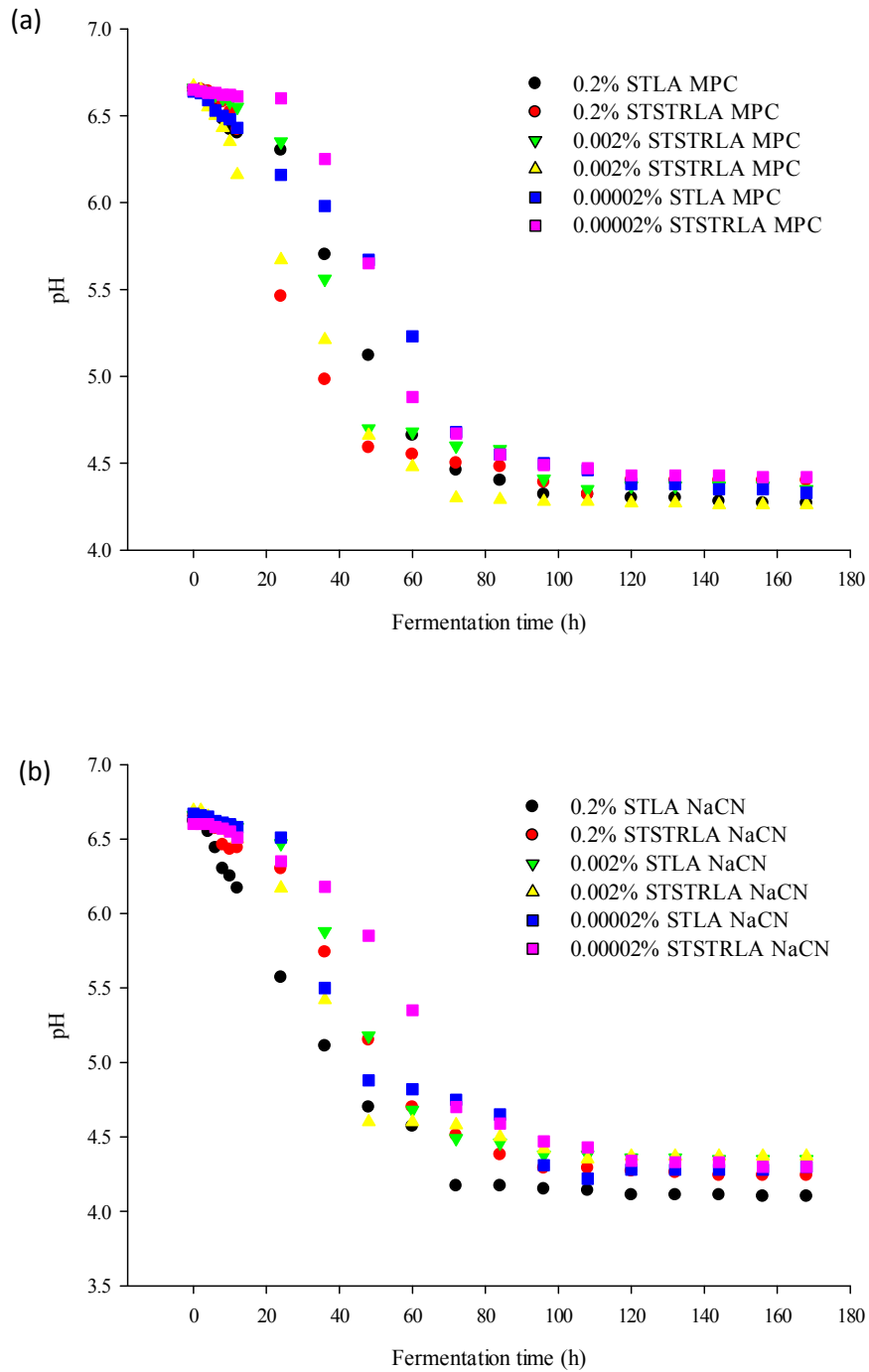


Figure 7.4: pH profiles of set yoghurt fortified with (a) milk protein concentrate (MPC) and (b) sodium caseinate (NaCN) at three different inoculum levels (0.2, 0.002 and 0.00002% (v/v)) of STLA and STSTRLA at a fermentation temperature of 25°C.

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Table 7.2: Effects of fermentation temperature (FT), inoculum size (IS), starter culture (SC) and fortifying material (FM) on pH profile during fermentation, and the final pH, titratable acidity, starter culture count and firmness of yoghurt at 168 h of fermentation.

Effect	df	pH profile			Final pH			Titratable acidity			<i>S. thermophilus</i>			<i>S. thermophilus</i> (ropy)			<i>L. acidophilus</i>			Firmness		
		MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	
Fermentation temperature (FT)	2	59.610	0.000	2.6952	0.000	0.1752	0.000	5.7329	0.000	0.4405	0.004	1.4073	0.00	11.129	0.000							
Inoculum size (IS)	2	0.8324	0.000	0.1246	0.000	0.0041	0.000	0.4549	0.000	0.4763	0.003	0.3488	0.00	0.0128	0.255							
Starter culture (SC)	1	0.1717	0.002	0.0000	0.954	0.0000	0.815	0.0009	0.778	n.a.	n.a.	0.0241	0.03	0.1362	0.001							
Fortification material (FM)	1	0.0227	0.266	0.0950	0.002	0.0032	0.004	0.0879	0.012	0.0162	0.341	0.0182	0.06	0.1750	0.000							
FT x IS	4	0.2032	0.000	0.0271	0.023	0.0016	0.004	0.0665	0.004	0.0111	0.583	0.0153	0.03	0.0128	0.252							
FT x SC	2	0.0927	0.007	0.0072	0.383	0.0001	0.673	0.0134	0.322	n.a.	n.a.	0.0030	0.52	0.1362	0.000							
FT x FM	2	0.0284	0.213	0.1425	0.000	0.0021	0.005	0.0364	0.063	0.0073	0.626	0.0101	0.13	0.1750	0.000							
IS x SC	2	0.2289	0.000	0.0073	0.381	0.0000	0.871	0.0480	0.031	n.a.	n.a.	0.0062	0.28	0.0051	0.562							
IS x FM	2	0.1893	0.000	0.0538	0.005	0.0001	0.676	0.0044	0.674	0.0030	0.812	0.0169	0.04	0.0015	0.838							
SC x FM	1	0.1638	0.003	0.0000	0.923	0.0000	0.696	0.0000	0.951	n.a.	n.a.	0.0084	0.18	0.0535	0.024							

n.a. not analyzed

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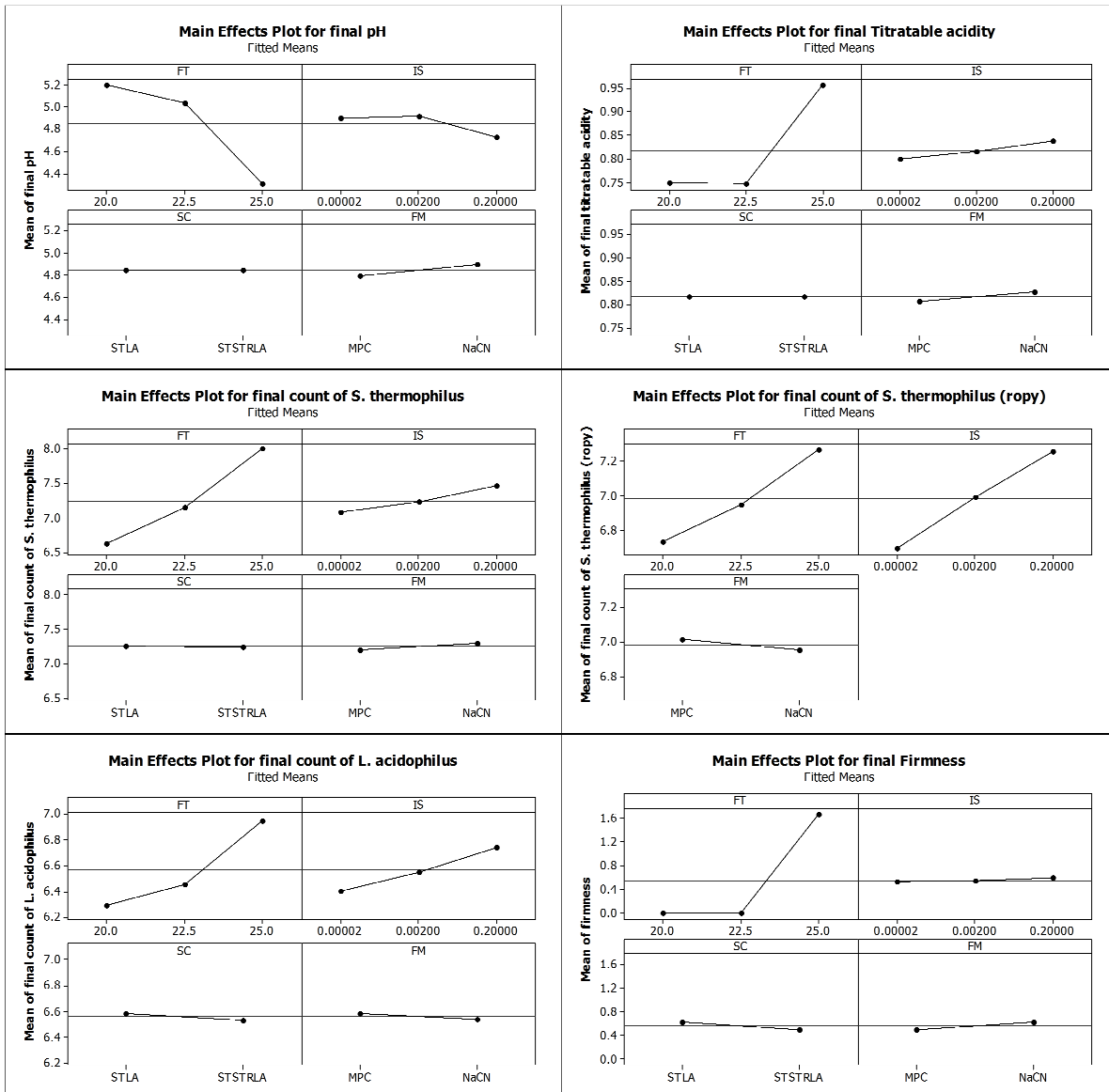


Figure 7.5: Main effects of fermentation temperature (FT), inoculum size (IS), starter culture composition (SC) and fortifying material (FM) for final pH, titratable acidity, *S. thermophilus* count, *S. thermophilus* (ropy) count, *L. acidophilus* count and firmness of set yoghurt as an MIT product.

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The rate of acidification can be expressed as $[\Delta\text{pH} = \text{pH}_{\text{zero time}} - \text{pH}_{\text{at time}}]$ as a function of time (Ayad et al., 2004). Figure 7.6 shows the acidification rates observed in the 36 yoghurts produced in this study. The rate curves fall into three groups according to fermentation temperature. This confirms the significant ($p < 0.05$) effect of fermentation temperature for yoghurt manufacture (Table 7.2 and Figure 7.5). Only the pH of yoghurt fermented at 25°C fell to the acceptable pH for yoghurt (pH 4.2 to 4.6).

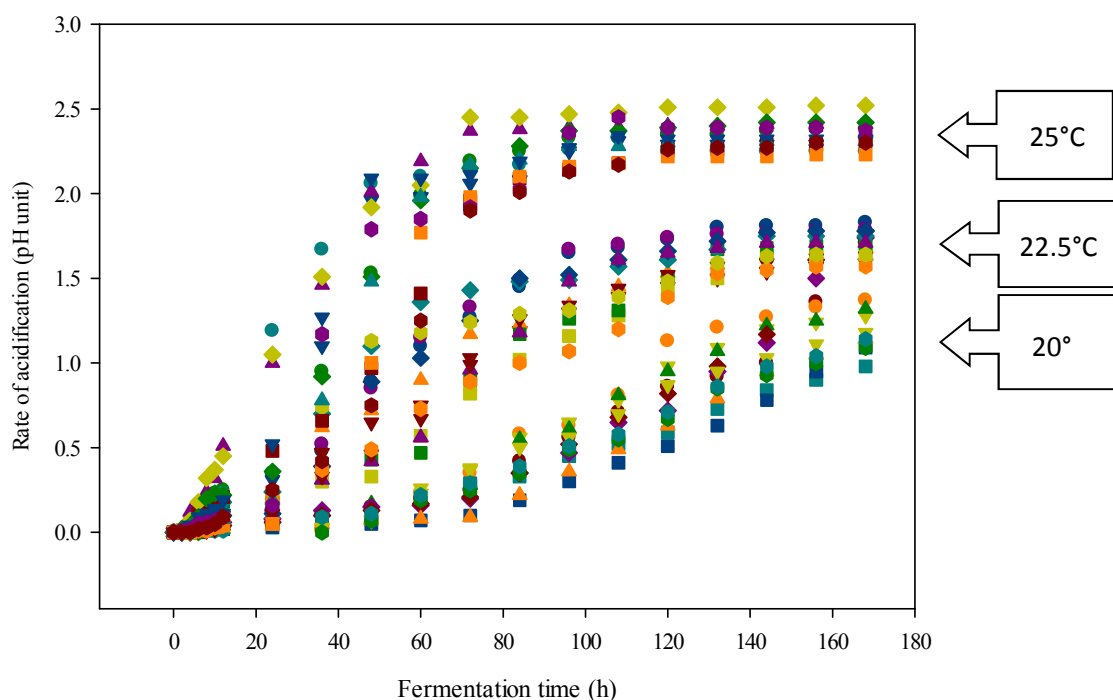


Figure 7.6: Rate of acidification, expressed as $[\Delta\text{pH} = \text{pH}_{\text{zero time}} - \text{pH}_{\text{at time}}]$ as a function of time, in set yoghurt as an MIT product fortified with milk protein concentrate (MPC) or sodium caseinate (NaCN), inoculated at three inoculum levels (0.2, 0.002 or 0.00002% (v/v)) of STLA or STSTRLA and fermented at 20, 22.5 or 25°C.

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A comparison of the final pHs observed in all 36 experiments is shown in Figure 7.7. The final pH was in the range 4.95 to 5.72 at the fermentation temperature of 20°C, 4.93 to 5.13 for 22.5°C and 4.10 to 4.40 for 25°C. The final pH at 20°C fell into two groups, whereas at the other temperatures, the final pH fell into only one group at each temperature. Higher inoculum size is tending to compensate for the fact that the fermentation temperature of 20°C is the lower limit for the bacteria growth. At the higher fermentation temperatures, inoculum size has little effect on final pH. At 20 and 22.5°C, the final pH was above the acceptable range for yoghurt, pH 4.2 to 4.6 (Tamime & Robinson, 2007; Soukoulis et al., 2007). However, at 25°C the pH obtained was within this acceptable range. This result is confirmed by the statistical analysis results in Table 7.2 and Figure 7.5, which shows that fermentation temperature significantly ($p < 0.05$) affected the final pH at 168 h of fermentation. This was probably due to the starter culture growing better at 25°C than at 20 or 22.5°C. The inoculum size and fortifying material were also found to have significant ($p < 0.05$) effects on the final pH of yoghurt, but these effects were smaller than that of fermentation temperature (Figure 7.5). Starter composition had no significant effect ($p < 0.05$) on the final pH of the yoghurt.

A comparison of titratable acidity at 168 h of fermentation for the 36 experiments is shown in Figure 7.8. The final titratable acidity for yoghurt was in the range 0.7110 to 0.7920 mmol (100 g)⁻¹ at the fermentation temperature of 20°C, 0.7050 to 0.8010 mmol (100 g)⁻¹ at 22.5°C and 0.9360 to 0.9990 mmol (100 g)⁻¹ at 25°C. The final titratable acidity for all yoghurts was above the minimum requirement for yoghurt which is 0.7 mmol (100 g)⁻¹ (IDF, 1992). Fermentation temperature significantly ($p < 0.05$) affected yoghurt titratable acidity at 168 h of fermentation (Table 7.2). Inoculum size and fortifying material were found also to significantly ($p < 0.05$) effect the titratable acidity of yoghurt and, as for final pH, these two factors had smaller effects than that of fermentation temperature (Figure 7.5). Starter culture had no significant effect ($p < 0.05$) on titratable acidity. Amatayakul et al. (2006) reported that starter culture type did not affect lactic acid concentration.

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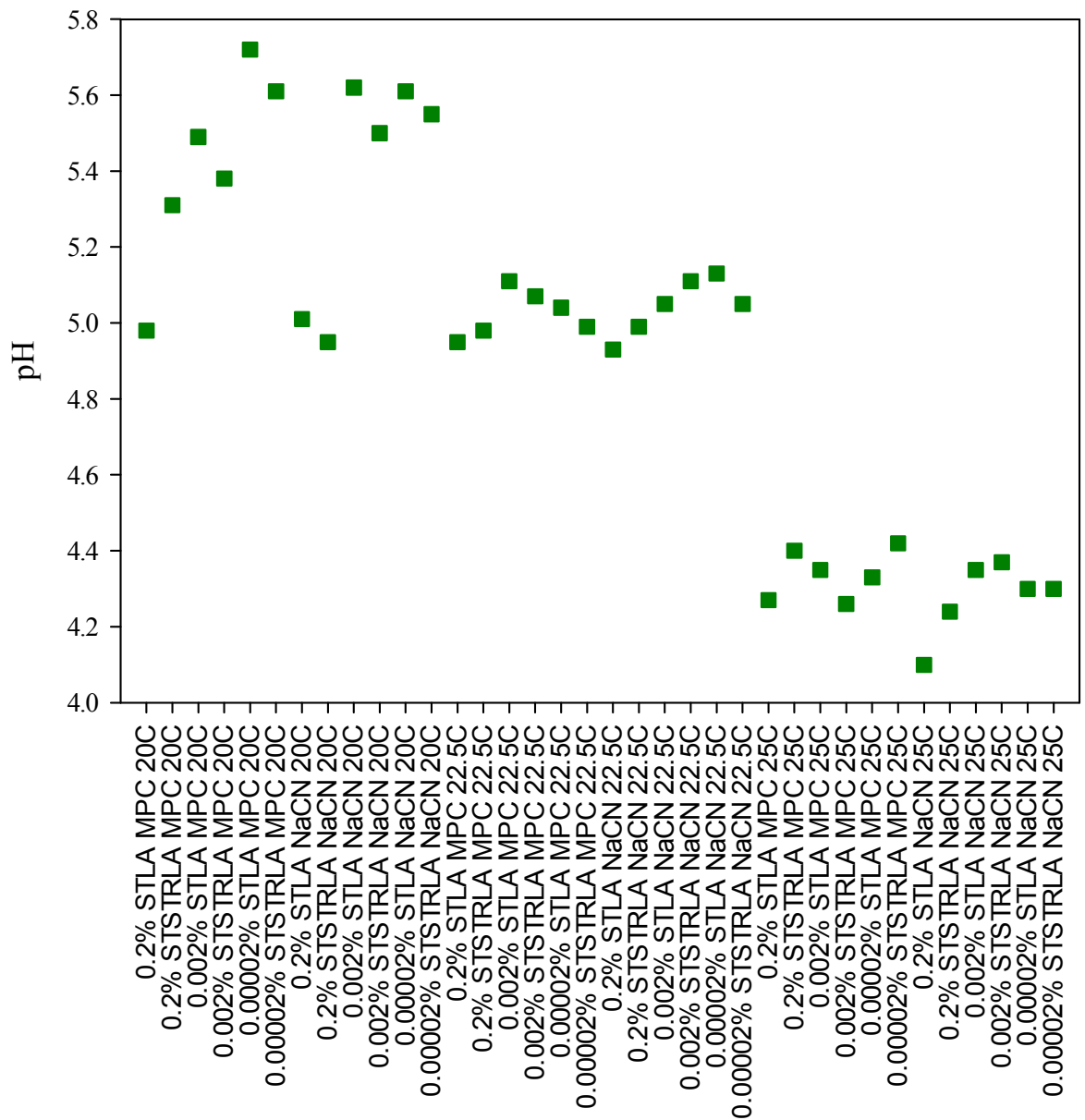


Figure 7.7: Final pH of set yoghurt as an MIT product fortified with milk protein concentrate (MPC) and sodium caseinate (NaCN) and inoculated with three inoculums levels (0.2, 0.002 and 0.00002% (v/v)) of STLA and STSTRLA at fermentation temperatures of 20, 22.5 and 25°C.

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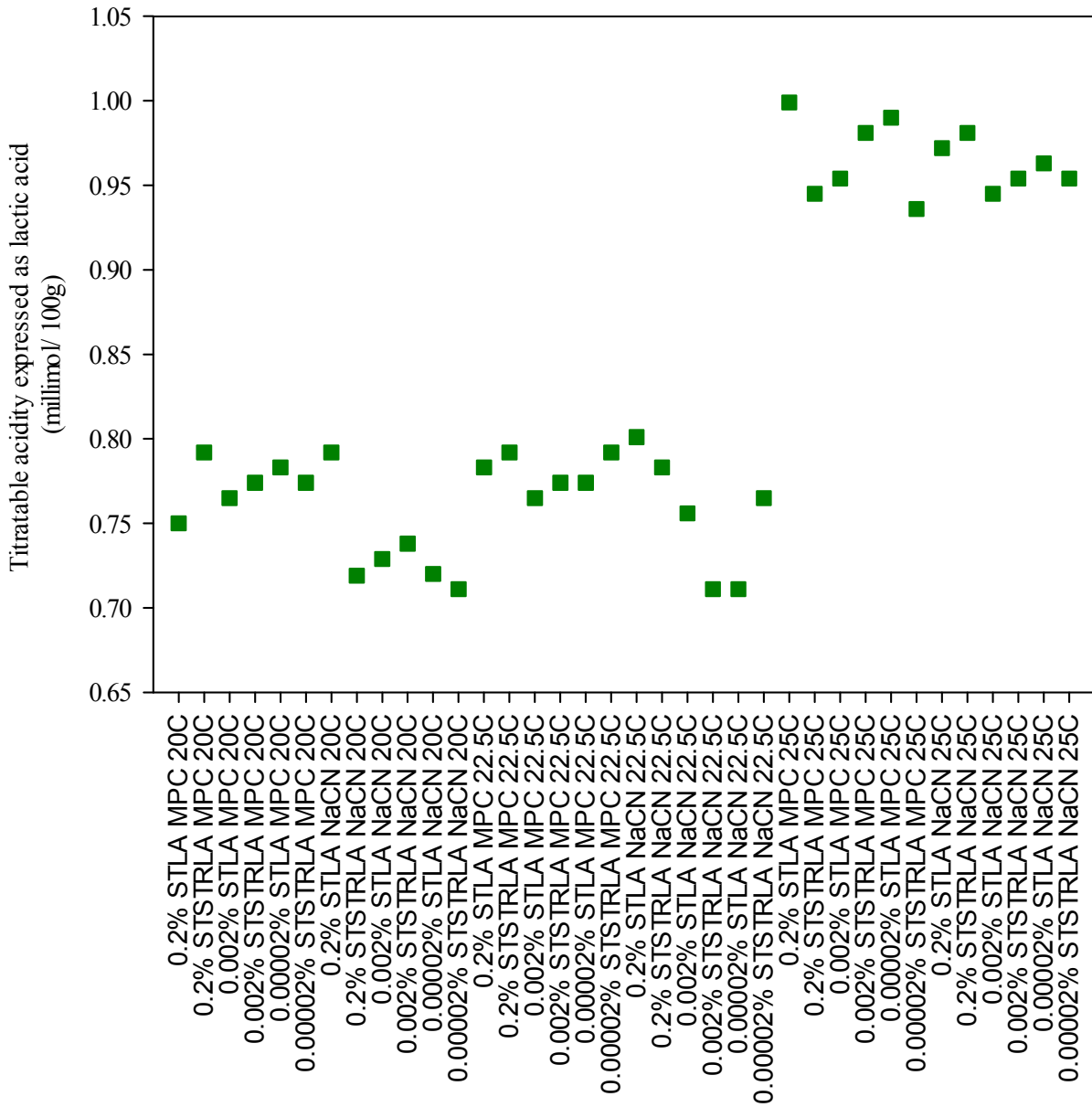


Figure 7.8: Final titratable acidity of set yoghurt as an MIT product fortified with milk protein concentrate (MPC) and sodium caseinate (NaCN) and inoculums levels (0.2, 0.002 and 0.00002% (v/v)) of STLA and STSTRLA at fermentation temperatures of 20, 22.5 and 25°C.

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The comparison of *Streptococcus thermophilus* STM5 count on 168 h of fermentation for the 36 experiments is shown in Figure 7.9. The *S. thermophilus* STM5 count for yoghurt was in the range of 6.62 to 6.89 log cfu g⁻¹ for STLA composition and 6.35 to 6.79 log cfu g⁻¹ for STSTRLA composition at fermentation temperature of 20°C, 6.71 to 7.67 log cfu g⁻¹ for STLA composition and 6.85 to 7.55 log cfu g⁻¹ for STSTRLA composition at 22.5°C and 7.75 to 8.22 log cfu g⁻¹ for STLA composition and 7.95 to 8.16 log cfu g⁻¹ for STSTRLA composition at 25°C. A very small difference of *S. thermophilus* STM5 count in two starter culture composition; STLA and STSTRLA was obtained. Confirming this, the starter culture compositions had no significant effect ($p < 0.05$) on the *S. thermophilus* STM5 count. Similar outcome was found by Amatayakul et al. (2006). At the end of fermentation (168 h), the *S. thermophilus* STM5 counts for some yoghurt fermented at 22.5°C and all yoghurt fermented at 25°C were found to be above the minimum requirement for starter culture count, 10⁷ cfu g⁻¹ in Codex Standard (FAO/WHO, 2003). This was not the case for yoghurt fermented at 20°C. The *S. thermophilus* STM5 count is significantly ($p < 0.05$) affected by the fermentation temperature, inoculum size and fortification materials (Table 7.2 and Figure 7.5), with the bigger effect was observed for the fermentation temperature.

The *Streptococcus thermophilus* (ropy strain) ST10 counts at 168 h of fermentation for all 36 experiments are shown in Figure 7.10. The *S. thermophilus* (ropy strain) ST10 count for yoghurt was in the range 6.40 to 7.05 log cfu g⁻¹ at the fermentation temperature of 20°C, 6.55 to 7.25 log cfu g⁻¹ at 22.5°C and 6.95 to 7.57 log cfu g⁻¹ at 25°C. The count was significantly ($p < 0.05$) affected by fermentation temperature and inoculum size, while fortifying material had no effect (Table 7.2 and Figure 7.5).

The *Lactobacillus acidophilus* LA5 count on 168 h of fermentation for 36 experiments is shown in Figure 7.11. The *L. acidophilus* LA5 count for yoghurt was in the range of 6.12 to 6.52 log cfu g⁻¹ for STLA composition and 6.05 to 6.50 log cfu g⁻¹ for STSTRLA composition for a fermentation temperature of 20°C, 6.15 to 6.68 log cfu g⁻¹ for STLA composition and 6.28 to 6.52 log cfu g⁻¹ for STSTRLA composition at 22.5°C and 6.77 to 7.25 log cfu g⁻¹ for STLA composition and 6.68 to 7.21 log cfu g⁻¹ for STSTRLA composition at 25°C. A small difference of *L. acidophilus* LA5 count in two starter culture composition; STLA and STSTRLA was obtained, although the effect of starter culture compositions on *L. acidophilus* LA5 was significant (Table 7.2). Yet referring to the main effect plot in Figure 7.5, the effect of starter culture

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composition was much less than the effect of fermentation temperature and inoculum size. Fortification had no significant effect ($p < 0.05$) on the count of *L. acidophilus* LA5 (Table 7.2). The final *L. acidophilus* LA5 count in all yoghurts was above the minimum requirement for *L. acidophilus* (10^6 cfu g⁻¹) in Codex Standard (FAO/WHO, 2003). Mainly, the *L. acidophilus* count was observed slightly lower than *S. thermophilus*. This is in line with Oliveira, Sodini, Remeuf, and Corrieu (2001) and Damin, Minowa, Alcântara, and Oliveira (2008).

Values of firmness at 168 h of fermentation are shown in Figure 7.12. Only yoghurt fermented at 25°C had completely gelled by the end of fermentation, with firmness in the range 1.308 to 2.29 N. Firmness was significantly ($p < 0.05$) affected by fermentation temperature, starter culture composition and fortifying material, with temperature having the biggest influence; at the fermentation temperatures 20 and 22.5°C, firmness was essentially zero. Yet, yoghurt firmness was not significantly affected by the inoculum size. A similar result was found by Ronnegard & Dejmek (1993), where they found (using oscillatory rheometry) that inoculum size has no effect on set yoghurt rigidity.

Yoghurt texture can be affected by fermentation temperature and type of starter culture (Guzel-Seydim, Sezgin & Seydim, 2005; Haque, Richardson & Morris, 2001). The non-ropy starter culture (STLA) produced firmer yoghurt than did the ropy starter culture (STSTRLA). This result is in agreement with Amatayakul et al. (2006), Hassan et al. (1996), Hess, Roberts, and Ziegler (1997), Hassan and Frank (1997) and Rohm and Kovac (1994), who found that the use of a ropy strain rather than a non-ropy strain resulted in a weaker gel than the non-ropy strain starter culture. This could be due to the incompatibility between the EPS (produced by the ropy strain) and milk proteins. EPS tend to segregate within pores (Hassan et al., 2003) and this interfere protein micelles interactions during yoghurt acidification (Robitaille et al., 2009).

Protein fortification with NaCN produced slightly better (higher) yoghurt firmness than did fortification with MPC (Figure 7.5). Damin et al. (2009) reported that fortification with NaCN resulted in a more elastic product with greater firmness than in the case of fortification with skim milk powder (SMP) or whey protein concentrate (WPC). A similar result was obtained by Tamime, Kalab, and Davies (1984), who found that firmness of yoghurt made

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with NaCN was 30% higher than that of SMP-fortified yoghurt, although the former yoghurt had a lower total solids content (12.8%, rather than 16%).

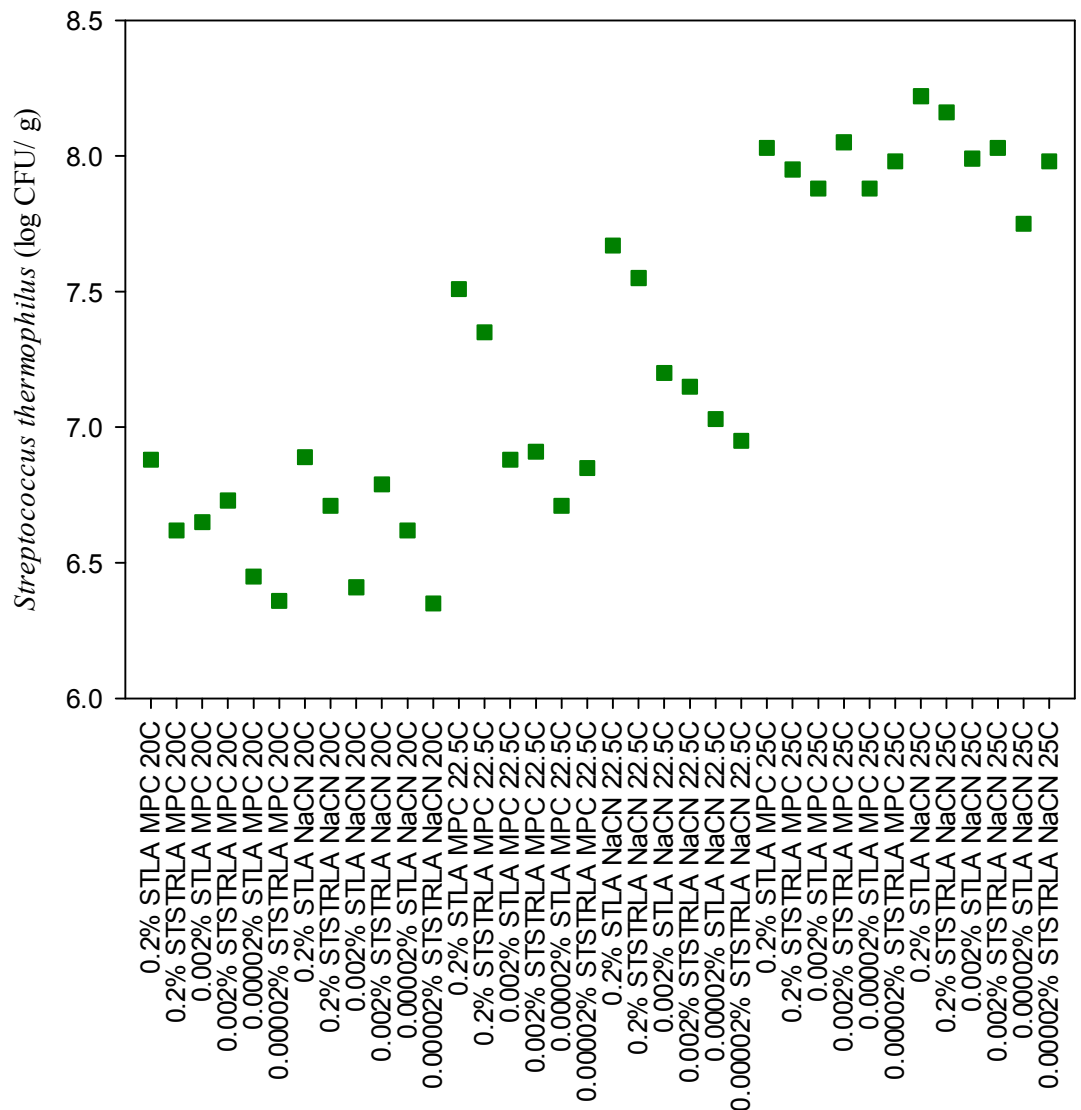


Figure 7.9: Final *S. thermophilus* STM5 count of set yoghurt as an MIT product fortified with milk protein concentrate (MPC) and sodium caseinate (NaCN) and inocula of (0.2, 0.002 and 0.00002% (v/v) of STLA and STSTRLA at fermentation temperatures of 20, 22.5 and 25°C.

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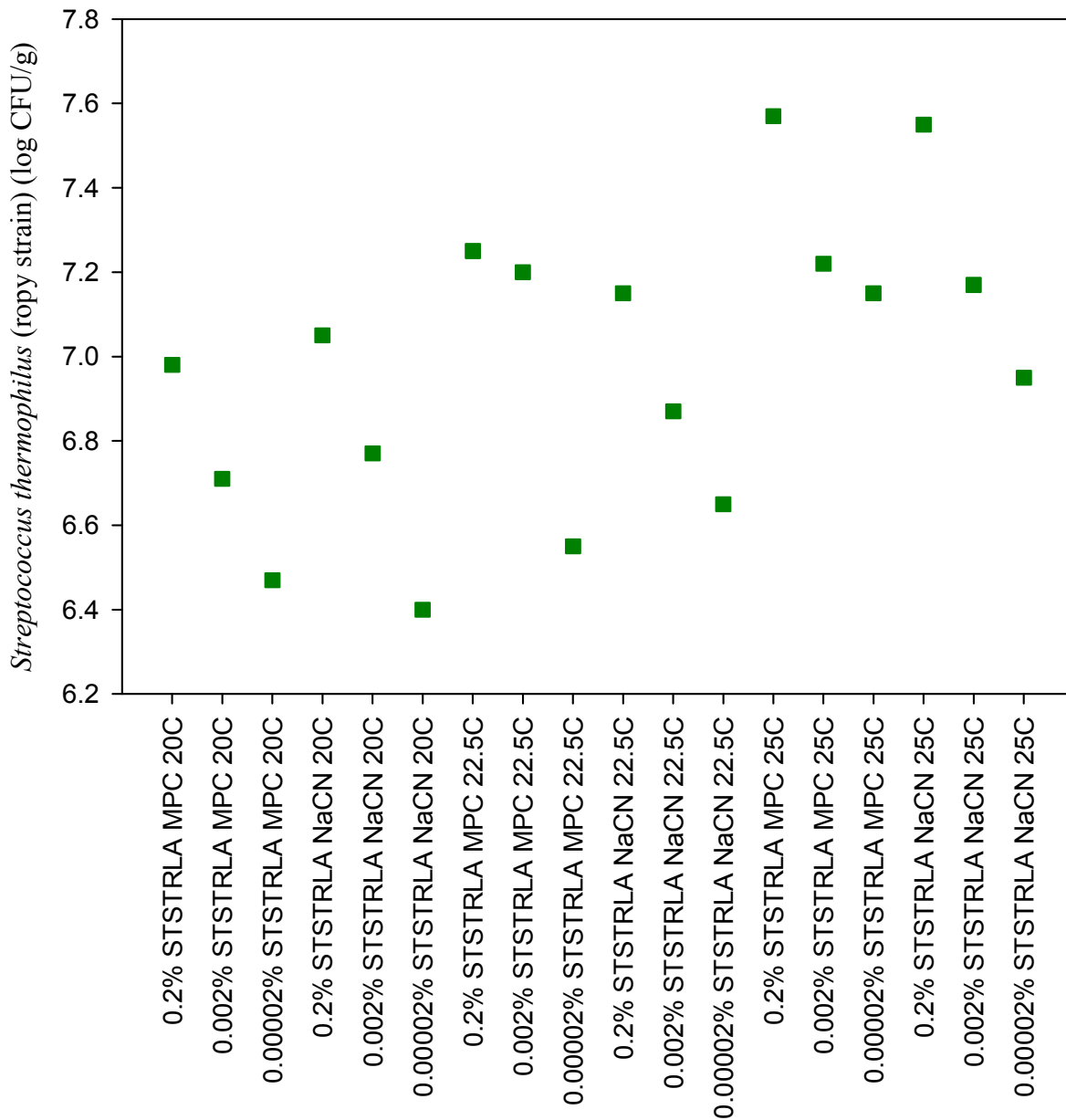


Figure 7.10: Final *Streptococcus thermophilus* (ropy strain) ST10 of set yoghurt as an MIT product fortified with milk protein concentrate (MPC) and sodium caseinate (NaCN) and inoculated at inocula (0.2, 0.002 and 0.00002% (v/v)) of STSTRLA at fermentation temperatures of 20, 22.5 and 25°C.

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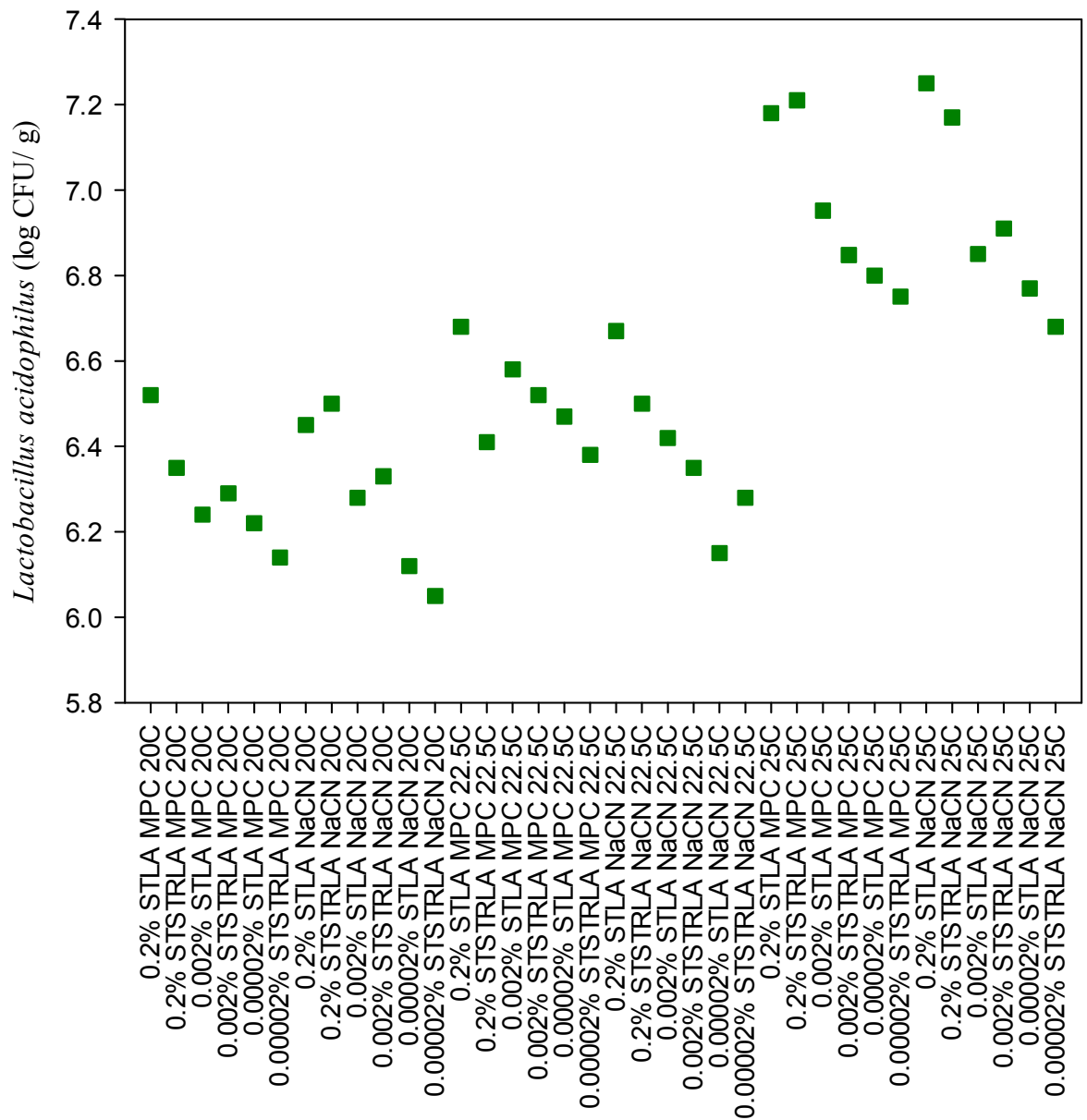


Figure 7.11: Final *L. acidophilus* LA5 count of set yoghurt as an MIT product fortified with milk protein concentrate (MPC) and sodium caseinate (NaCN) and inocula of (0.2, 0.002 and 0.00002% (v/v)) of STLA and STSTRLA at fermentation temperatures of 20, 22.5 and 25°C.

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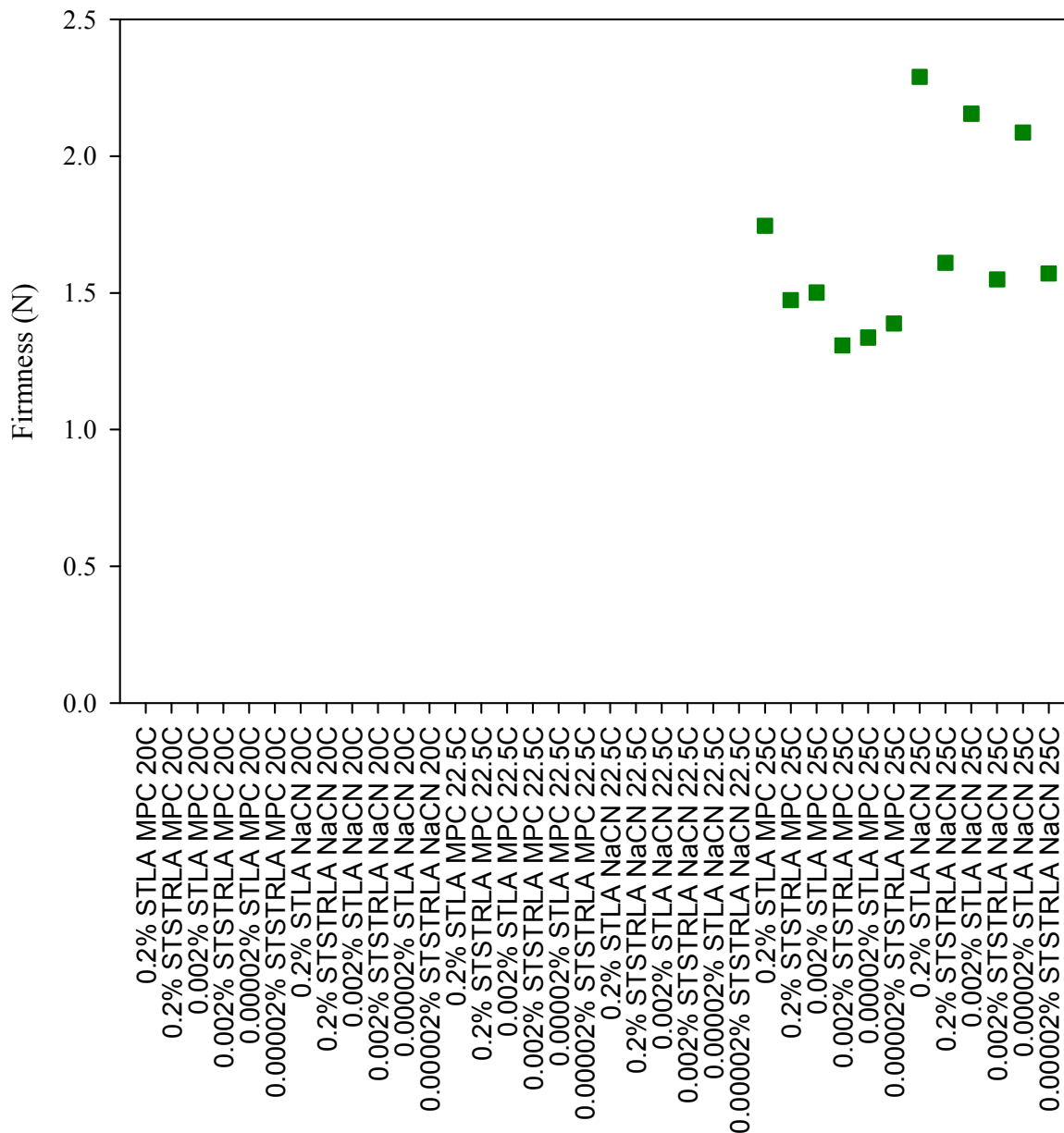


Figure 7.12: Final firmness of set yoghurt as an MIT product fortified with milk protein concentrate (MPC) and sodium caseinate (NaCN) and inoculums levels (0.2, 0.002 and 0.00002% (v/v)) of STLA and STSTRLA at fermentation temperatures of 20, 22.5 and 25°C.

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7.5 Conclusions

A fermentation temperature of 25°C was found to be the best for producing set yoghurt as a made-in-transit product. Low (0.2% (v/v)) and medium low (0.002% (v/v)) inocula may be used to inoculate the yoghurt milk base. Fortification with sodium caseinate (NaCN) generally produced a slightly firmer yoghurt texture in terms of firmness than fortification with milk protein concentrate (MPC). The starter culture containing a ropy strain *S. thermophilus* (STSTRLA) produced less firm yoghurt than that made with STLA. Based on the finding of the work described in this chapter, two preferred sets of conditions for producing MIT set yoghurt in terms of acceptable final pH and firmness, are (1) 0.002% (v/v) of STLA, fortified with NaCN and fermented at 25°C, and (2) 0.2% (v/v) of STLA, fortified with MPC and fermented at 25°C. The microstructure, sensory characteristics and storage stability of set yoghurt produced using these two sets of conditions were studied, and the results are presented in Chapter 8.

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8.1 Abstract

The effect of two manufacturing methods for MIT set yoghurt on microstructure, sensory and storage stability were investigated. Manufacturing method (1) consisted of a skim milk base fortified with milk protein concentrate (MPC) inoculated with a 0.2% (v/v) inoculum of *S. thermophilus* STM5 and *L. acidophilus* LA5 (STLA) in a ratio of 1:1. Manufacturing method (2) consisted of a skim milk base fortified with sodium caseinate (NaCN) inoculated with a 0.002% (v/v) inoculum of STLA. In both manufacturing methods, fermentation was at 25°C for 168 h. Sensory evaluation of the yoghurts manufactured by each method was compared with standard set yoghurt prepared with a skim milk base inoculated with a 2.0% (v/v) inoculum of *S. thermophilus* STM5 and *L. delbruekii* subsp. *bulgaricus* (STLB) fermented at 42°C for 12 h. The pH and microstructure were monitored throughout the 168 h of fermentation. Sensory evaluation was carried out 48 h after storage. Yoghurt was stored at 4 to 6°C after fermentation. The pH, titratable acidity, starter culture count, firmness and spontaneous syneresis were analyzed at the end of fermentation (168 h) and during 12 weeks storage. The microstructure of the set yoghurt fortified with NaCN appeared denser than yoghurt fortified with MPC. There were no significant differences ($p > 0.05$) between the two MIT set yoghurts on sensory evaluation (descriptive test) yet they were significantly different ($p < 0.05$) from the standard set yoghurt. MIT set yoghurts scored better than standard set yoghurt for overall acceptance. Yoghurts produced with 0.002% (v/v) STLA fortified with NaCN and with 0.2% (v/v) STLA fortified with MPC produced acceptable products for up to 6 weeks and 4 weeks of storage, respectively, at 4 to 6°C.

8.2 Introduction

Two formulations for MIT set yoghurt, described in Chapter 7, consisted of (1) skim milk base fortified with milk protein concentrate (MPC) inoculated with a 0.2% (v/v) inoculum (about 10^8 cfu mL⁻¹ in total) of *S. thermophilus* STM5 and *L. acidophilus* LA5 (STLA) in a ratio of 1:1 and (2) skim milk base fortified with sodium caseinate (NaCN) inoculated with a 0.002% (v/v) inoculum of STLA. Microstructure characterization, sensory evaluation and storage stability of these yoghurts is the subject of this chapter.

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The quality and acceptance of yoghurt depends upon the flavor and texture of the product (Soukoulis, et al., 2007). These two attributes are affected by the milk base composition (Rohm, 1993), heat treatment (Soukoulis, et al., 2007), starter culture and fermentation temperature (Tamime & Robinson, 2007b).

The milk base composition is likely to have the greatest effect on yoghurt texture. Fortification of the skim milk base with milk protein concentrate (MPC) or sodium caseinate (NaCN) improves the firmness of yoghurt, and syneresis decreases as the protein content increases (Schkoda, Hechler, & Hinrichs, 2001). Soukoulis et al. (2007) found yoghurt fortified with MPC produced a product with a better texture than that produced through the addition of whey protein. Isleten & Karagul-Yuceer (2006) reported that yogurts fortified with NaCN exhibited better physical and sensory properties than control yoghurts. Yoghurts made with other casein-based ingredients were found firmer, with less syneresis, than yoghurts that were fortified at the same protein level with whey protein-based ingredients (Modler et al., 1983).

The objective of this study was to evaluate the microstructure, sensory characteristics and storage stability of two MIT set yoghurts produced using two formulations: skim milk base fortified with (1) MPC and (2) NaCN.

8.3 Materials and Methods

8.3.1 *Experimental design*

An experiment was carried out to study the microstructure, storage stability and sensory characteristics of two set yoghurts compared with standard yoghurt. Yoghurt was manufactured with three different formulations (Table 8.1). The pH, titratable acidity, starter culture count, firmness and spontaneous syneresis were measured at the end of fermentation (168 h) and every 2 weeks during 12 weeks of subsequent storage. The pH was also measured every 2 h for the first 12 h of fermentation and every 12 h for the remaining 156 h. Microstructure was examined at 0 h and every 24 h during the 168 h of fermentation. Sensory evaluation was carried out after the final yoghurt had been stored at 4 to 6°C for 48 h.

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Table 8.1: Set yoghurt formulations and processing conditions

Formulation	Fortifying material	Starter culture composition	Inoculum size (% (v/v))	Fermentation temperature (°C)
1	Milk protein concentrate (MPC)	STLA ^a	0.2%	25°C
2	Sodium caseinate (NaCN)	STLA	0.002%	25°C
Standard yoghurt	None	STLB ^b	2.0%	42°C

^a *S. thermophilus* STM5 and *L. acidophilus* LA5 ^b *S. thermophilus* STM5 and *L. bulgaricus*

8.3.2 Cultures

The lactic acid starter bacteria used were *S. thermophilus* STM5 (ST) and *L. acidophilus* LA5 (LA) supplied freeze dried and *L. delbrueki* subsp. *bulgaricus* supplied frozen, by the Fonterra Research Centre (Palmerston North, New Zealand). The cultures were activated twice in skim milk media (10% w/v skim milk in distilled water, sterilized at 110°C for 10 min) at 37 °C for 18 h before use (see Figure 7.1 in Chapter 7). This procedure produced a consistent concentration of starter culture within log 10⁸ CFU ml⁻¹ in each starter bacterium: 8.75 ± 0.63 log cfu mL⁻¹ (log colony forming units mL⁻¹) for *S. thermophilus* STM5, 8.30 ± 0.75 log cfu mL⁻¹ for *L. acidophilus* LA5 and 8.27 ± 0.35 log cfu mL⁻¹ for *L. bulgaricus* confirmed by plate counting. A 1:1 mixture of STLA was then used as a combined starter culture at a level in the milk base of 0.2% and 0.002% (v/v) for MIT yoghurt, while a 1:1 mixture of STLB at 2.0% (v/v) inoculum size was used for standard yoghurt.

8.3.3 Preparation and fortification of the yoghurt milk base

Skim milk powder (SMP) was obtained from Fonterra (Fonterra Co-operative Group, New Zealand). The typical composition of SMP is as follows: lactose 54.1%, protein 33.4%, minerals 7.9%, moisture 3.8% and fat 0.8%. Experimental yoghurt milk bases were prepared using reconstituted skim milk powder (4.0% protein (w/v)) fortified with MPC or NaCN to bring the final protein content to 5.0% (w/v). For the standard set yoghurt, the milk base contained only SMP. The dried ingredients were mixed and reconstituted in distilled water with agitation using a magnetic stirring unit (Heidolph MR 3001) for 3 h at 25°C (Lee and Lucey, 2004). This reconstitution method is a gentle process and does not significantly damage the casein micelles compared with high shear and/or homogenization processes (Bock *et al.* 2008). The milk bases were stored in a refrigerator (4°C) overnight before use (Lee and Lucey, 2004).

8.3.4 Heat treatment of milk base

The reconstituted skim milk was taken from the refrigerator and transferred to the Institute of Food, Nutrition and Human Health (IFNHH) Pilot Plant for ultra-high-temperature (UHT) processing using an indirect UHT plant (Alfa Laval, Lund, Sweden). Prior to an experimental run, the plant was pre-sterilized by circulating water at 120°C outlet temperature for 1h and by treating the milk collection area with UV light. The holding temperature was set at 138°C and the flow rate at 0.833 L min⁻¹ to obtain a holding time of 6 s. These UHT conditions, 138°C for 6 s, are based on a previous study by Schmidt et al. (1985). The UHT milk was collected by hand aseptically into sterile Schott Duran bottles at 6-10°C in a laminar flow cabinet.

The yoghurt milk base for standard set yoghurt was subjected to a high-temperature long-time (HTLT) heat treatment (90°C for 30 min). This treatment was carried out batch wise using a temperature controlled water bath (Model ED, Julabo Labortechnik GMBH Germany). The treated milk was dispensed into sterile Schott Duran bottles (autoclaved at 121°C for 15min) and stored at 4°C until used for yoghurt making.

8.3.5 Yoghurt processing

Yoghurt manufacture was carried out in the Massey University IFNHH Product Development Lab. The milk was taken from the refrigerator and allowed to come to the fermentation temperature (~25°C for MIT set yoghurt and ~42°C for standard set yoghurt) in an incubator. The UHT milk base was then inoculated with 0.2 and 0.002% (v/v) starter culture at ratio 1:1 of *S. thermophilus* STM5 and *L. acidophilus* LA5 (STLA) for milk bases fortified with MPC and NaCN, respectively. The HTLT treated milk for standard set yoghurt was inoculated with 2.0% (v/v) of a starter culture of *Streptococcus thermophilus* STM5 and *Lactobacillus delbrueki* subsp. *bulgaricus* in the ratio 1:1 (STLB). The milk base was shaken manually for 60 s and left in the fermentation bottle for 10 min after inoculation before being poured into sterile plastic containers (55 mm height; 40 mm diameter) (LabServ, Auckland, New Zealand). The containers were filled to a headspace of approximately 4.0 cm (sample volume ≈ 50 mL). The containers were then sealed with screw lids and placed in an incubator set at the fermentation temperature of 25°C for MIT set yoghurt and 42°C for standard set yoghurt.

8.3.6 Measurement of pH

See Section 3.2.4 (Chapter Three)

8.3.7 Microbiological analysis

See Section 3.2.5 (Chapter Three)

8.3.8 Determination of titratable acidity

See Section 3.2.6 (Chapter Three)

8.3.9 Measurement of firmness

See Section 3.2.7 (Chapter Three)

8.3.10 Confocal laser scanning microscopy (CLSM)

The confocal laser scanning microscope (CLSM) was operated in fluorescence mode as described by Blonk and van Aalst (1993). The CLSM sample was prepared using the method of Lucey, Munro, & Singh (1998) with some modification. The fluorescent protein dye Fast Green FCF (Merck, Darmstadt, Germany) was used to stain the protein matrix of the acid yoghurt gels. A few grains (~5 mg) of Fast Green FCF were added to 50 mL yoghurt milk base that had been fortified with MPC or NaCN and inoculated with STLA prior to the addition of dye. After addition of the dye, the milk base was stirred by shaker for at least 1 h to disperse the dye. A few drops of the mixture were transferred to a microscope slide that had a shallow depression. A cover slip was placed over the sample which was then incubated at 25°C for 168 h. The slide was examined in the CLSM at 0 h and every 24 h during 168 h of fermentation.

The samples were scanned on a Leica TCS 4D confocal microscope (Leica Lasertechnik GmbH, Heidelberg, Germany) with a 100x oil immersion objective (numerical aperture = 1.4). The CLSM had an air-cooled Ar/Kr laser which was used at an excitation wavelength of 568 for Fast Green FCF. Each individual sample was prepared in duplicate.

8.3.11 Determination of spontaneous syneresis of undisturbed set yoghurt

The level of spontaneous syneresis in undisturbed set yoghurt was determined by using a modified version of the aspiration method of Lucey (2001) (Amatayakul, et al., 2006). A cup of set yoghurt was taken out from the cold room (4-6°C), weighed and kept at approximately 45° to allow the whey to be collected on the side of the cup. A needle connected to a syringe was used to withdraw the liquid whey from the surface of the sample, and the cup of yoghurt was weighed again. The process was carried out within a period of 10 s to prevent further leakage of whey from the curd affecting the result. Syneresis was expressed as the mass of whey as a percentage of the initial mass of the yoghurt sample.

8.3.12 Sensory Evaluation

Two sensory evaluations were carried out for MIT and standard set yoghurt using trained panellists for descriptive evaluation and consumer panellists for acceptance evaluation. Descriptive evaluation is for the description of both qualitative and quantitative sensory aspects of a product by trained panels, and acceptance evaluation is to assess personal preference by current or potential customers of a product (Meilgaard, Civille, & Carr, 2007).

8.3.12.1 Descriptive evaluation

Advertisements were posted and "Sensory Evaluation Panel Interest Survey" forms (Appendix 8a) were distributed to students and staff at Massey University to gauge the interest of people in taking part in descriptive evaluation. Nineteen out of thirty people (called subjects in the following) who were interested and could commit to attending all four sessions (three training and one evaluation session) were selected by means of a screening test. Each session was carried out in the Food Sensory Laboratory, IFNHH.

Screening test

A screening test was used to select suitable panelists. The screening test comprised 1) a screening questionnaire (Appendix 8b), 2) identification of basic tastes (Appendix 8c) and 3) ranking of basic tastes (Appendix 8d). First, subjects were asked to answer all the questions presented in the screening questionnaire, which comprised questions on health, food habits and knowledge of flavour. Next, the subjects were instructed to (1) identify and (2) rank the basic taste in the individual booth.

For the first test, subjects were asked to identify four solutions of basic taste: sweet, sour, salty and bitter. The basic taste solutions were prepared according to American Standard Test Method (Hootman, 1992) with 2.0% (w/v) sucrose for sweet solution, 0.2% (w/v) sodium chloride for salty solution, 0.07% (w/v) citric acid for sour solution and 0.07% (w/v) caffeine for bitter solution. Distilled water was used as blank solution with the basic taste solutions.

For the second test, subjects were requested to rank the basic taste (sweet, sour, salty and bitter) with four intensities, from low to high. Each of the basic taste solutions was prepared as follows: 0.5, 1.0, 2.0 and 4.0% (w/v) sucrose for sweet solution; 0.9, 1.8, 3.6 and 6.3% (w/v) for salty solution; 0.315, 0.63, 0.9 and 1.26% (w/v) for both citric acid and caffeine

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for sour and bitter solutions, respectively. In the ranking test, a total of 16 solutions (4 basic tastes x 4 intensities) were presented to the subject.

Both test samples were assigned with a 3-digit random code and presented in two trays at the same time to the subject. Subjects were provided with a score sheet for identify (Appendix 8c) and rank (Appendix 8d) test to record their finding. Based on the screening tests, subjects with minimum score of 75% on both the identification and ranking of basic taste were selected as the descriptive test panelist.

Training sessions

The 8 selected panelists were requested to attend three training sessions and one product evaluation session. In the first training session, the panelists were asked to generate possible attributes by examining three yoghurt samples (2 MIT set yoghurts and 1 standard set yoghurt) one at a time. The attributes generated consisted of yoghurt appearance, aroma, texture and taste. For appearance and aroma, all possible attributes were listed by examining the yoghurt surface and smell, respectively. For texture, panelists were required to generate texture attributes assessed with a spoon and in the mouth for two yoghurt conditions: before and after stirring. Ten stirs with a spoon were chosen instead of twenty stirs (Folkenberg, Dejmek, Skriver, & Ipsen, 2005), because ten were sufficient to break the yoghurt structure producing a smooth texture. For yoghurt taste, the panelists were required to taste a spoonful of yoghurt and list taste attributes.

A consensus was reached on the attributes that were possessed by yoghurt samples by eliminating ambiguous and synonymous attributes. The final attributes were ranked into an order suitable for yoghurt evaluation: appearance; aroma; texture assessed with spoon before stirring; texture assessed in mouth before stirring; appearance after stirring; texture assessed with spoon after stirring; texture assessed in mouth after stirring; and taste. Training sessions involved roundtable discussions, and testing of yoghurts in individual sensory booths for evaluation training.

Panelists were trained to evaluate yoghurt samples using the generated attributes and, for each attribute, a 15 cm line scale anchored on the left with 'absent' and on the right with 'intense' (Appendix 8e). To facilitate training, the absent, low, low moderate, moderate, moderate high and high to intense (Figure 8.1) were used during the training sessions but not during the actual yoghurt evaluation. Certain attributes were anchored differently; for

example, colour was anchored on the left with 'white' and on the right with 'cream', as shown in Appendix 8e.

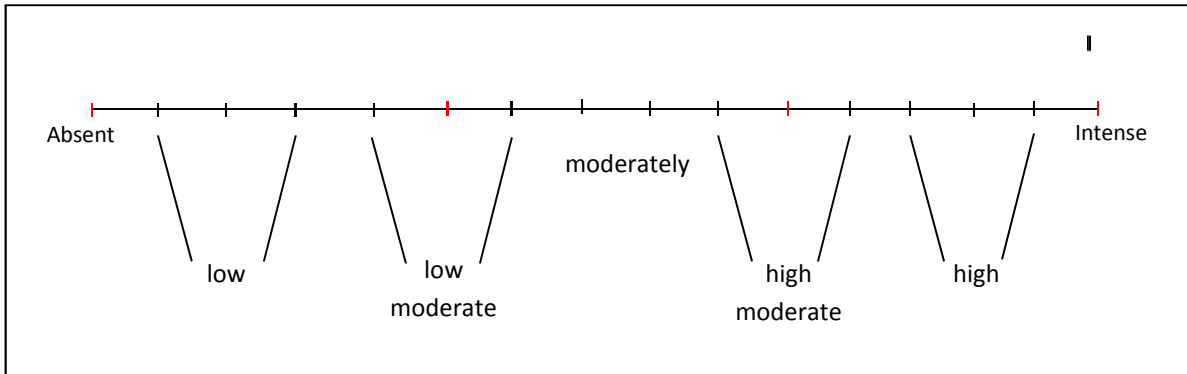


Figure 8.1: Legends to define intensity of attributes were used during evaluation training only.

Yoghurt evaluation

The yoghurt evaluation was carried out after the panelists had received about 8 h of training (session 4). The trained panelists were asked to evaluate six yoghurt samples (3 yoghurt type in duplicate) in the individual sensory booths. Duplicate samples were presented as replication increases the reliability and the possibility of trained panelists being able to detect differences (Kemp, Hollowood, & Hort, 2009). Six yoghurt samples were labeled with three-digit randomized codes and were served monadically, with one sample evaluated for five minutes before the next sample was served. Plain water and plain crackers were provided for the panelists to cleanse their palates. The trained panelists evaluated the samples using 15 cm intensity scales for 19 attributes (Table 8.2). These attributes were developed earlier during the training session. Panelists were provided with a descriptive test score sheet (Appendix 8e) to record their findings.

In the training and evaluation sessions, yoghurt samples that had been kept at 4 to 6°C for 48 h after manufacture were presented to the panellists. The sessions were scheduled every Monday, for 4 weeks, from 9 am to 12 noon, as sensory evaluation should not be conducted after meals or tea breaks to prevent the introduction of bias (Meilgaard, et al., 2007).

8.3.12.2 Acceptance evaluation

Advertisements were posted around Massey University inviting students and staff to participate in yoghurt acceptance evaluation. Three yoghurt samples (2 MIT set yoghurts and 1 standard yoghurt) were presented to each of 30 participants participant (n = 30) in individual booths. This number of participant was sufficient, as Stone, Sidel and Bliebaum (2004) suggested that the ideal number of participants for acceptance testing conducted in the laboratory environment is 25 to 50. The 30 consumers who participated in the acceptance test comprised 25% males and 75% females, with 80% aged 21 - 30, 13% aged 31- 4, and 6% aged 51 and above. The frequency of yoghurt consumption amongst the panelists was 30% daily, 33% every three days, 17% weekly, 10% fortnightly, 6% monthly and 3% who never consume yoghurt.

Participants were requested to evaluate three yoghurts (2 MIT yoghurts and 1 standard yoghurt) samples. For each sample, seven attributes were evaluated which are (1) color and (2) syneresis for appearance, (3) sour for aroma, (4) softness assessed using spoon and (5) thickness assessed in mouth for texture, (6) sour for taste and (7) overall acceptability. All these attributes were evaluated based on 9-point hedonic scale, anchored on the left with “dislike extremely” and on the right with “like extremely” (Appendix 8f)

Yoghurt samples were labelled with 3-digit random codes and served simultaneously to the participants. Samples were served with plain water and plain crackers to allow panellists to cleanse their palate between samples. Yoghurt samples presented to the participants had been stored at 4 to 6°C for 48 h after manufacture. Participants were provided with acceptance evaluation test core sheet (Appendix 8f) to record their finding.

8.3.13 Statistical Analysis

Analysis of variance was performed using MINITAB 15 (Minitab Inc., State College, PA, USA) to determine any differences between the two set yoghurts on the basis of pH, titratable acidity, starter culture growth, firmness and spontaneous syneresis at the end of fermentation and during 12 weeks storage. Two-way ANOVA (sample and panellist) with interaction was used for the analysis of sensory scores.

8.4 Results and Discussion

8.4.1 *Microstructure of set yoghurt as an MIT product*

The effect of two different set yoghurt formulations on microstructure was studied. Representative CLSM micrographs of the two set yoghurts, 0.2% (v/v) of STLA with milk protein concentrate (MPC), and 0.002% (v/v) of STLA with sodium caseinate (NaCN), are shown in Figures 8.2 and 8.3 respectively. It was hard to observe any differences between these two yoghurts. Both yoghurts appeared similar from 0-168 h fermentation, although with a less compact network developing in the MPC fortified yoghurt between 120 h (Figure 8.2d) and 168 h (Figure 8.2f) of fermentation.

No structure appeared in either yoghurt after 24 h fermentation, and this was reflected in the liquid nature of the product (data not shown). Both yoghurts exhibited structure after 48 h of fermentation (Figure 8.2b and 8.3b). The pH of the yoghurt at this time was pH 5.5 and pH 5.3 for yoghurt with MPC and with NaCN, respectively. Yoghurt gelation is dependent on acidification (Lee & Lucey, 2010). Acidification of milk leads to the disruption of the internal structure of casein micelles due to the solubilization of colloidal calcium phosphate (Dalglish & Law, 1989). Heated milk starts to gel at about pH 5.3, the isoelectric point of β -lactoglobulin. When the pH of milk reaches the isoelectric point of casein (pH 4.6), there is a decrease in electrostatic repulsion between casein molecules due to the decrease in their net negative charge (Lee & Lucey, 2010). This is associated with an increase in casein-casein attractions owing to increasing hydrophobic and electrostatic interaction (Horne, 1998). The acidification process results in the formation of chains and clusters of micelles that are linked together to form a three-dimensional network (Davies, Shankar, Brooker, & Hobbs, 1978).

From 48 to 168 h, the yoghurt gels were observed to become more dense, with a decrease in pore size and an increase in the degree of interconnectivity between clusters towards the end of fermentation. In general, however, it appears that the extent of interconnectivity of protein clusters was slightly greater in gels containing NaCN than in those containing MPC (Figure 8.2f and 8.3f).

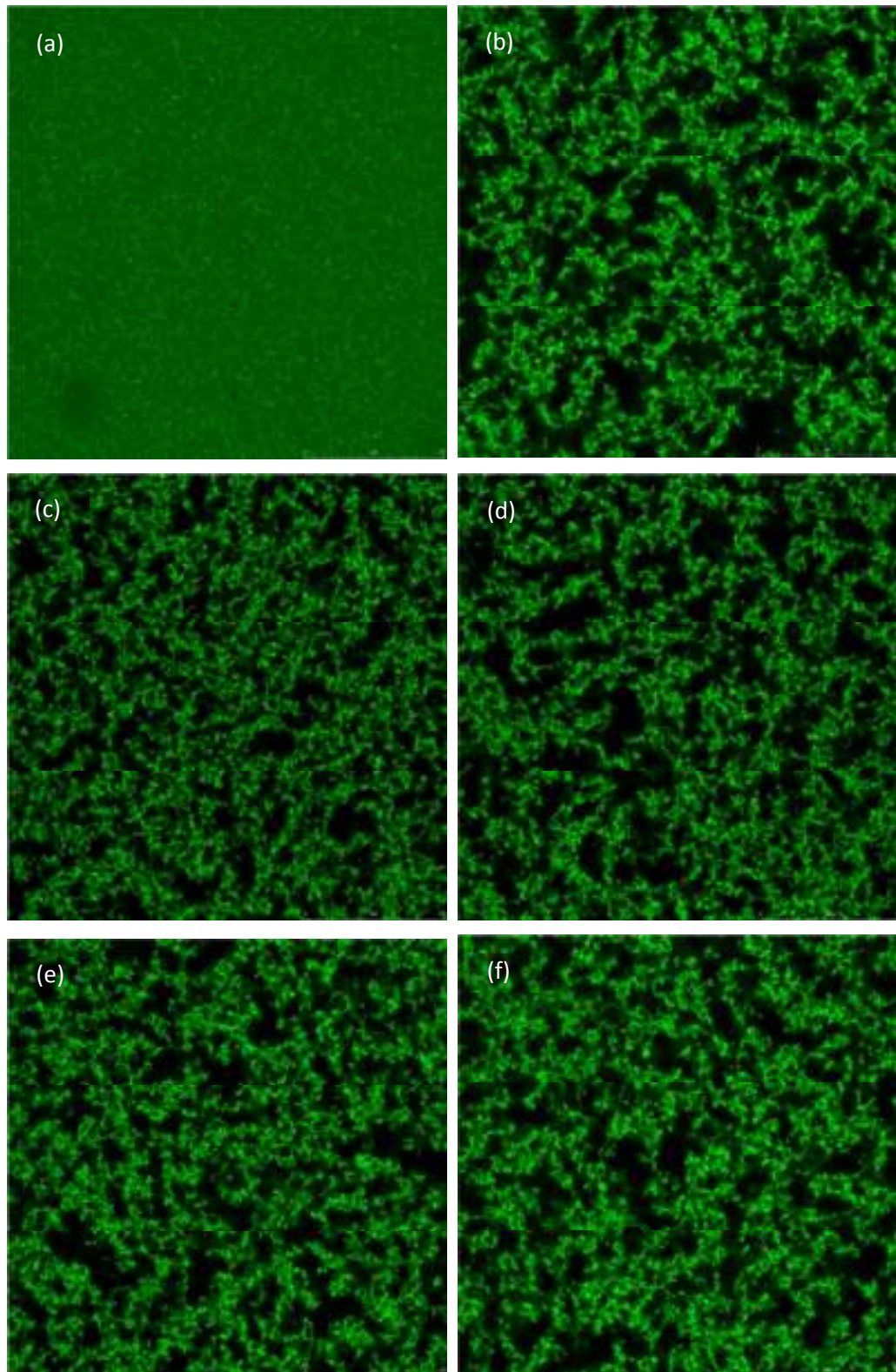


Figure 8.2: Confocal laser scanning micrographs of set yoghurt at 0 h (a), 48 h (b), 96 (c), 120 (d), 144 h (e) and 168 h (f) of fermentation for the formulation: 0.2% (v/v) of STLA using milk protein concentrate (MPC). The green colour indicates the protein network.

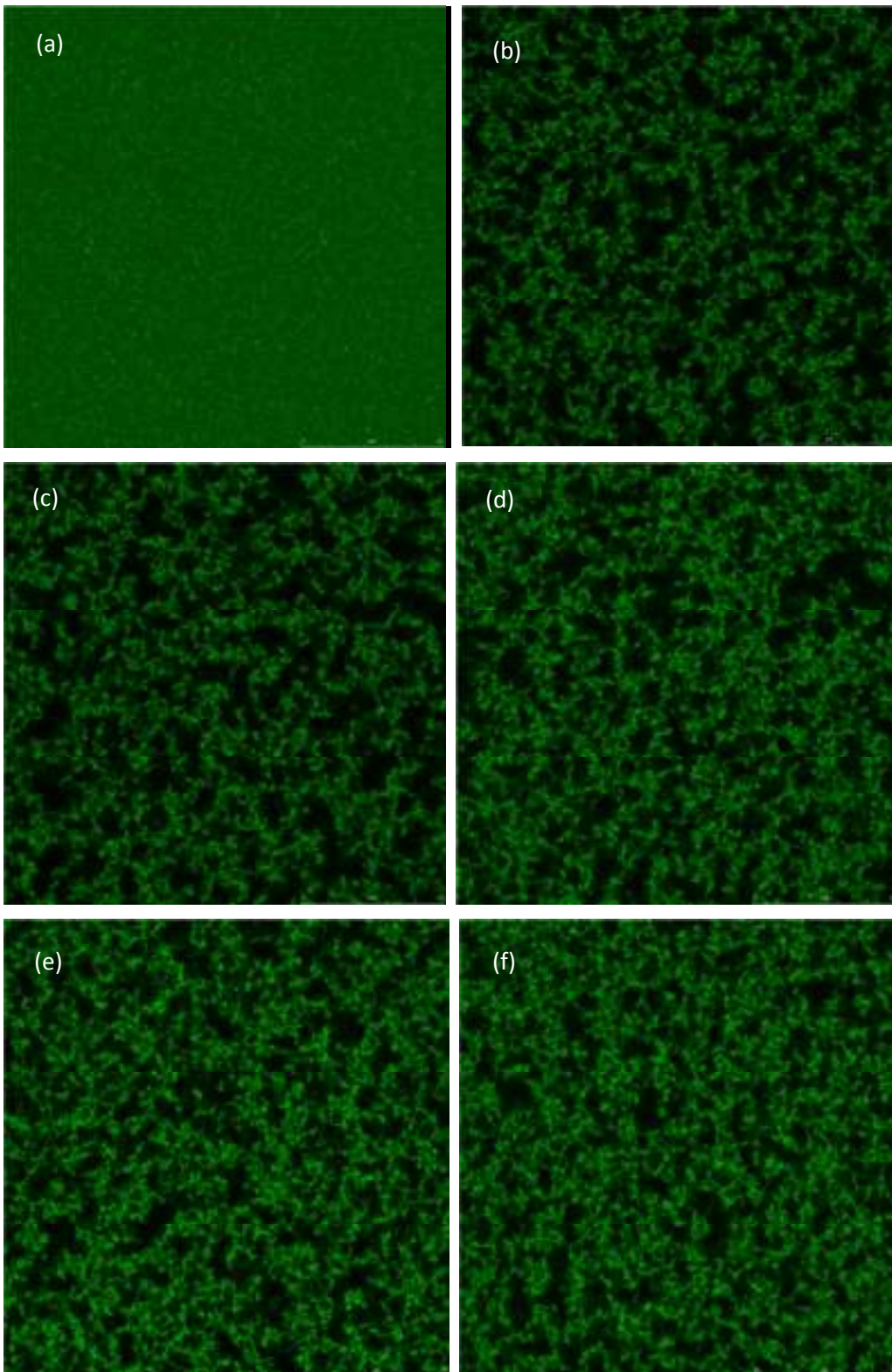


Figure 8.3: Confocal laser scanning micrographs of set yoghurt at 0 h (a), 48 h (b), 96 (c), 120 (d), 144 h (e) and 168 h (f) of fermentation for the formulation: 0.002% (v/v) of STLA using sodium caseinate (NaCN). The green colour indicates the protein network.

8.4.2 Sensory evaluation of set yoghurt as a MIT product

8.4.2.1 Descriptive evaluation

Based on the sensory screening test, ten panelists were chosen for the descriptive evaluation. However, two panelists were unavailable due to illness. This resulting in a final total of eight panelists: 5 male and 3 female, aged from 20 to 40 years. The panelists were required to achieve a minimum correct response of 75%. Stone et al. (2004) have stated a minimum of 65% correct responses in a screening test as a guideline for selecting participants suitable for training.

In the first training session, set yoghurt attributes with definitions and suitable reference materials were discussed and developed with the panelists. The final 19 attributes were listed in Table 8.2. The reference materials for specific attributes were presented physically to the panelists during the training session. Generally the attributes are based on previous yoghurt sensory studies (Folkenberg, et al., 2005; Isleten & Karagul-Yuceer, 2006). In the second and third training sessions, panelists were required to evaluate three yoghurts (2 MIT set yoghurts and 1 standard yoghurt) using the attributes that had been developed earlier.

Most yoghurt attributes were found to be significantly different ($p < 0.05$) among the three yoghurt samples except for flowability before stirring, lumpiness after stirring, syneresis after stirring and softness after stirring (Table 8.3). Significant differences ($p < 0.05$) between panelists were found for only seven of the nineteen attributes (Table 8.3). This indicates that panelists were able to agree, for most of the attributes, on their differentiation between the three set yoghurts. Variation between panelists was probably due to several factors such as individual differences in the interpretation of attribute scales, and differences in sensitivity (Bayarri, et al., 2011). There was a significant ($p < 0.05$) interaction effect between product and panelist for eight attributes. This indicates that differences in the way panelists assessed each of these attributes depended on yoghurt type (Stone, et al., 2004).

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Table 8.2: Attributes and definitions used for sensory profiling of set yoghurts

Main attributes	Specific attributes	Definition	Reference / intensity (on a scale of 0 to 15)
Appearance	Syneresis	The amount of free whey on the surface of the yoghurt cup	
	Smoothness	Quantity of particles quantified by visual inspection of the yoghurt surface	
	Colour	Visual appearance of the product ranging from white to cream	Milk - 2; Cream - 10
Aroma	Sourness	Basic taste stimulated by acids	
Texture with spoon (before and after stirring)	Syneresis	The amount of free whey observed in the yoghurt when sample is scooped and being hold for 5 seconds	
	Flowability	Ability of yoghurt to flow	Cream - 15
	Softness	Force required to scoop the sample	Cream - 15
Texture in mouth (before and after stirring)	Thickness	Mouth coating	
	Stickiness	Stickiness perceived in between upper and lower teeth (bite between teeth)	Milk Powder - 15
	Graininess	Irregularity / grains texture perceived when tongue is push against the palate	Humus - 15
Appearance after stirring	Lumpy	Lumps observed by visual inspection of the yoghurt	Porridge - 15 (reference not presented)
Taste	Sourness	Basic taste stimulated by acids	Citric acid solution (0.08%) - 5; Citric acid solution (0.15%) - 10
	Astringent	The shrinking or drying effect on the tongue surface	Tea solution (soak 2 tea bags in 200ml of water for 1 hour) – 10

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Table 8.3: p-values for the effect of yoghurt formulation on yoghurt attribute, for differences between panelists in assessing attributes, and for yoghurt-panelist interactions

Attribute	Yoghurt	Panelist	Yoghurt x Panelist
Appearance			
Syneresis	<0.0001*	0.0584 ^{ns}	0.2451 ^{ns}
Smoothness	0.0103*	0.0642 ^{ns}	0.0026*
Colour	<0.0001*	0.3171 ^{ns}	0.0025*
Aroma			
Sourness	<0.0001*	0.1435 ^{ns}	0.3226 ^{ns}
Texture assessed with spoon before stirring			
Syneresis	0.0023*	0.3224 ^{ns}	0.005*
Flowability	0.5218 ^{ns}	0.0012*	<0.0001*
Softness	0.0009*	<0.0001*	0.0525 ^{ns}
Texture assessed in mouth before stirring			
Thickness	0.0001*	0.3278 ^{ns}	0.3677 ^{ns}
Stickiness	<0.0001*	0.0011*	0.3017 ^{ns}
Graininess	<0.0001*	0.3617 ^{ns}	0.1264 ^{ns}
Appearance after stirring			
Lumpy	0.4059 ^{ns}	0.0008*	0.1050 ^{ns}
Texture assessed with spoon after stirring			
Syneresis	0.0502 ^{ns}	<0.0001*	0.1105 ^{ns}
Flowability	0.0123*	0.0623 ^{ns}	0.0002*
Softness	0.1368 ^{ns}	0.0001*	0.1188 ^{ns}
Texture assessed in mouth after stirring			
Thickness	<0.0001*	0.1028 ^{ns}	0.4973 ^{ns}
Stickiness	<0.0001*	0.0071*	0.5942 ^{ns}
Graininess	0.0023*	0.0679 ^{ns}	<0.0001*
Taste			
Sourness	<0.0001*	0.1261 ^{ns}	0.0002*
Astringent	<0.0001*	0.9311 ^{ns}	<0.0001*

An asterisk (*) indicates significant differences are present at $p < 0.05$ (95% confidence interval); ns = not significant.

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A comparison of the mean values of attributes of the three set yoghurts is presented in Table 8.4. The two set yoghurts produced as a MIT products were observed by the trained panellists to be significantly different ($p < 0.05$) compared with standard set yoghurt for all attributes except flowability before stirring, flowability after stirring (in the case of MIT yoghurt (NaCN) only), lumpiness after stirring, syneresis after stirring and softness after stirring. These results are in accord with those presented in Table 8.3, and show that the MIT set yoghurts were different from standard set yoghurt.

The two MIT set yoghurts were similar to one another: 15 of the 19 attributes evaluated were not significantly different ($p > 0.05$) between them. The four attributes observed to be different were apparent syneresis, syneresis before stirring, thickness before and thickness after stirring, and flowability after stirring. On the basis of these differences, the set yoghurt fortified with NaCN was observed to be better than the yoghurt fortified with MPC. Yoghurt containing NaCN exhibited less apparent syneresis, less syneresis before stirring, a lower flowability after stirring, and a thicker gel both before and after stirring than did the MPC fortified yoghurt. Syneresis is one of the challenges in set yoghurt production (Lee & Lucey, 2010). The more syneresis that is present the lower the yoghurt quality. The flowability and softness of both MIT yoghurts increased on stirring. This result is in line with Folkenberg et al. (2005), who reported decreasing gel strength caused by the partial breakdown of the protein network due to stirring, as the integrity and coherence of the protein network contributes to gel strength.

The difference between the three set yoghurts is visualized by means of a radar chart in Figure 8.4. The red line (0.2% STLA MPC) and green line (0.002% STLA NaCN) have similar patterns, confirming the similarity between the two MIT yoghurts. In contrast, the blue line (standard set yoghurt) has a very different pattern for many of the attributes. The two MIT set yoghurts were observed to produce more syneresis, and to have a smoother appearance and a whiter colour than the standard set yoghurt. The attributes of texture measured before stirring with a spoon appear not to differ among the three set yoghurts according to the radar chart (Figure 8.4), but actually the MIT set yoghurts produced somewhat more syneresis, and were softer and had greater flowability than the standard set yoghurt (Table 8.4). The same observation can be made in regard to the textural attributes evaluated after stirring with a spoon.

Texture assessment in mouth before and after stirring showed that both MIT set yoghurts had less thickness, stickiness and graininess than standard yoghurt. The aroma and taste assessments revealed that the MIT set yoghurts were less sour than standard set yoghurt. This is in accord with pH measurement: the MIT set yoghurts had a higher pH (pH 4.38 for both) than the standard set yoghurt (pH 3.68). The lumpiness of the three set yoghurts after stirring was almost the same. Lumpiness is due to protein aggregates of 1 to 5 mm present in yoghurt (Isleten & Karagul-Yuceer, 2006).

The fifteen attributes that were found to differ significantly among the three set yoghurts (Table 8.3) were mapped using principal component analysis (PCA) (Figure 8.5). PCA is a useful multivariate analysis procedure for simplifying and describing the interrelationships between multiple variables. Differences between samples can be visualized using PCA (Jaworska, Waszkiewicz-Robak, Kolanowski, & Swiderski, 2005).

The first component, on the x-axis of Figure 8.5, explained 97.8% of the total variability, while the second component, on the y-axis, accounted for 2.2% of the total variability. Standard set yoghurt exhibited greater sourness, aroma and taste, and was less soft and less smooth, than the two MIT set yoghurts. Standard set yoghurt also showed higher stickiness before and after stirring, as well as higher astringency. MIT set yoghurt (0.002% (v/v) STLA NaCN) was perceived to have the smoothest appearance compared with the other two set yoghurts. MIT set yoghurt (0.2% (v/v) STLA MPC) was perceived to have more apparent syneresis and more syneresis when assessed with a spoon before stirring.

PCA confirms that the two MIT yoghurts were very different from the standard yoghurt, and different from each other to a much lesser extent, on the basis of the sensory attributes identified and evaluated. The attributes that had a major influence on differentiation of the three yoghurts were sourness and aroma, colour, thickness before stirring, apparent syneresis and flowability after stirring.

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Table 8.4: Mean values of sensory attributes (ranging from 0 to 15, arbitrary units), and Fisher's least significant differences between means (indicated by superscripts).

Attributes	Samples		
	Standard Set Yoghurt	MIT Set Yoghurt (MPC)	MIT Set Yoghurt (NaCN)
Appearance			
Syneresis	3.03 ^c	10.10 ^a	7.89 ^b
Smoothness	11.60 ^b	12.6 ^a	12.99 ^a
Colour	11.24 ^a	3.20 ^b	3.28 ^b
Aroma			
Sourness	12.91 ^a	3.03 ^b	4.64 ^b
Before stirring: texture assessed with spoon			
Syneresis	1.66 ^c	4.34 ^a	3.11 ^b
Flowability	0.12 ^a	0.30 ^a	0.24 ^a
Softness	7.72 ^b	10.21 ^a	9.81 ^a
Before stirring: texture assessed in mouth			
Thickness	11.22 ^a	4.48 ^c	6.76 ^b
Stickiness	7.62 ^a	1.80 ^b	2.66 ^b
Graininess	5.99 ^a	1.93 ^b	2.23 ^b
After stirring: Appearance			
Lumpy	9.20 ^a	8.60 ^a	9.60 ^a
After stirring: texture assessed with spoon			
Syneresis	0.80 ^a	1.18 ^a	1.33 ^a
Flowability	7.62 ^b	10.10 ^a	7.46 ^b
Softness	10.47 ^a	11.38 ^a	10.57 ^a
After stirring: texture assessed in mouth			
Thickness	11.33 ^a	5.20 ^c	6.97 ^b
Stickiness	8.23 ^a	2.50 ^b	3.11 ^b
Graininess	6.23 ^a	2.73 ^b	3.76 ^b
Taste			
Sourness	13.65 ^a	2.68 ^b	3.10 ^b
Astringent	9.25 ^a	2.17 ^b	2.81 ^b

^{a b c} Means within a row with common superscripts are not significantly different ($p < 0.05$)

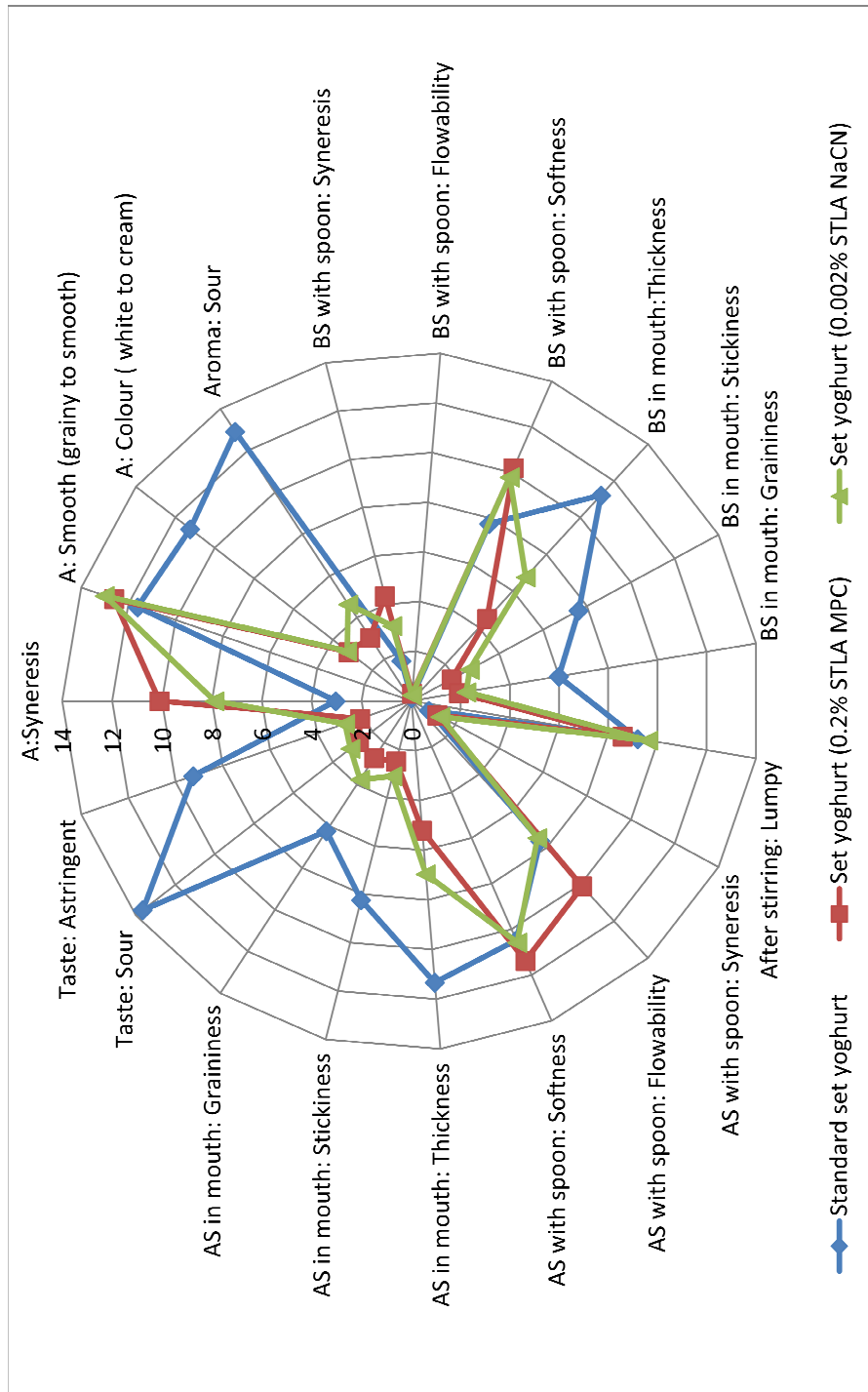


Figure 8.4: Radar plot of evaluated sensory attributes of three set yoghurts: standard set yoghurt (2.0% (v/v) of STLB; blue), MIT set yoghurt (0.2% (v/v) of STLA using milk protein concentrate (MPC); red) and MIT set yoghurt (0.002% (v/v) of STLA using sodium caseinate (NaCN); green). "A" denotes appearance, "BS with spoon" denotes texture assessed with spoon before stirring; "BS in mouth" denotes texture assessed in mouth before stirring; "AS with spoon" denotes texture assessed with spoon after stirring; "AS in mouth" denotes texture assessed in mouth after stirring.

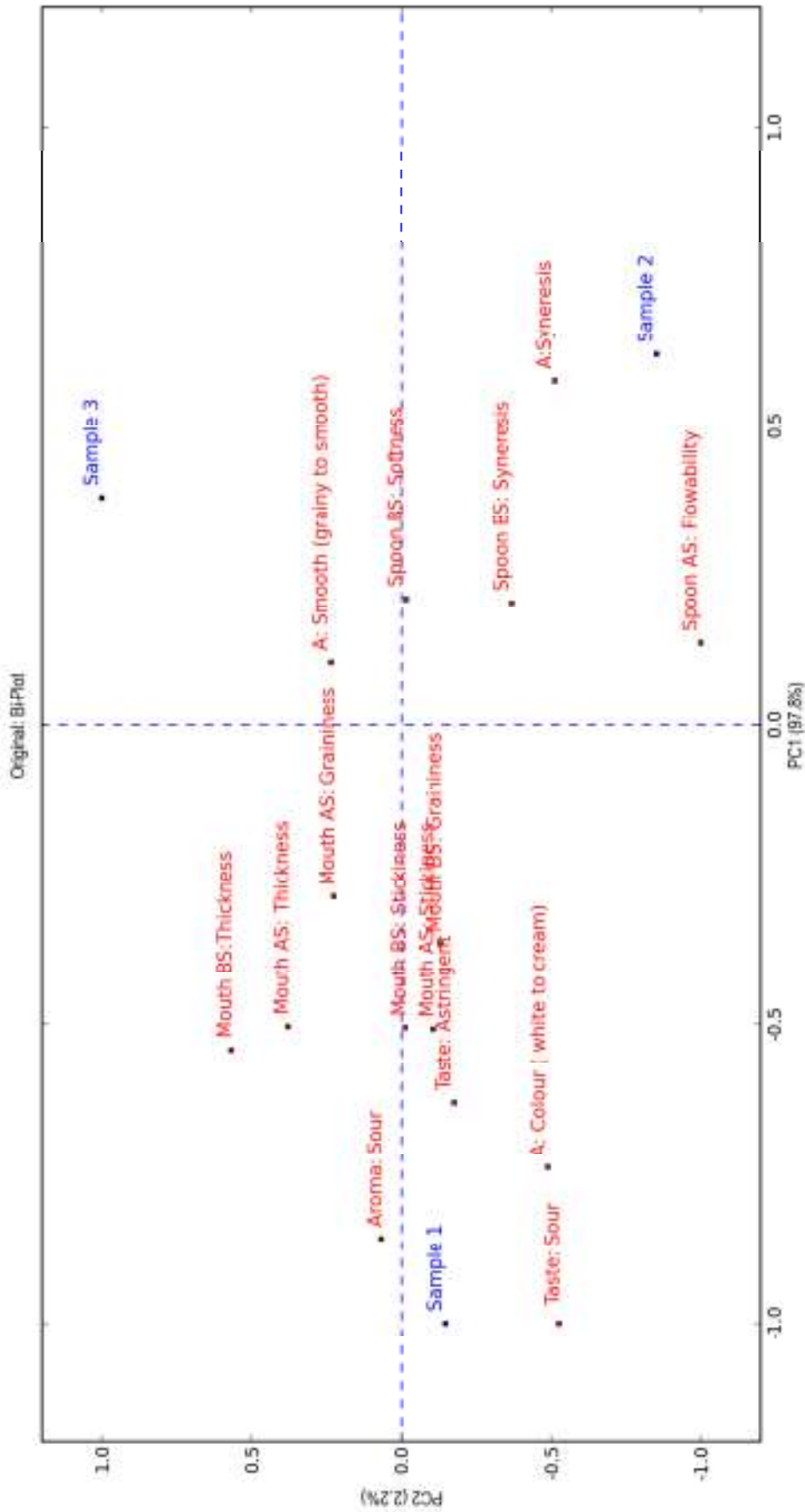


Figure 8.5: Principle Component Analysis biplots of standard set yoghurt and MIT set yoghurts for significant attributes. Sample 1: Standard set yoghurt, Sample 2: MIT set yoghurt (0.2% (v/v) STLA MPC) and Sample 3: MIT set yoghurt (0.002% (v/v) STLA NaCN)

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8.4.2.2 Acceptance test

The sensory acceptance test was carried out for the same three set yoghurts: the standard set yoghurt and the two MIT set yoghurts (Figure 8.6). The attributes used for this test were mainly derived from the PCA result, which highlighted the attributes that had a major influence in differentiating the three yoghurts. The thirty consumers who participated in the acceptance test comprised 25% males and 75% females, with 80% aged 21 - 30, 13% aged 31- 4, and 6% aged 51 and above. The frequency of yoghurt consumption amongst the panelists was 30% daily, 33% every three days, 17% weekly, 10% fortnightly, 6% monthly and 3% who never consume yoghurt.

The results are presented in Figure 8.6. The degree of liking for all seven attributes was found to be similar for the two set yoghurts for MIT product except for the sour aroma, where the yoghurt fortified with MPC produced a significantly ($p < 0.05$) higher liking score. The comparison of the attribute based on preference between the two MIT set yoghurts and the standard set yoghurt, produced no significant difference ($p < 0.05$) except for two attributes: the sour taste and overall acceptability. The MIT yoghurts were significantly more acceptable than the standard yoghurt in term of these two characteristic. The overall acceptability was scored a bit higher for yoghurt fortified with MPC yet there was no significant difference ($p < 0.05$) between both MIT set yoghurts. In one study by Isletan and Karagul-Yuceer (2006), yoghurt fortified with NaCN received a higher preference rating than yoghurt fortified with whey protein isolate (WPI) and a control yoghurt (fortified with SMP).

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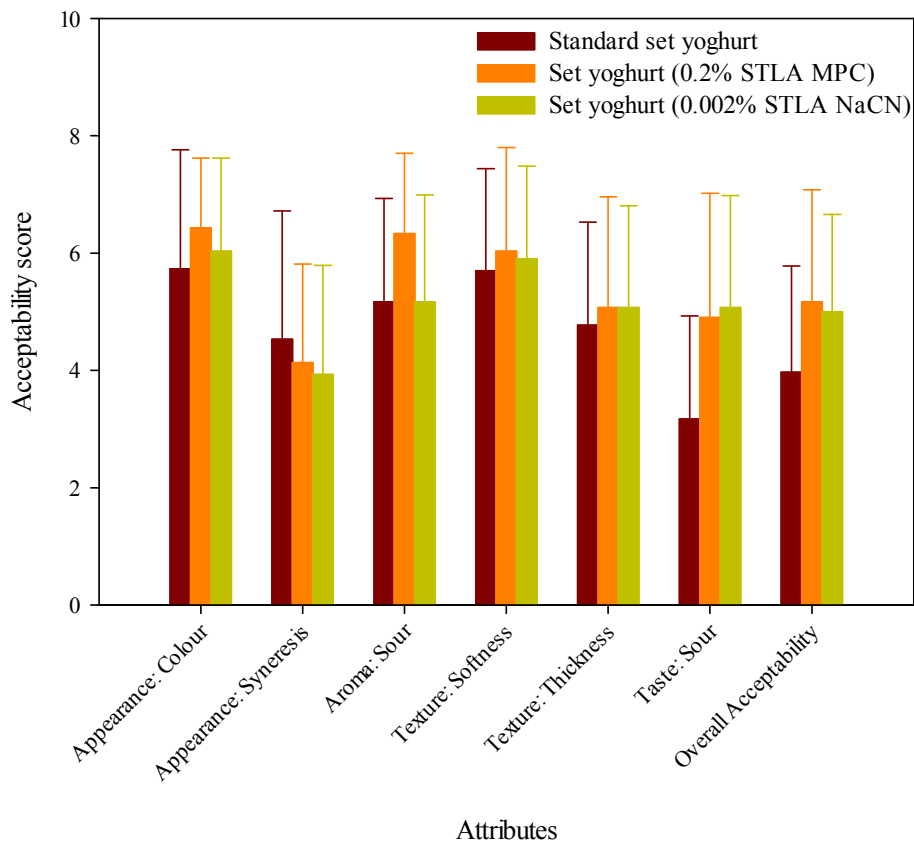


Figure 8.6: Acceptability scores^a for three set yoghurts: standard set yoghurt (2.0% (v/v) of STLB), MIT set yoghurt (0.2% (v/v) of STLA using milk protein concentrate (MPC)) and MIT set yoghurt (0.002% (v/v) of STLA using sodium caseinate (NaCN)).^aAll values are based on a 9-point hedonic scale, where 1 = dislike extremely and 9 = like extremely. Internal bar = \pm (2 x sample standard deviation).

8.4.3 Storage stability of set yoghurt for MIT product

During the 168 h of fermentation, yoghurt produced with 0.2% (v/v) STLA fortified with MPC showed a faster pH drop in the first 24 h than did yoghurt produced with 0.002% (v/v) STLA fortified with NaCN (Figure 8.7). The pH drop for both yoghurts was fastest between 24 and 60 h of fermentation, with only a small pH drop from 60-168 h.

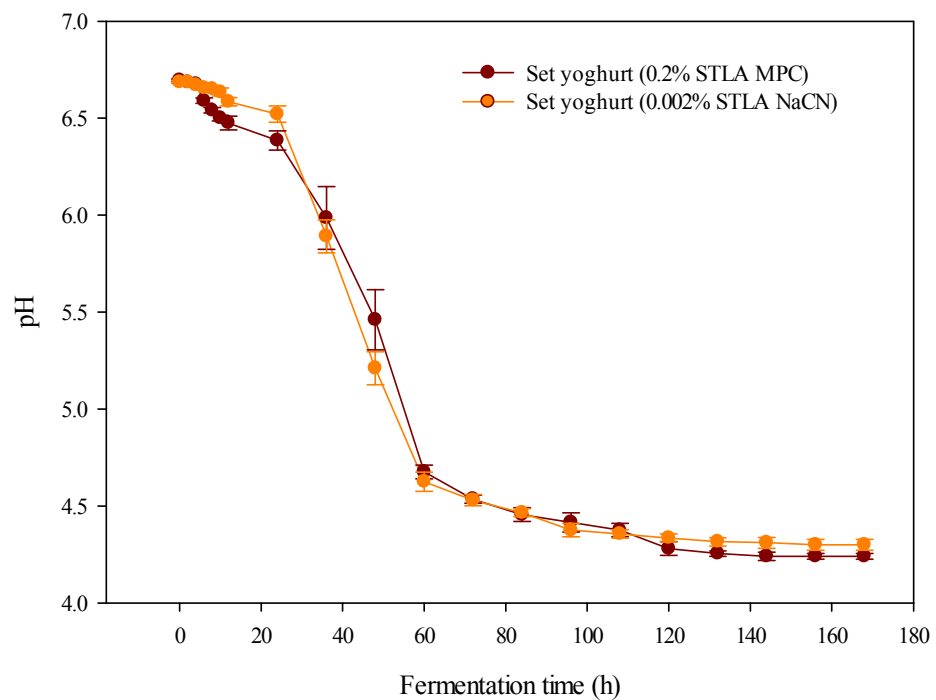


Figure 8.7: pH profiles of two set yoghurts for MIT (0.2% (v/v) of STLA using milk protein concentrate (MPC) and 0.002% (v/v) of STLA using sodium caseinate (NaCN)) during 168 h of fermentation. Internal bar = ± (2 x sample standard deviation).

During 12 weeks of post-fermentation storage at 4-6°C, both fortified MIT yoghurts showed a slow reduction in pH (Figure 8.8). The horizontal red line in the Figure 8.7 indicates the limit of yoghurt acceptability in terms of pH (Tamime & Robinson, 2007). On the basis of this line, both yoghurts were above the minimum pH until week 4 for yoghurt produced with 0.2% (v/v) STLA fortified with MPC and week 8 for yoghurt produced with 0.002% (v/v) STLA fortified with

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NaCN. Yoghurt fortified with NaCN was observed to have a slightly higher pH than yoghurt fortified with MPC throughout the 12 weeks of chilled storage.

The pH data shown in Figure 8.8 is mirrored by the titratable acidity data presented in Figure 8.9. In this study, the yoghurt was stable up to 4 and 8 weeks for yoghurt fortified with MPC and NaCN, respectively. No sign of contamination was found during the long storage. This was and is an advantage gained from using UHT sterilized milk as the yoghurt milk base.

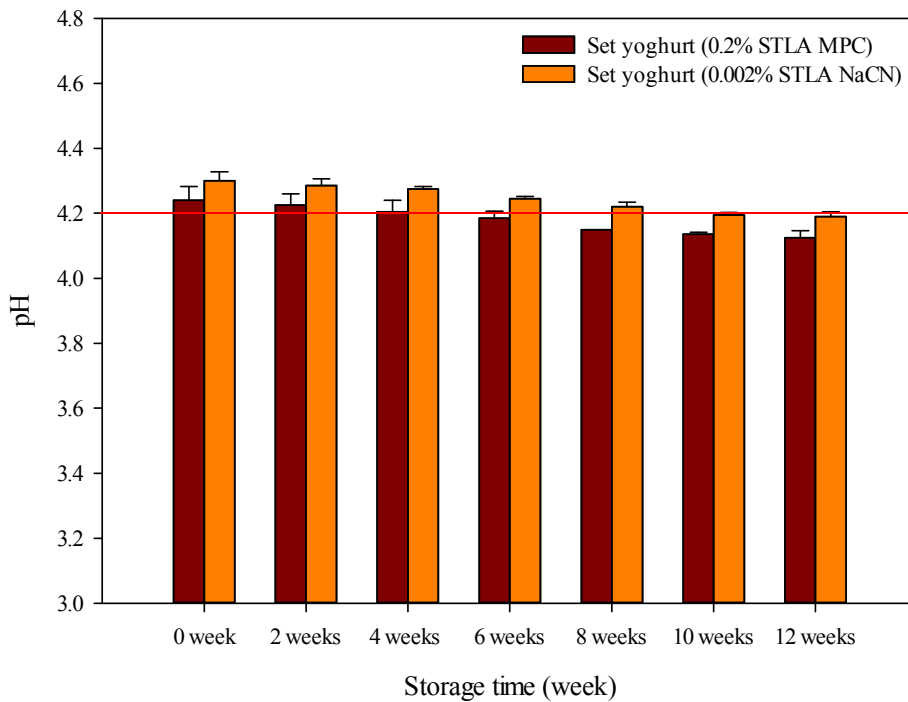


Figure 8.8: pH profiles of two set yoghurts (0.2% (v/v) of STLA using milk protein concentrate (MPC) and 0.002% (v/v) of STLA using sodium caseinate (NaCN)) during 12 weeks of storage at 4-6°C. Internal bar = \pm (2 x sample standard deviation).

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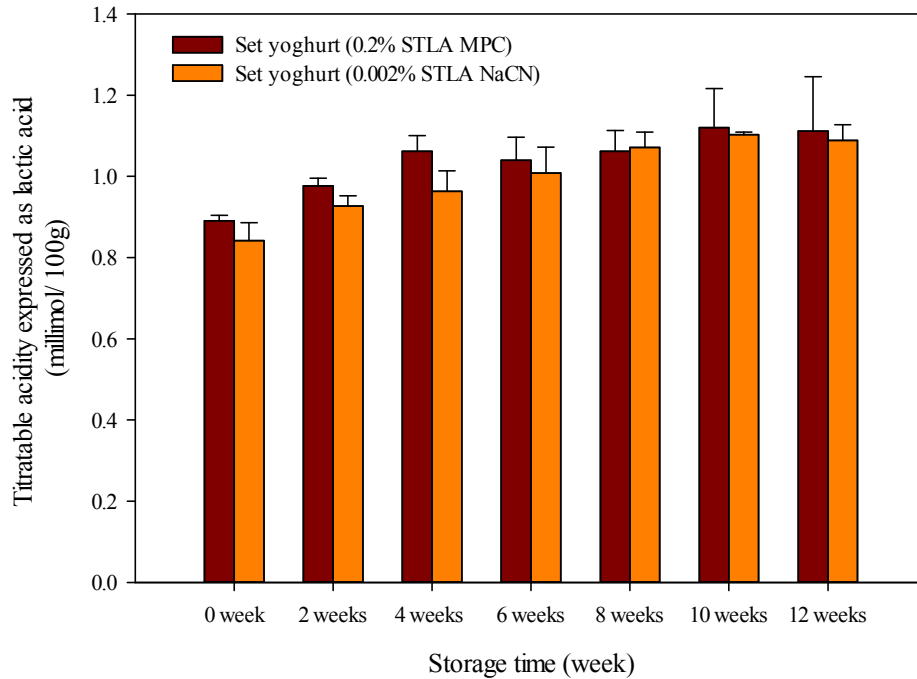


Figure 8.9: Titratable acidity profiles of two set yoghurts (0.2% (v/v) of STLA using milk protein concentrate (MPC) and 0.002% (v/v) of STLA using sodium caseinate (NaCN)) during 12 weeks of storage at 4-6°C. Internal bar = ± (2 x sample standard deviation).

Changes in starter bacteria counts during storage are shown in Figure 8.10. The *S. thermophilus* STM5 count was higher than that of *L. acidophilus* LA5 throughout the 12 weeks of storage. Both starter bacteria showed a decrease in viability, with *S. thermophilus* decreasing from 7.70 to 6.69 log cfu g⁻¹ in yoghurt fortified with MPC and from 7.54 to 6.58 log cfu g⁻¹ in yoghurt fortified with NaCN. Both yoghurts met the minimum required total count of starter bacteria (1.0 x 10⁷ cfu g⁻¹) after week 6 of storage.

The count of *L. acidophilus* LA5 decreased from 6.59 to 5.33 log cfu g⁻¹ in yoghurt fortified with MPC and from 6.65 to 5.44 log cfu g⁻¹ in yoghurt fortified with NaCN. This agrees with Sodini et al. (2002), who noticed a reduction in *L. acidophilus* in yoghurt during 5 weeks of storage at 4°C. They found that the *L. acidophilus* count was above 10⁶ cfu g⁻¹ until week 3 yet in the current study, the count was observed close to 10⁶ cfu g⁻¹ at week 6 of storage.

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The slow pH decline during storage affects the viability of the starter culture. The required number for a probiotic species count must exceed the 1.0×10^6 cfu g⁻¹ as specified in the Codex Standard (FAO/WHO, 2003).

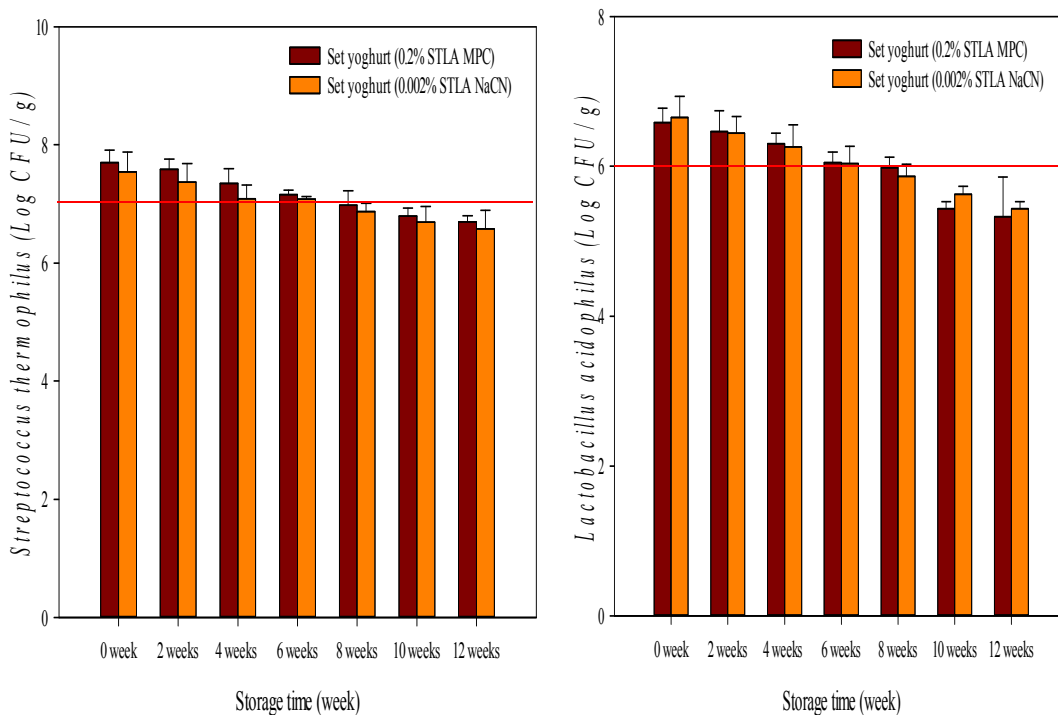


Figure 8.10: *S. thermophilus* STM5 and *L. acidophilus* LA5 counts for two set yoghurts (0.2% (v/v) of STLA using milk protein concentrate (MPC) and 0.002% (v/v) of STLA using sodium caseinate (NaCN)) during 12 weeks of storage at 4-6°C. Internal bar = ± (2 x sample standard deviation).

Yoghurt firmness throughout the storage shows yoghurt fortified with NaCN is higher than yoghurt fortified with MPC (Figure 8.11). Initially, yoghurt firmness was 1.73 N and 1.96 N for yoghurt fortified with MPC and fortified with NaCN, respectively. During the first 6 weeks of storage, the firmness of both yoghurts increased. This could have been due to protein rearrangement continuing during storage and leading to more extensive protein-protein interactions (Abu-Jdayil & Mohamed, 2002). After week 6, there was little change in yoghurt firmness. Yoghurt fortified with NaCN had a higher firmness than yoghurt fortified with MPC

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throughout the 12 weeks of storage. Isleten and Karagul-Yuceer (2006) found a better texture in yoghurt fortified with NaCN than in yoghurt fortified with SMP or with whey protein isolate during 12 days of storage at 5°C.

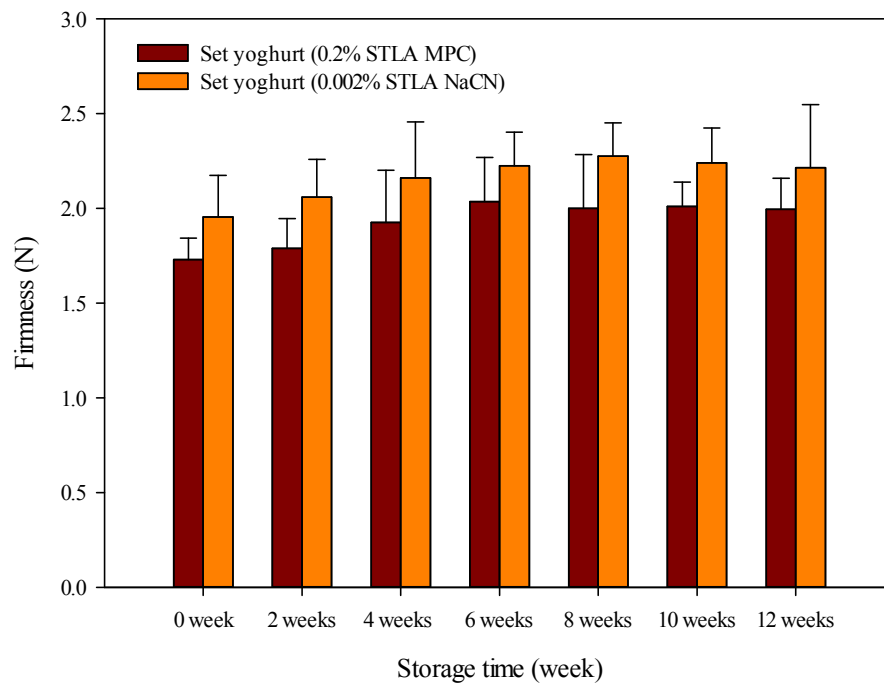


Figure 8.11: Firmness profiles of two set yoghurts (0.2% (v/v) of STLA using milk protein concentrate (MPC) and 0.002% (v/v) of STLA using sodium caseinate (NaCN)) during 12 weeks storage at 4-6°C. Internal bar = \pm (2 x sample standard deviation). Internal bar = \pm (2 x sample standard deviation).

During the 12 weeks of storage, spontaneous syneresis (Figure 8.12) was measured. Syneresis or whey separation is due to rearrangements of casein-casein bonds after casein aggregation and whey protein denaturation (the latter resulting from heat treatment applied prior to fermentation) have been initiated. This leads to local stresses in the protein network and subsequent breaking of protein strands (Pareira et al., 2003). The amount of syneresis decreased during the 12 weeks of storage for yoghurt fortified with NaCN. Similarly, a decrease in syneresis was observed for yoghurt fortified with MPC over the first 6 weeks of storage. After week 6, a small amount of whey appeared on the surface of yoghurt fortified with MPC. Isleten and

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Karagul-Yuceer (2006) observed a decrease in syneresis during 12 days of storage in set type yoghurt.

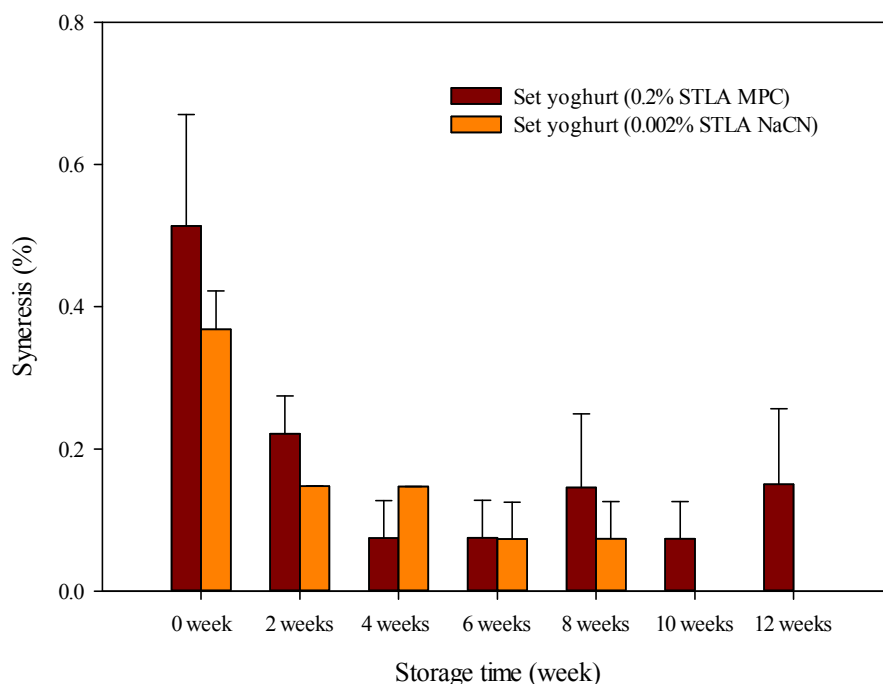


Figure 8.12: Spontaneous syneresis in two set yoghurts (0.2% (v/v) of STLA using milk protein concentrate (MPC) and 0.002% (v/v) of STLA using sodium caseinate (NaCN)) during 12 weeks of storage at 4-6°C. Internal bar = \pm (2 x sample standard deviation).

8.5 Conclusion

Set yoghurt fortified with NaCN had a denser microstructure than yoghurt fortified with MPC. Sensory evaluation based on descriptive evaluation revealed similar attributes for the two MIT set yoghurts, yet these were significantly different ($p < 0.05$) from the standard set yoghurt. MIT set yoghurts scored better than the standard set yoghurts for overall acceptance. Yoghurt produced with 0.002% (v/v) STLA fortified with NaCN and with 0.2% (v/v) STLA fortified with MPC were acceptable products that lasted for up to 6 weeks and 4 weeks of storage, respectively, at 4 to 6°C, based on the final pH and concentration of the starter bacteria.

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CHAPTER NINE: PREDICTIVE MODEL DEVELOPMENT AND VALIDATION FOR FERMENTATION OF SET CULTURE YOGHURT AS A MADE-IN-TRANSIT PRODUCT

CHAPTER NINE : PREDICTIVE MODEL DEVELOPMENT AND VALIDATION FOR FERMENTATION OF SET CULTURE YOGHURT AS A MADE-IN-TRANSIT PRODUCT

9.1 Abstract

Yoghurt acidification (based on pH profile), starter bacteria growth (*S. thermophilus* STM5 and *L. acidophilus* LA5) and firmness of set yoghurt during 168 h of fermentation at four fermentation temperatures (22.5, 25, 27.5 and 30°C) were studied. Among the primary models used to model yoghurt acidification, the modified logistic model was better than the modified Gompertz and Baranyi models to describe the experimental (observed) data. For starter bacteria growth using *S. thermophilus* STM5 and *L. acidophilus* LA5, the modified Gompertz model was found to be better than the other two models. Modified Gompertz also described the firmness of the yoghurt during fermentation. The derived growth kinetic parameters, such as maximum specific growth rate (μ_{max}) and lag phase duration (λ), were modelled using the square root equation as the secondary model. The selection of the best models was based on R^2 (the coefficient of multiple determinations). The validation of the developed models was carried out by fermenting set yoghurt within (interpolation) and outside (extrapolation) the fermentation temperatures used to develop the models. The possibility for the prediction of yoghurt acidification, starter bacteria growth and firmness will be useful for the quality assurance of made-in-transit products.

9.2 Introduction

A milk base fortified with sodium caseinate (NaCN) inoculated at 0.002% (v/v) with *S. thermophilus* STM5 and *L. acidophilus* LA5 (STLA) in the ratio 1:1 (total inoculum concentration of about 10^8 cfu mL⁻¹) and incubated at 25°C produced set yoghurt as an MIT product with a long fermentation time, good texture, acceptable sensory characteristics and stable during chilled storage (Chapter 8). A mathematical model for predicting pH decline, starter bacteria growth and firmness of yoghurt based on these conditions was developed. Several studies have reported empirical mathematical models, based on the modified Gompertz equation, for predicting the extent of yoghurt acidification (De Brabandere & De Baerdemaeker, 1999; Soukoulis, Panagiotidis, Koureli, & Tzia, 2007) but these have not been applied to a product made by extended fermentation as an MIT product.

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Empirical models are commonly used for modelling fermentation owing to the complexity of the system and number of variables involved (Soukoulis, et al., 2007). The sigmoidal shape of the pH profile during fermentation makes it suitable to be modelled using the modified Gompertz or similar equations (De Brabandere & De Baerdemaeker, 1999). For modelling bacterial growth, the modified Gompertz, modified logistic and Baranyi (Baranyi-Roberts) equations have been used (Ding et al., 2011; Mataragas, Dimitriou, Skandamis, & Drosinos, 2011).

The objectives of this study were to model yoghurt acidification (the pH-time profile), starter bacteria growth and firmness as functions of fermentation temperature, and to validate the developed models using interpolation and extrapolation data.

9.3 Materials and Methods

9.3.1 Experimental design

An experiment was carried out to develop an empirical model. The yoghurt was manufactured with 0.002% (v/v) of STLA in the ratio 1:1 (total inoculum concentration of about 10^8 cfu mL⁻¹) in a milk base fortified with NaCN. The milk base was fermented at four different fermentation temperatures; 22.5, 25, 27.5 and 30°C (Sanyo Incubator). The fermentation temperature was selected as the primary factor influencing set yoghurt manufacture (Chapter 7). The temperature levels chosen were based on temperature fluctuations that might be expected to occur during fermentation in yoghurt transport. The pH was measured during fermentation (168 h) every 2 h for the first 12 h and every 12 h for the remaining 156 h (20 measurements). The starter culture count was measured in samples taken every 2 h for the first 12 h and every 24 h for the remaining 156 h (14 measurements). Firmness was measured at 12 h, at 24 h and every 24 h for the remaining 156 h (9 measurements).

For model validation, independent experiments were carried out within (23 and 27°C; interpolation) and outside (20 and 32°C; extrapolation) the fermentation temperature range used to develop the model. Data collection for model development was replicated using five different batches. Duplicate experiments were carried out for model validation.

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9.3.2 Cultures

See Section 3.1.1 (Chapter Three).

This procedure produced a consistent concentration of starter culture within 10^8 cfu mL⁻¹ in each starter bacteria: 8.66 ± 0.75 log cfu mL⁻¹ (log colony forming units mL⁻¹) for *S. thermophilus* STM5, 8.25 ± 0.98 log cfu mL⁻¹ for *L. acidophilus* LA5.

9.3.3 Preparation and fortification of the yoghurt milk base

See section 8.3.3 (Chapter 8) for preparation of yoghurt milk base using skim milk powder (SMP) fortified with sodium caseinate (NaCN).

9.3.4 Heat treatment of milk base

See Section 3.2.2 (Chapter Three).

9.3.5 Yoghurt processing

Yoghurt manufacture was carried out in the Massey University IFNHH Product Development Lab. The milk was brought out from the refrigerator and allowed to come to the fermentation temperature (~25°C) in an incubator. The UHT milk was then inoculated with 0.002% (v/v) at ratio 1:1 of *Streptococcus thermophilus* STM5 and *Lactobacillus acidophilus* LA5 (STLA) starter culture. The milk was shaken manually for 60 s and left in the fermentation bottle for 10 min after inoculation before it was poured into sterile plastic containers (55 mm height; 40 mm diameter) (LabServ, Auckland, New Zealand). The containers were filled to a headspace of approximately 4.0 cm (sample volume \approx 50 mL). The containers were then sealed with screw lids and placed into an incubator set at fermentation temperature of 25°C.

9.3.6 Measurement of pH

See Section 3.2.4 (Chapter Three).

9.3.7 Microbiological analysis

See Section 3.2.5 (Chapter Three).

9.3.8 Measurement of firmness

See Section 3.2.7 (Chapter Three).

9.3.9 Mathematical modelling

9.3.9.1 Introduction

The modified Gompertz (Eq. 1), modified logistic (Eq. 2) and Baranyi (Eq. 3) equations were used to develop the primary model for yoghurt acidification based on the pH-time profile, the growth of two starter bacteria (*Streptococcus thermophilus* STM5 and *Lactobacillus acidophilus* LA5) and yoghurt firmness development, at four different fermentation temperatures.

9.3.9.2 Primary models

The primary models trialed were as follows:

- a) Modified Gompertz equation (Gibson, Bratchell, & Roberts, 1987):

$$\log \frac{N}{N_o} = D \exp \left\{ -\exp \left[\frac{\mu_{max} e}{D} (\lambda - t) + 1 \right] \right\} \quad (\text{Eq. 1})$$

- b) Modified logistic equation (Gibson, et al., 1987)

$$\log \frac{N}{N_o} = \frac{D}{1 + \exp \left[\frac{\mu_{max}}{D} (\lambda - t) + 2 \right]} \quad (\text{Eq. 2})$$

- c) Baranyi equation (Baranyi & Roberts, 1994)

$$\log \frac{N}{N_o} = \mu_{max} A_B(t) - \ln \left(1 + \frac{\exp(\mu_{max} A_B(t)) - 1}{\exp \left(\log \left(\frac{N_{max}}{N_o} \right) \right)} \right) \quad (\text{Eq. 3})$$

where,

$$A_B(t) = t + \frac{1}{\mu_{max}} \log[\exp(-\mu_{max}t) + \exp(-h_o) - \exp(-\mu_{max}t - h_o)]$$

(Eq. 4)

h_o is dimensionless parameter quantifying, in the case of starter culture growth, the initial physiological state of the microbial cells. It is defined as:

$$h_o = \lambda\mu_{max} \quad \text{(Eq. 5)}$$

Ideally, h_o is a measured physical value, the use of which makes the Baranyi equation partly mechanistic rather than autonomous. If no such value is used, h_o is simply a constant whose value is found by curve fitting, along with the other constants in the equation. The equation is then autonomous, and is used in that form here for starter culture growth, pH decline and firmness development.

Identification of variables

t = time (h)

For starter culture growth,

N_o = cell concentration at t = 0 (cfu g⁻¹)

N = cell concentration at t = t (cfu g⁻¹)

N_{max} = final cell concentration (cfu g⁻¹)

For pH decline,

Log N/ N_o represents (pH-pH_o)

where pH_o = initial pH, and pH = pH at time t

N_{max} represents final pH

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For firmness,

Log N/N₀ represents firmness

N_{max} represents final firmness

Identification of constants

The constants in equations 1, 2, and 3 (Table 9.1) were estimated by fitting the equations to the experimental data (five replicates) using SigmaPlot 12.0 (Systat Software, Inc., Chicago, IL, USA).

Table 9.1: Definition of the constants in the modified Gompertz, modified Logistic and Baranyi equations.

Constants	pH decline	Starter culture growth	Firmness
	Maximum rate of pH decline expressed as	Maximum specific growth rate expressed as	Maximum rate of increase in firmness expressed as
μ_{max}	$\mu_{max,pH} = \frac{d(pH - pH_0)}{dt}$ (pH units h ⁻¹)	$\mu_{max,sc} = \frac{d\left(\log\left(\frac{N}{N_0}\right)\right)}{dt}$ (h ⁻¹)	$\mu_{max,F} = \frac{d(firmness)}{dt}$ (N h ⁻¹)
λ	$\lambda_{pH} = \text{Lag time (h)}$	$\lambda_{sc} = \text{Lag time (h)}$	$\lambda_F = \text{Lag time (h)}$
D	D_{pH} = maximum pH change (pH units)	$D_{sc} = \log\frac{N_{max}}{N_0}$ (dimensionless)	D_F = maximum firmness (N)
h_0	$h_{0,pH}$ (pH units)	$h_{0,sc}$ (dimensionless)	$h_{0,F}$ (N)

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9.3.10 Secondary models

The temperature dependence of the parameters μ_{max} , λ and D obtained from the best-fit primary models for pH decline, starter culture growth and firmness development was itself modelled using the square root equation as a secondary model (Ratkowsky, Olley, McMeekin, & Ball, 1982):

$$\sqrt{\mu_{max}} = a_{\mu}(\theta - \theta_{min, \mu}) \quad \text{(Eq. 6)}$$

$$\sqrt{\frac{1}{\lambda}} = a_{\lambda}(\theta - \theta_{min, \lambda}) \quad \text{(Eq. 7)}$$

$$\sqrt{D} = a_D(\theta - \theta_{min, D}) \quad \text{(Eq. 8)}$$

where a_{μ} , a_{λ} and a_D are the slopes of the linear regressions of $\sqrt{\mu_{max}}$, $\sqrt{\frac{1}{\lambda}}$ and \sqrt{D} against fermentation temperature and θ_{min} is equal to (-intercept/ α) (°C). In the case of starter culture growth, θ_{min} is the notional minimum temperature for growth (°C). The units of α are given in Table 9.2.

Table 9.2: Unit of constant, α , in equations 6 to 8 for pH decline, starter bacteria growth and firmness development.

	α_{μ}	α_{λ}	α_D
pH decline	(pH units) ^{1/2} h ^{-1/2} °C ⁻¹	h ^{-1/2} °C ⁻¹	(pH units) ^{1/2} °C ⁻¹
Starter bacteria growth	h ^{-1/2} °C ⁻¹	h ^{-1/2} °C ⁻¹	-
Firmness	N ^{1/2} h ^{-1/2} °C ⁻¹	h ^{-1/2} °C ⁻¹	N ^{1/2} °C ⁻¹

*A secondary model relating D to temperature could not be established in the case of starter bacteria growth. Instead, the mean of the values of D determined at the four temperatures concerned is reported in section 9.4.2.2.

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In establishing secondary models for μ_{max} and D in the case of pH decline, the fact that these constants had negative values was ignored, since the square root of a negative number cannot be found. In applying the resulting secondary models, it would of course necessary to make predicted values negative before substituting them into the primary model.

9.3.11 Statistical analysis

Curve fitting of experimental data was carried out using SigmaPlot 12 software. The statistical index used to compare and discriminate between primary models, and to evaluate the adequacy of the secondary models, was the coefficient of determination (R^2) (Giannuzzi, Pinotti, & Zaritzky, 1998; Ross, 1996; te Giffel & Zwietering, 1999; van Boekel, 2009):

The R^2 is the fraction of the square of the deviations of the observed values about their mean explained by the equation fitted to the experimental data. R^2 ranges in value from 0 to 1, with 1 indicating that the regression curve fits the experimental data exactly. It is calculated by the equation:

$$R^2 = 1 - \frac{\sum_{i=1}^n (\text{observed}_i - \text{predicted}_i)^2}{\sum_{i=1}^n (\text{observed}_i - \text{mean})^2} \quad (\text{Eq. 9})$$

where n is the total number of data points; $mean$ is the average observed value.

9.4 Results and Discussion

9.4.1 Modelling the effect of time and fermentation temperature on yoghurt acidification

9.4.1.1 Primary model

The pH data obtained during the making of yoghurt at different fermentation temperatures (22.5, 25, 27.5 and 30°C) (Figure 9.1) were used to generate primary models based on the modified Gompertz (Figure 9.2a), modified logistic (Figure 9.2b) and Baranyi (Figure 9.2c) equations. The fits of the three equations to the experimental data are shown in Figure 9.2. The model parameters are presented in Table 9.2. $\mu_{max,pH}$ and λ_{pH} decreased with increasing fermentation temperature, as would be expected. Higher fermentation temperatures resulted in

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faster yoghurt acidification through enhanced starter culture growth. This is in agreement with De Brabandere and De Baerdemaeker (1999), who found that fermentation temperature affected the parameters of their Gompertz equation based model, though not markedly over the relatively narrow temperature range (39 to 45°C) investigated.

The parameter D was fairly constant at 25, 27.5 and 30°C, but was noticeably lower at 22.5°C (Figure 9.1 and Table 9.3). This latter temperature is very close to the minimum temperature for the starter culture growth in which lies in the range 20 to 22°C (Cogan, 1996; Holt et al., 1994). Model discrimination was carried out on the basis of the statistical index R^2 to select the best primary model, whose parameters could then be used to develop secondary models. The R^2 values obtained for the three models used were very close to one another (Table 9.3), with the modified logistic model producing the best fit overall (generally the highest R^2 values) over the four fermentation temperatures.

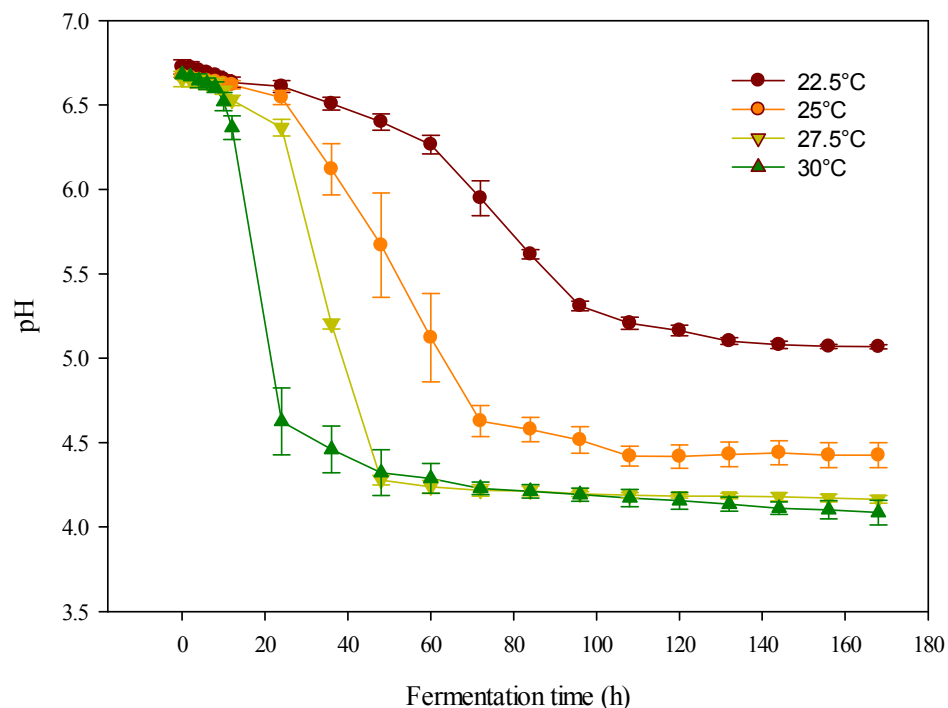


Figure 9.1: pH profiles of set culture yoghurt at four different fermentation temperatures (22.5, 25, 27.5 and 30°C).

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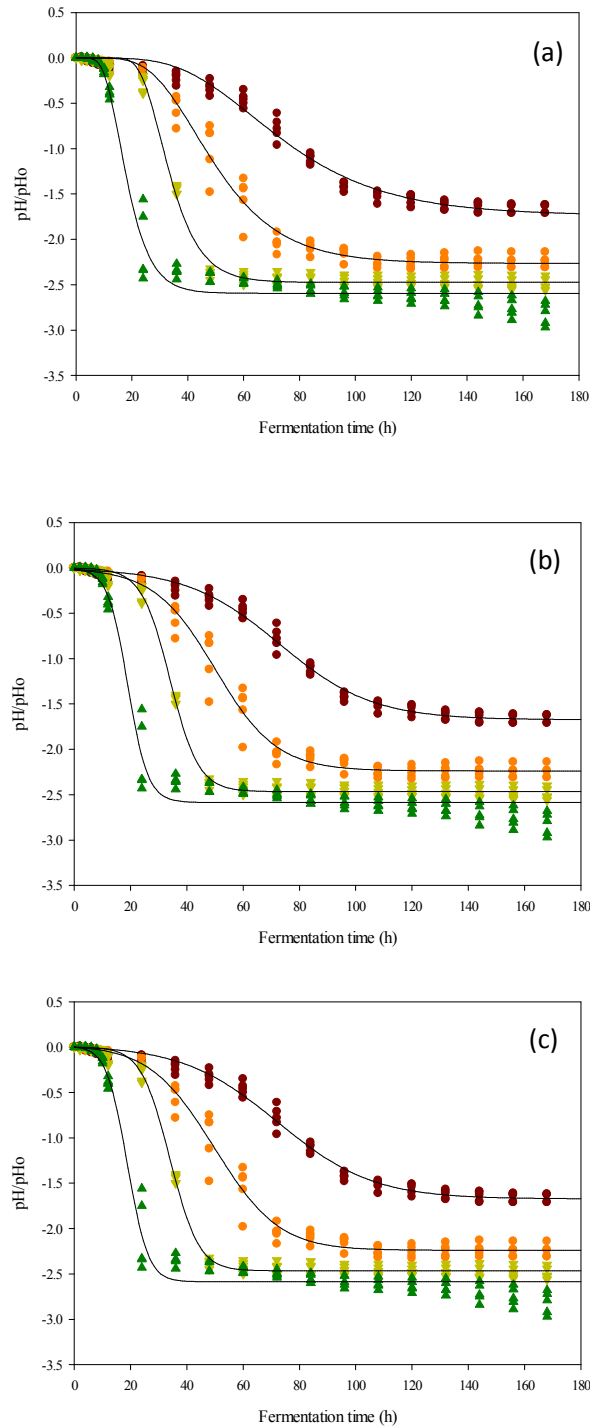


Figure 9.2: Observed pH profiles (symbols) fitted with the primary models (lines) (a) modified Gompertz equation, (b) modified logistic equation and (c) Baranyi equation (brown: 22.5°C; orange: 25°C; olive green: 27.5°C and green : 30°C).

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Table 9.3: Primary model parameters and R^2 values obtained by fitting the modified Gompertz, modified logistic and Baranyi equations to the experimental pH profiles for yoghurt made by fermentation at 22.5, 25, 27.5 and 30°C.

Model	Fermentation temperature (°C)	$\mu_{max, pH}$ (pH units h ⁻¹)	λ_{pH} (h)	D_{pH} (pH units)	h_o (pH units)	R^2
Modified Gompertz	22.5	-0.025	36.97	-1.735		0.987
	25	-0.051	26.35	-2.265		0.989
	27.5	-0.120	22.86	-2.474		0.996
	30	-0.163	9.945	-2.595		0.987
Modified Logistic	22.5	-0.025	39.89	-1.674		0.993
	25	-0.051	28.35	-2.238		0.990
	27.5	-0.122	23.95	-2.466		0.998
	30	-0.182	11.95	-2.586		0.986
Baranyi	22.5	0.063	85.89	-1.673	5.385	0.992
	25	0.096	61.60	-2.240	5.901	0.990
	27.5	0.217	39.69	-2.465	8.625	0.998
	30	0.305	23.13	-2.586	7.057	0.986

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9.4.1.2 Secondary models for pH decline

The $\mu_{max,pH}$, λ_{pH} and D_{pH} values from the modified logistic equation at the four fermentation temperatures (Table 9.3) were used in developing secondary models of the temperature dependence of these parameters, and thus the temperature dependence of pH decline. The square root equation was selected as a general secondary model, as it was found to describe adequately the experimental data.

The $\mu_{max,pH}$, λ_{pH} and D_{pH} values based on the modified logistic equation are compared in Figures 9.3 to 9.5 with the fitted square root equations (Table 9.4). No large deviations of the observed values from the fitted curves are apparent. Overall, the square root model is suitable for the prediction of $\mu_{max,pH}$, λ_{pH} and D_{pH} within the experimental temperature range.

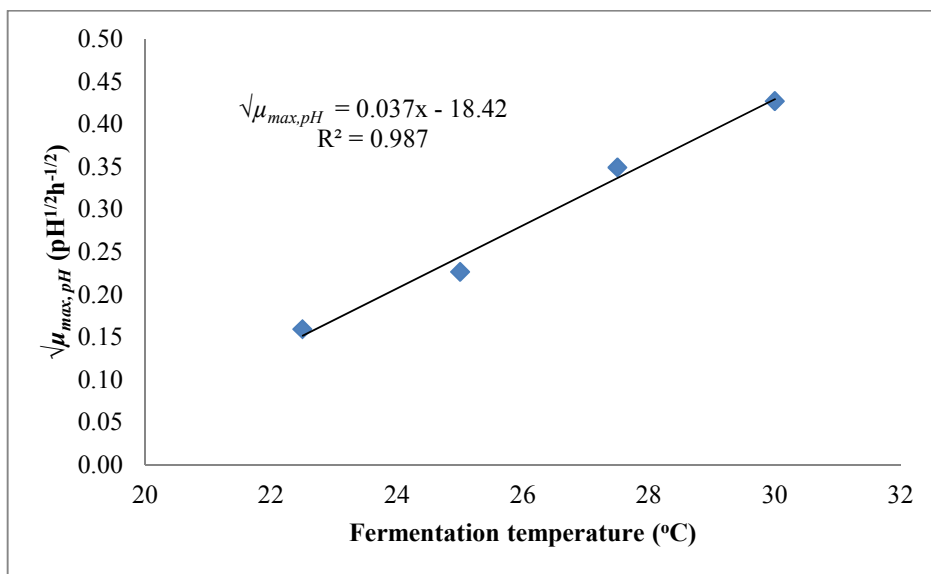


Figure 9.3: Observed values of $\sqrt{\mu_{max,pH}}$ (symbol) compared with square root predictive equation (line).

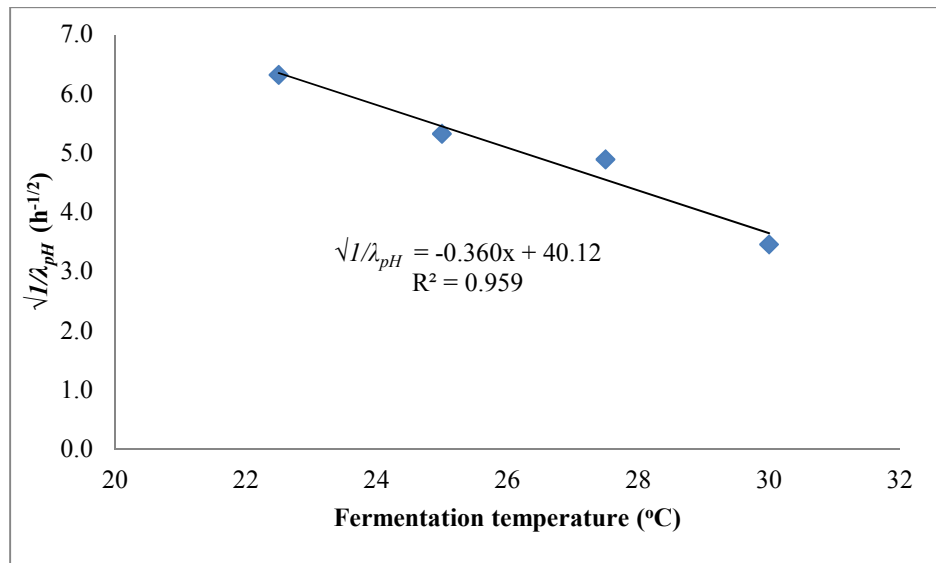


Figure 9.4: Observed values of $\sqrt{1/\lambda_{pH}}$ (symbol) compared with square root predictive equation (line).

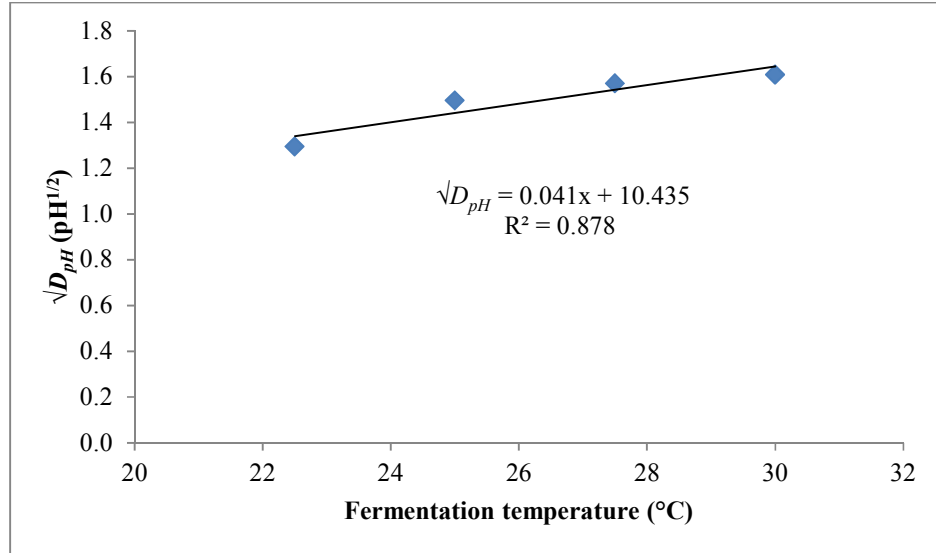


Figure 9.5: Observed values of $\sqrt{D_{pH}}$ (symbol) compared with square root predictive equation (line).

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Table 9.4: Parameters of the secondary models for the effect of temperature on pH decline.

Constants	α	θ_{min} (°C)	R^2
$\sqrt{\mu_{max, pH}}$	0.037 ((pH units) ^{1/2} h ^{-1/2} °C ⁻¹)	18.416	0.987
$\sqrt{1/\lambda_{pH}}$	-0.360 (h ^{-1/2} °C ⁻¹)	-40.117	0.959
$\sqrt{D_{pH}}$	0.041 ((pH unit) ^{1/2} °C ⁻¹)	-10.435	0.878

9.4.1.3 Validation

Validation of a mathematical model is crucial to confirm authenticity and provide confidence in the application of the model (Ding, et al., 2011). Validation here was based on pH data collected at temperatures other than those used in the development of the primary and secondary models: two temperatures within the fermentation temperature range (23 and 27°C; interpolation) and two temperatures outside the range (20 and 32°C; extrapolation). The pH profile at each of these four temperatures predicted using the primary model, in which the model parameters were estimated using the secondary models, was close to that observed, with a high R^2 (Figure 9.6). This validates the models.

When fermentation was carried out at below 25°C, the R^2 values for predicted versus observed pH were slightly lower, 0.9389 for fermentation temperatures of 23°C (interpolation, Figure 9.6a) and 20°C (extrapolation, Figure 9.6c) compared with the R^2 values for the two higher temperatures. At these lower temperatures, there were clear trends in the discrepancies between predicted and observed values, rather than just random discrepancies. The models developed may not be ideal for these lower temperatures; however the quality of product produced at these low temperatures is, in any case, unacceptable. The model should be limited to use within the temperature range 25 to 30°C.

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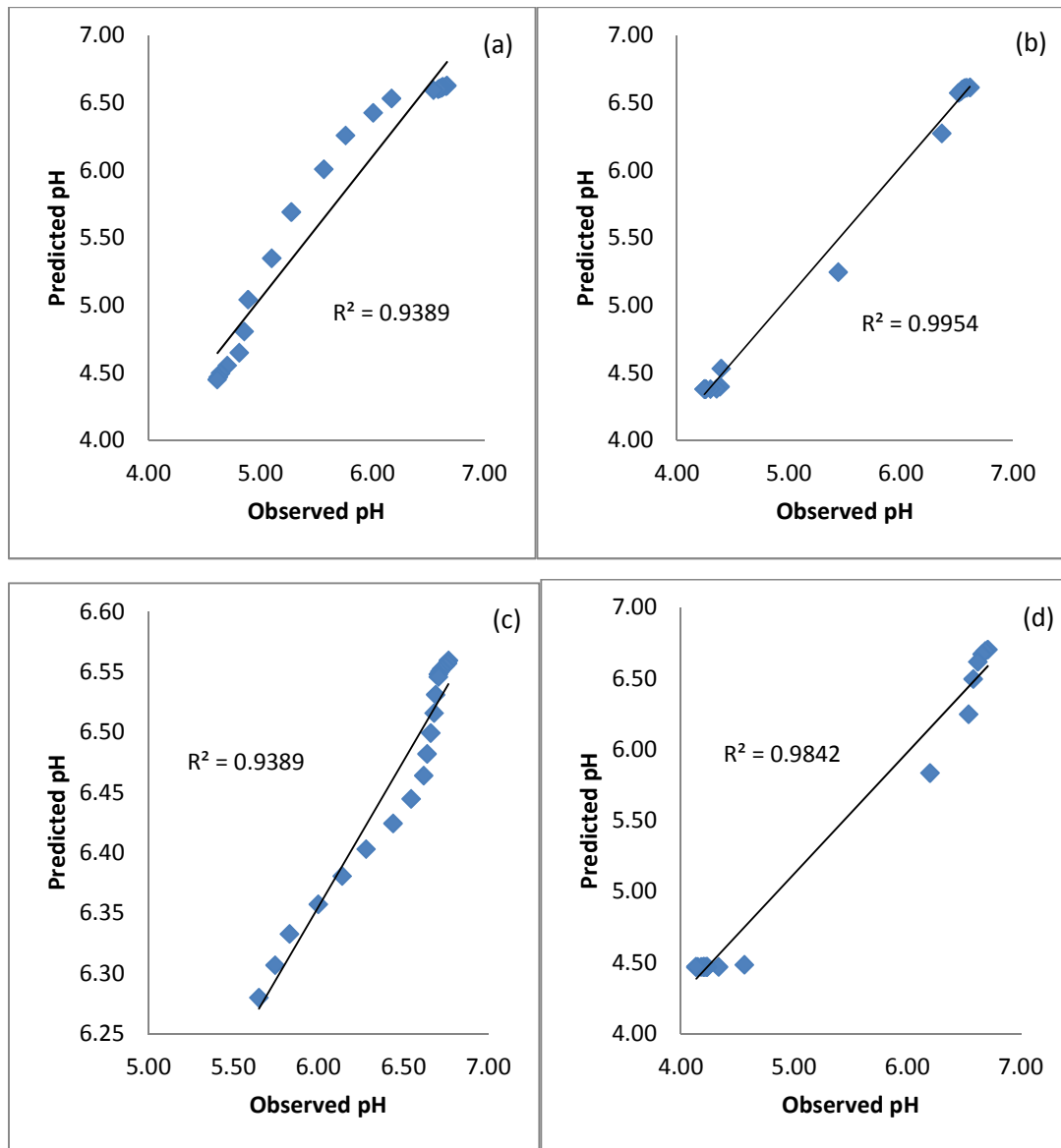


Figure 9.6: Predicted versus observed pH profiles during 168 h of fermentation at temperatures of (a) 23°C and (b) 27°C (interpolation), and (c) 20°C and (d) 32°C (extrapolation).

9.4.2 Modelling the effects of time and fermentation temperature on the growth of starter bacteria (*S. thermophilus* STM5 and *L. acidophilus* LA5) in yoghurt

9.4.2.1 Primary model

Starter bacteria (*S. thermophilus* STM5 and *L. acidophilus* LA5) growth in yoghurt was examined at four different fermentation temperatures (22.5, 25, 27.5 and 30°C) (Figure 9.7). As expected, $\mu_{max, sc}$ increased and λ_{sc} decreased with increasing fermentation temperature. The data sets were fitted with the modified Gompertz equation (Figure 9.8a), the modified logistic equation (Figure 9.8b) and the Baranyi equation (Figure 9.8c) as primary models. The fits of these three models were very similar (Figure 9.8 and Table 9.5). The parameter D_{sc} , for a given organism-equation, combination did not vary substantially (Table 9.5) with temperatures. Mean values were calculated in next section (section 9.4.2.2)

R^2 values were compared to find the best primary model on the basis of goodness of fit (Table 9.5). The values for the three models used were very close to each other, with those for the modified Gompertz model being slightly higher overall than those for the modified logistic and Baranyi models. The modified Gompertz equation was thus selected as the best primary model.

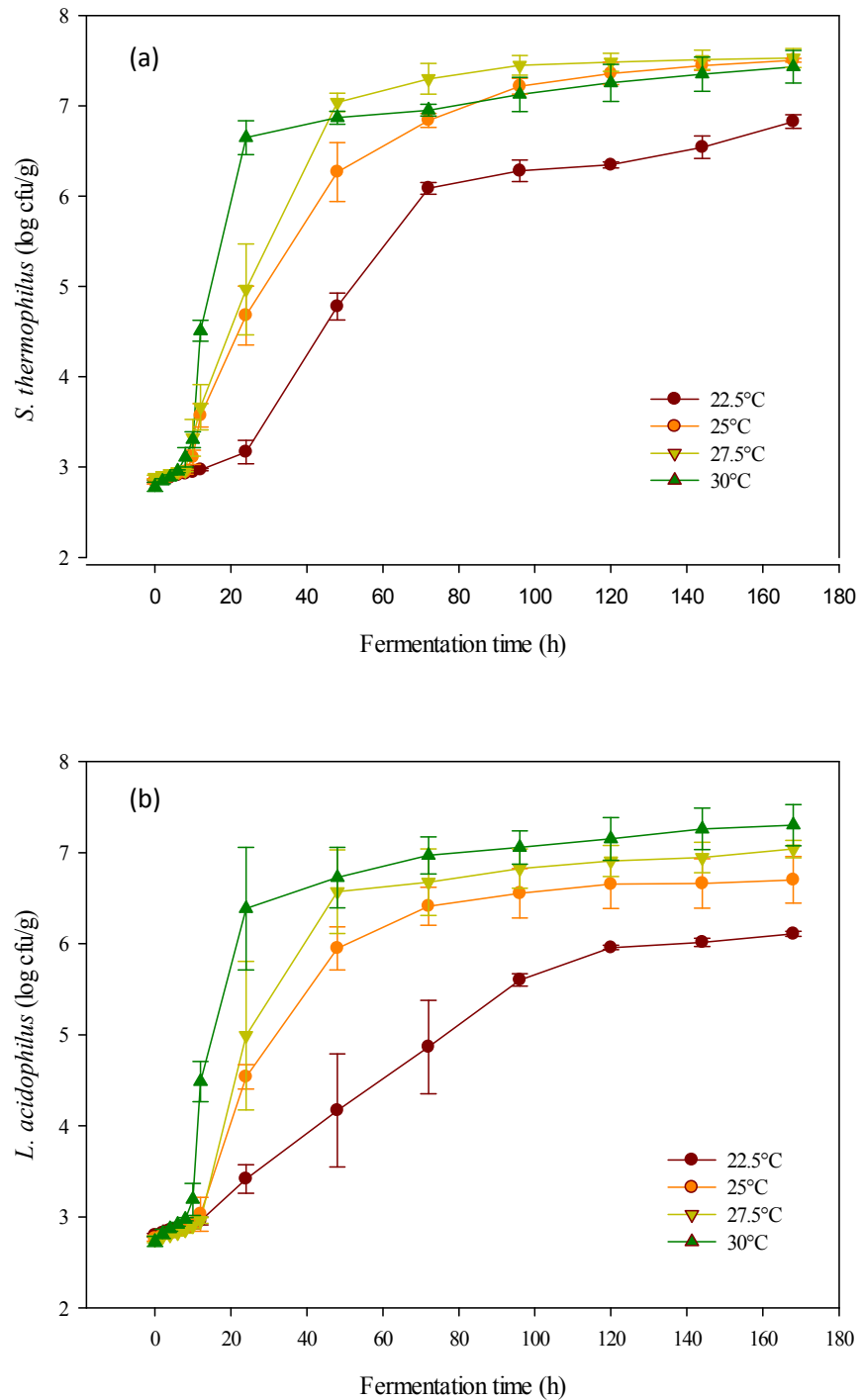


Figure 9.7: *S. thermophilus* STM5 growth (a) and *L. acidophilus* LA5 growth (b) in set culture yoghurt at four different fermentation temperatures: 22.5, 25, 27.5 and 30°C.

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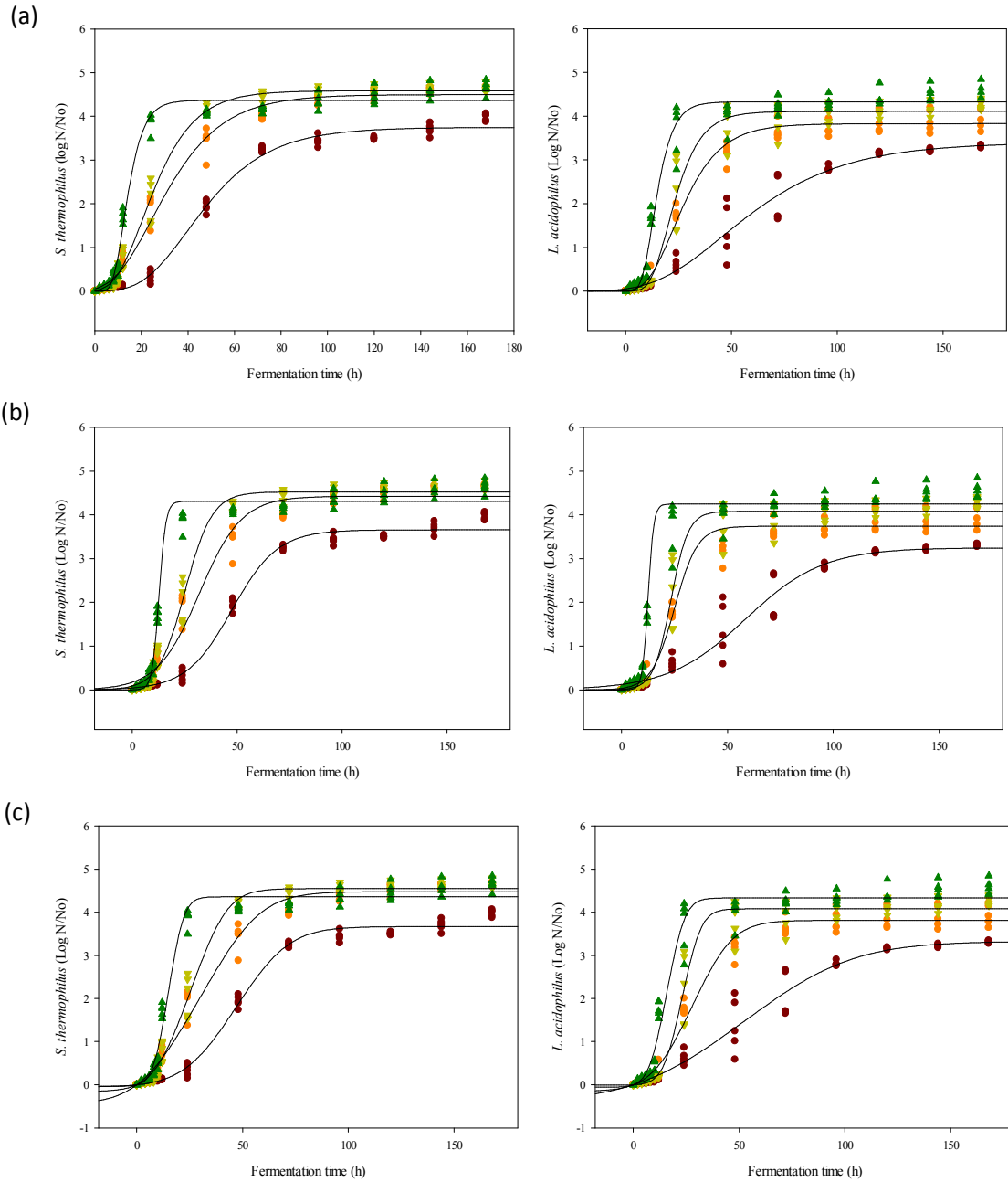


Figure 9.8: Experimental data (symbols) for starter bacteria (*S. thermophilus* STM5 and *L. acidophilus* LA5) growth in yoghurt compared with the fitted primary models (lines) (a) modified Gompertz equation , (b) modified logistic equation and (c) Baranyi equation (brown: 22.5°C; orange: 25°C; olive green: 27.5°C and green: 30°C).

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Table 9.5: Primary model parameters and R^2 values obtained by fitting the modified Gompertz, modified logistic and Baranyi equations to the experimental growth profiles of *S. thermophilus* STM5 and *L. acidophilus* LA5 in yoghurt made by fermentation at 22.5, 25, 27.5 and 30°C.

Model	Fermentation temperature (°C)	$\mu_{max,sc}$ (h^{-1})	λ_{sc} (h)	D_{sc}	h_o	R^2
<i>S. thermophilus</i> STM5						
Modified Gompertz	22.5	0.072	20.54	3.749		0.994
	25	0.099	7.951	4.499		0.990
	27.5	0.139	8.690	4.588		0.993
	30	0.342	7.735	4.367		0.989
Modified Logistic	22.5	0.084	25.76	3.658		0.994
	25	0.110	11.92	4.425		0.981
	27.5	0.175	12.28	4.525		0.990
	30	0.668	9.508	4.310		0.987
Baranyi	22.5	0.101	29.17	3.668	2.937	0.994
	25	0.101	9.862	4.476	0.994	0.988
	27.5	0.161	11.93	4.547	1.925	0.992
	30	0.369	9.146	4.357	3.376	0.986
<i>L. acidophilus</i> LA5						
Modified Gompertz	22.5	0.039	13.87	3.399		0.975
	25	0.114	10.10	3.829		0.989
	27.5	0.179	11.64	4.109		0.980
	30	0.300	7.476	4.328		0.980
Modified Logistic	22.5	0.045	22.14	3.249		0.970
	25	0.172	14.12	3.740		0.984
	27.5	0.250	15.06	4.080		0.979
	30	0.799	9.848	4.247		0.977
Baranyi	22.5	0.049	22.43	3.325	1.095	0.976
	25	0.134	14.31	3.810	1.923	0.985
	27.5	0.290	16.10	4.079	4.674	0.979
	30	0.316	9.096	4.331	2.878	0.977

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9.4.2.2 Secondary model

The parameter D_{sc} in the modified Gompertz equation did not vary substantially with temperature for either *S. thermophilus* STM5 or *L. acidophilus* LA5 (as was the case for the other equation-organism combinations) (Table 9.5). Secondary modeling was therefore not needed, and mean values were calculated: 4.30 for *S. thermophilus* STM5 and 3.92 for *L. acidophilus* LA5. The dependence of $\mu_{max, sc}$ and λ_{sc} on temperature was modelled by applying the square root equation to the Gompertz equation data for these parameters. Figures 9.9 and 9.10 show comparisons between observed values and the fitted models for *S. thermophilus* STM5 and *L. acidophilus* LA5, respectively. Table 9.6 shows the model parameters and goodness of fit in terms of R^2 values. The square root models fit the $\mu_{max, sc}$ data quite well, but the λ_{sc} data rather less well. McKellar and Knight (2000) also mentioned that the lag phase, λ , is difficult to determine accurately due to our poor understanding of the physiological events taking place during the adaptation of cells to new environments.

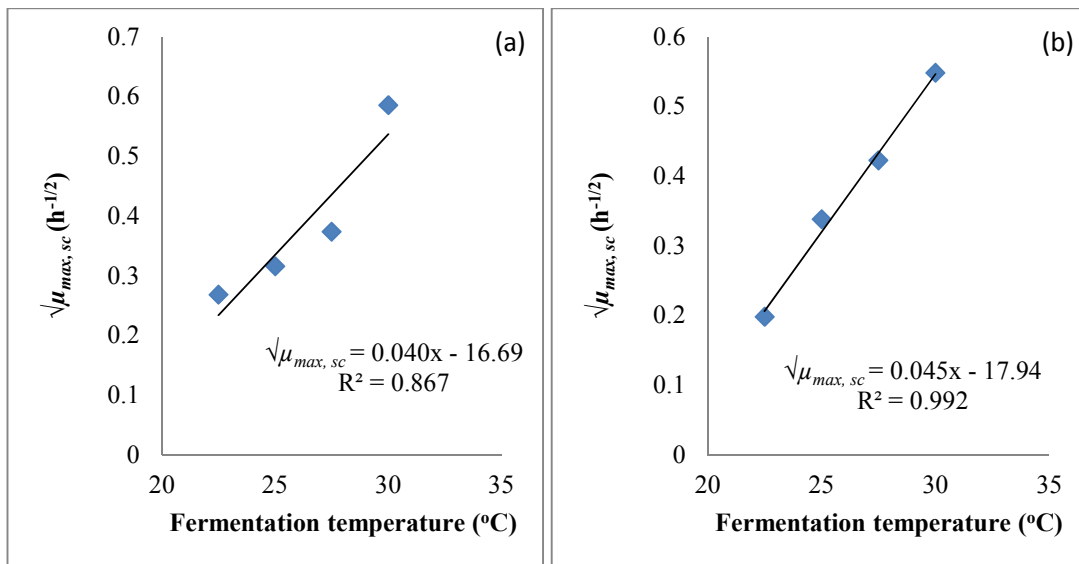


Figure 9.9: Observed values of $\sqrt{\mu_{max, pH}}$ (symbol) compared with square root predictive equation (line) for a) *S. thermophilus* STM5 and b) *L. acidophilus* LA5.

CHAPTER NINE: PREDICTIVE MODEL DEVELOPMENT AND VALIDATION FOR FERMENTATION OF SET CULTURE YOGHURT AS A MADE-IN-TRANSIT PRODUCT

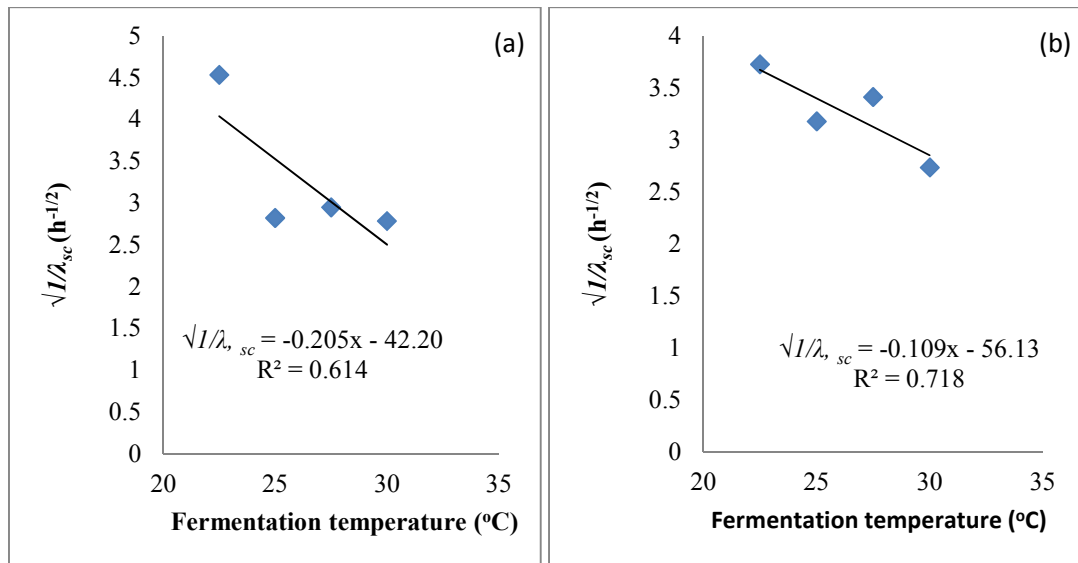


Figure 9.10: Observed values of $\sqrt{1/\lambda_{sc}}$ (symbol) compared with square root predictive equation (line) for a) *S. thermophilus* STM5 and b) *L. acidophilus* LA5.

Table 9.6: Constants value obtained from the regression equation.

Constants	$\alpha \text{ (h}^{-1/2}\text{oC}^{-1}\text{)}$	$\theta_{min} \text{ (}^\circ\text{C)}$	R^2
<i>S. thermophilus</i> STM5			
$\sqrt{\mu_{max, sc}}$	0.040	16.6881	0.867
$\sqrt{1/\lambda_{sc}}$	-0.205	42.201	0.614
<i>L. acidophilus</i> LA5			
$\sqrt{\mu_{max, sc}}$	0.045	17.941	0.992
$\sqrt{1/\lambda_{sc}}$	-0.109	56.131	0.718

9.4.2.3 Validation

Validation of the models for starter bacteria growth was carried out using growth data obtained in the same experiments as those used to provide validation data for the pH decline models (section 9.4.1.3). Thus, data were obtained at 23 and 27°C (interpolation) and at 23 and 27°C (extrapolation). The predicted *S. thermophilus* STM5 and *L. acidophilus* LA5 growth profiles during the 168 h of fermentation were close to the observed profiles, with high R^2 values for both interpolation (Figure 9.11) and extrapolation (Figure 9.12). *S. thermophilus* STM5 at temperatures of 23, 27 and 32°C and *L. acidophilus* LA5 at 23°C reveal clear trends in the discrepancies between predicted and observed values, rather than just random discrepancies.

The model (modified Gompertz with square root model) was observed not only to predict starter culture growth within the fermentation temperature used for model development but also produced a high R^2 (>0.9) for the data predicted and observed for extrapolation (20 and 32°C).

CHAPTER NINE: PREDICTIVE MODEL DEVELOPMENT AND VALIDATION FOR FERMENTATION OF SET CULTURE YOGHURT AS A MADE-IN-TRANSIT PRODUCT

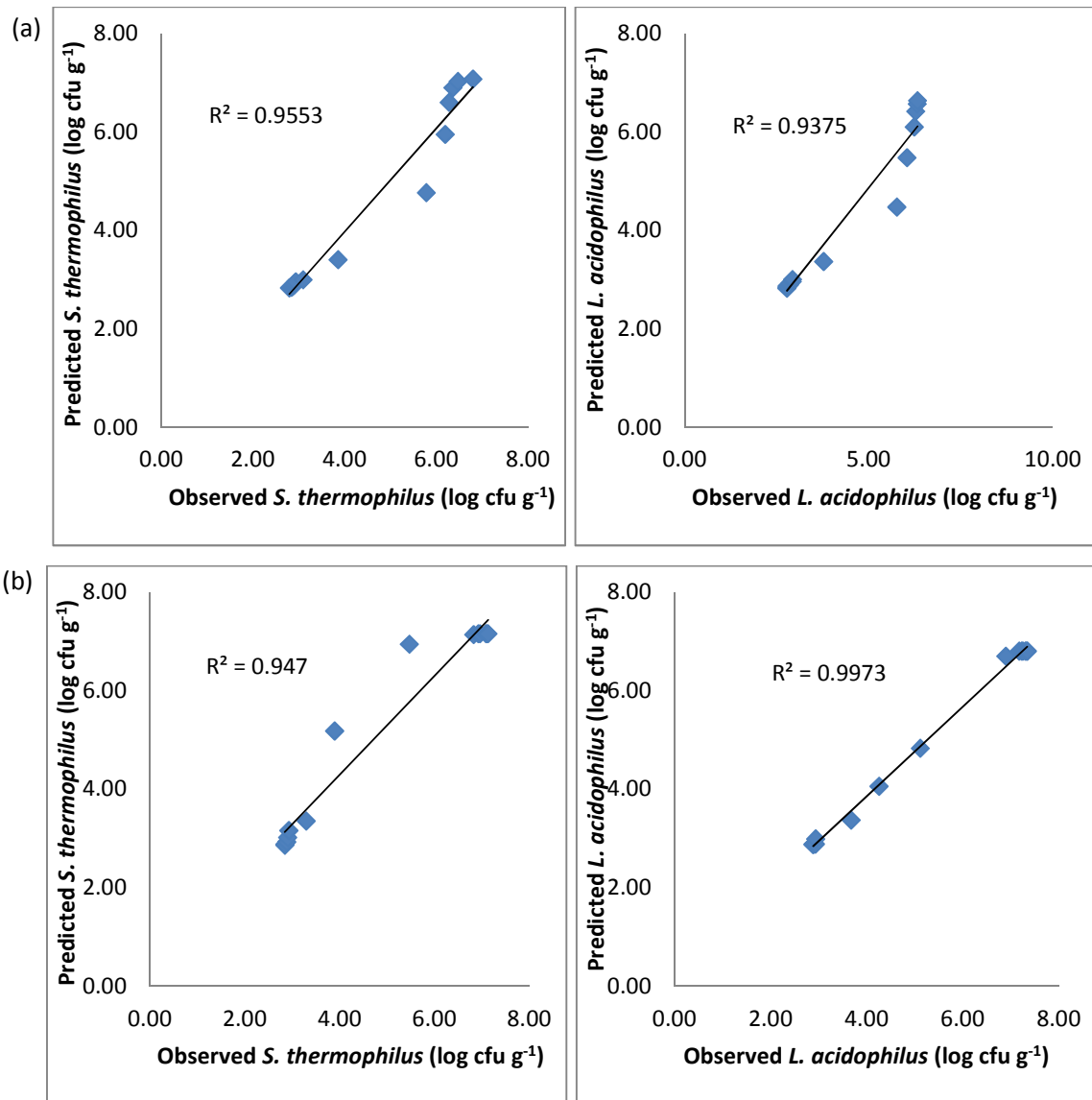


Figure 9.11: Predicted versus observed growth profiles for *S. thermophilus* ST5 and *L. acidophilus* LA5 during 168 h of fermentation at temperatures (a) 23°C and (b) 27°C (interpolation).

CHAPTER NINE: PREDICTIVE MODEL DEVELOPMENT AND VALIDATION FOR FERMENTATION OF SET CULTURE YOGHURT AS A MADE-IN-TRANSIT PRODUCT

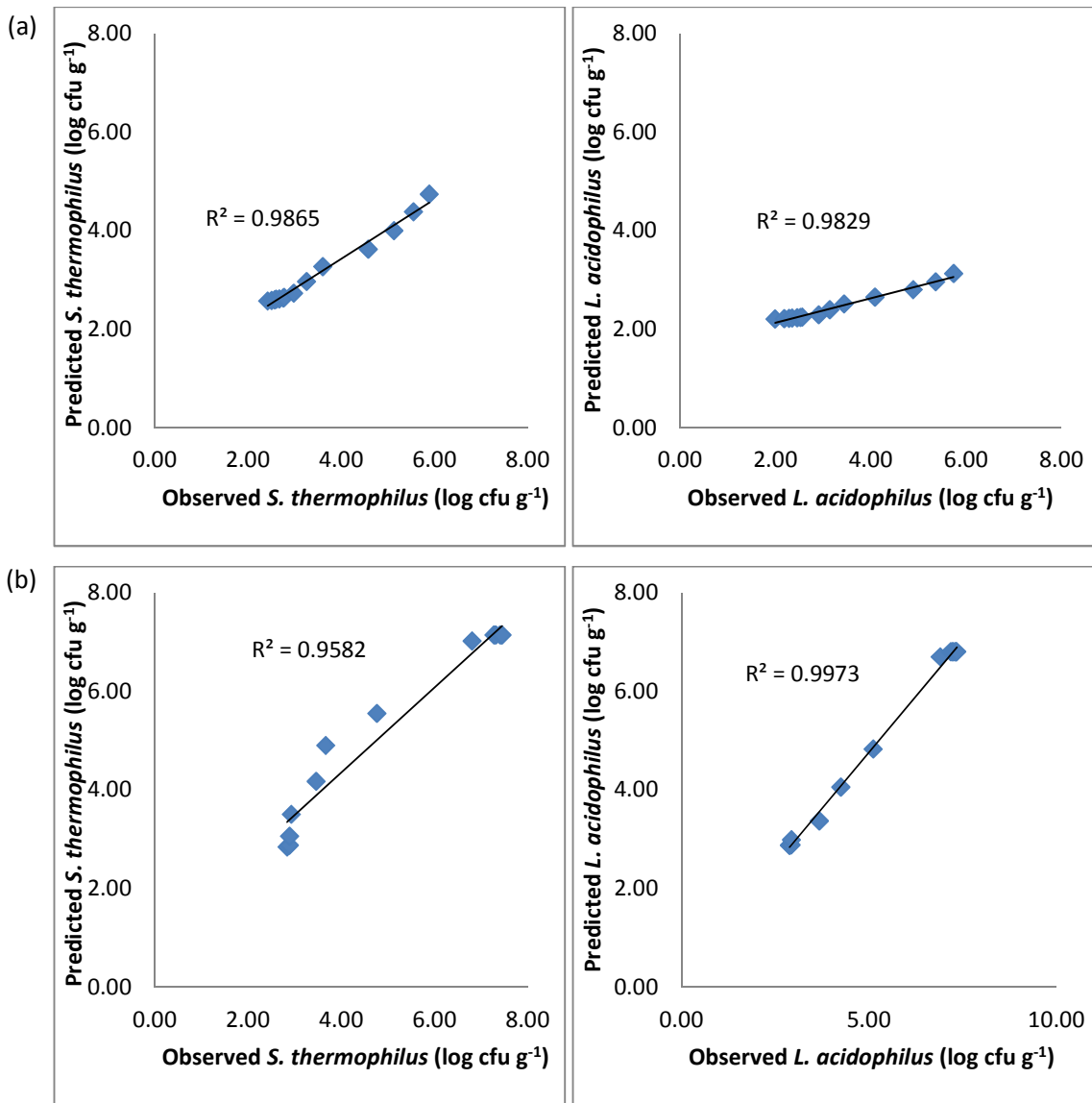


Figure 9.12: Predicted versus observed growth profiles for *S. thermophilus* STM5 and *L. acidophilus* LA5 during 168 h of fermentation at temperatures of (a) 20°C and (b) 32°C (extrapolation).

9.4.3 Modelling the effect of time and fermentation temperature on yoghurt texture in term of firmness.

9.4.3.1 Primary model

The firmness of yoghurt was measured at different fermentation temperatures (25, 27.5 and 30°C) (Figure 9.13). Milk bases fermented at 22.5°C did not completely gel after 168 h of fermentation. Data of yoghurt firmness were only fitted into the primary models of modified Gompertz and modified logistic (Figure 9.14). The Baranyi model was found not to work for these data, probably due to the less sigmoidal shape. The $\mu_{max,F}$ and λ_F increased and reduced, respectively, as expected with increasing of fermentation temperature (Table 9.7). This result was in line with the outcome for acidification and a starter culture growth model, since higher fermentation temperature induces the yoghurt acidification, which also influences the yoghurt firmness. The parameter also increased with the fermentation temperature especially at 30°C. Then, $\mu_{max,F}$, λ_F and D_F values were taken to the secondary model. High R^2 values, 0.994, 0.983 and 0.959 for 25, 27.5 and 30°C, respectively were obtained for data fitted into the modified Gompertz model compared to modified logistic (Table 9.7).

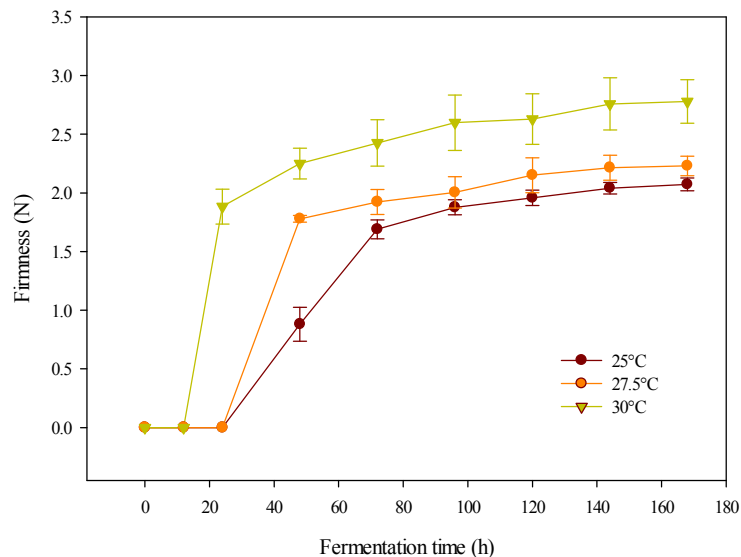


Figure 9.13: Firmness of set culture yoghurt at the different fermentation temperatures (22.5, 25, 27.5 and 30°C).

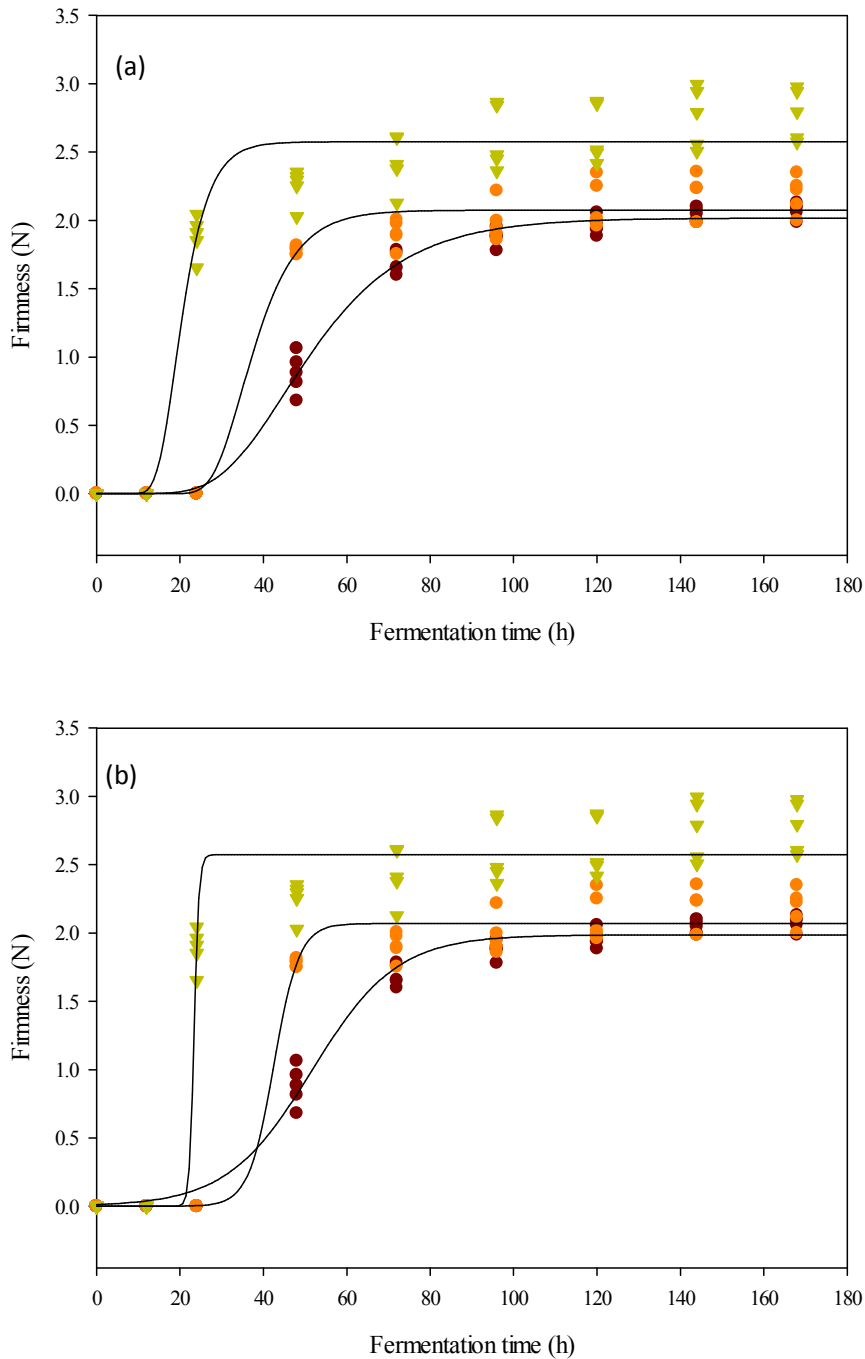


Figure 9.14: Experimental data (symbols) for firmness development in yoghurt compared with the fitted primary models (lines) (a) the modified Gompertz equation, (b) the modified logistic equation (brown: 25°C; orange: 27.5°C and olive green: 30°C).

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Table 9.7: Primary model parameters and R^2 values obtained by fitting the modified Gompertz and modified logistic equations to the experimental firmness development profiles in yoghurt made by fermentation at 25, 27.5 and 30°C.

Model	Fermentation temperature (°C)	$\mu_{max, F}$ (N h ⁻¹)	λ_F (h)	D_F (N)	R^2
Modified Gompertz	25	0.047	29.50	2.017	0.994
	27.5	0.107	27.80	2.074	0.983
	30	0.214	14.48	2.575	0.959
Modified Logistic	25	0.049	31.32	1.986	0.989
	27.5	0.172	36.50	2.069	0.983
	30	1.130	22.29	2.574	0.959

9.4.3.2 Secondary model

The $\mu_{max, F}$, λ_F and D_F values for yoghurt firmness at the three fermentation temperatures obtained from fitting the modified Gompertz were used (Table 9.7) to develop secondary models to describe the effect of fermentation temperature on yoghurt firmness. Again, square root equations were found adequate.

The observed $\mu_{max, F}$, λ_F and D_F values are compared with the fitted lines in Figures 9.15, 9.16 and 9.17. The model parameters are shown in Table 9.8.

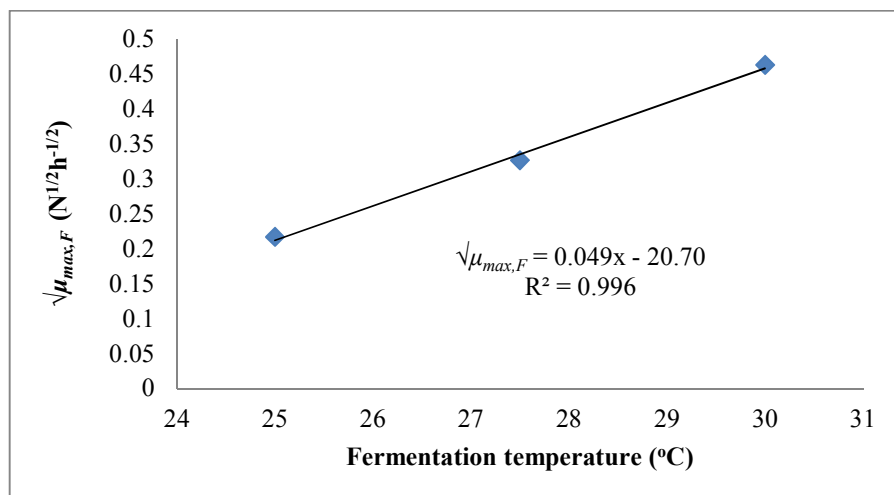


Figure 9.15: Observed values of $\sqrt{\mu_{max, F}}$ (symbol) compared with square root predictive equation (line).

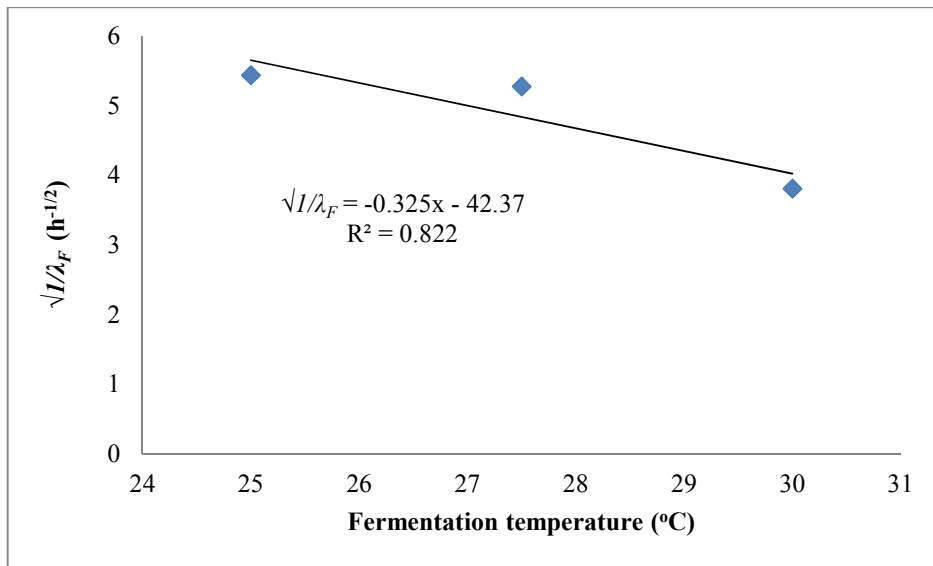


Figure 9.16: Observed values of $\sqrt{1/\lambda_F}$ (symbol) compared with square root predictive equation (line).

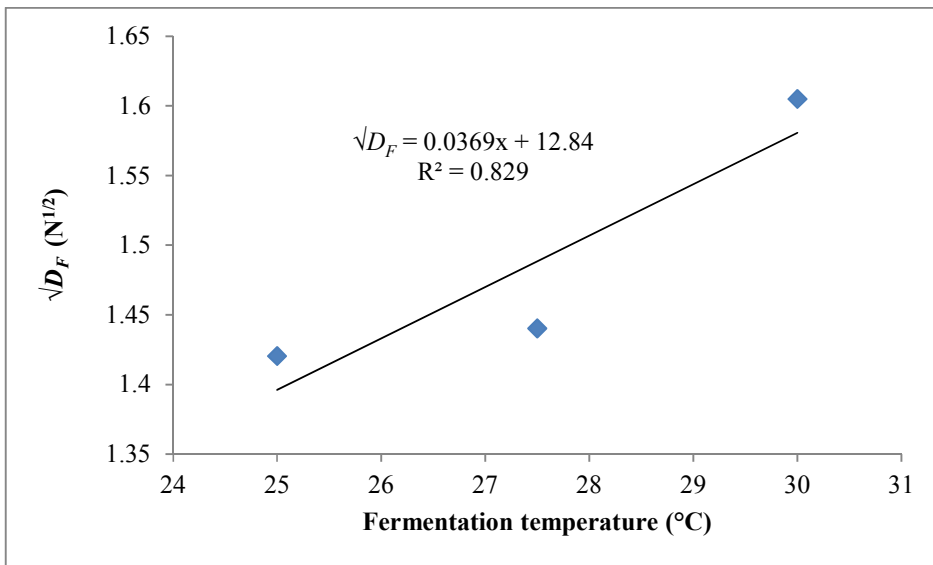


Figure 9.17: Observed values of $\sqrt{D_F}$ (symbol) compared with square root predictive equation (line).

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Table 9.8: Constants value obtained from the regression equation.

Constants	a	θ_{min} (°C)	R^2
$\sqrt{\mu_{max,F}}$	0.049 ($N^{1/2}h^{-1/2}C^{-1}$)	20.695	0.996
$\sqrt{1/\lambda_F}$	-0.325 ($h^{-1/2}C^{-1}$)	42.367	0.822
$\sqrt{D_F}$	0.036 ($N^{1/2}C^{-1}$)	-12.842	0.829

9.4.3.3 Validation

Validation of the models for firmness was carried out using firmness data obtained in the same experiments as those used to provide validation data for the pH decline models (section 9.4.1.3) (Figure 9.18). However, firmness development (gel development) did not occur at 20°C (extrapolation) or 23°C (interpolation). Thus the models could be tested only at 27°C (interpolation) and 32°C (extrapolation). The predicted firmness profiles at 27 and 32°C were close to the observed values, with high R^2 values (Figure 9.18). This demonstrates that the models' ability to predict firmness within and outside the fermentation temperature range used in developing the model provided fermentation temperature is above about 25°C.

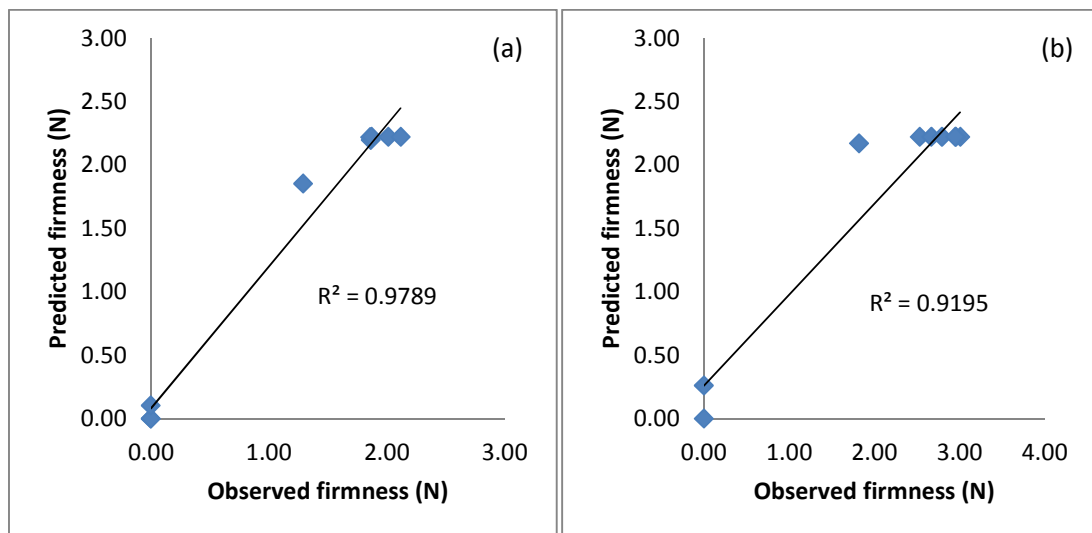


Figure 9.18: Predicted versus observed yoghurt firmness during 168 h of fermentation at temperatures of (a) 27°C and (b) 32°C.

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9.5 Conclusion

Yoghurt acidification and starter bacteria growth are best described by the modified logistic and modified Gompertz equations, respectively. Yoghurt firmness is best described by the modified Gompertz equation. Combining these primary models with the square root equation based secondary models provides satisfactory to good predictions of pH decline, starter bacteria growth and firmness development during fermentation as functions of temperature. Validation revealed that the models were capable of reasonably accurate predictions both within and beyond the temperature range used in model development. This suggests that the models would be suitable for setting temperatures to be used in the distribution of MIT yoghurt, and for predicting, at least approximately, the effects of temperature fluctuations during transit. The modelling of non-isothermal microbial growth, and the problems and solutions inherent in this, are discussed by van Boekel (2009).

This is the first time that the modelling of key fermentation parameters has been developed as a tool for an MIT food.

9.6 References

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CHAPTER NINE: PREDICTIVE MODEL DEVELOPMENT AND VALIDATION FOR FERMENTATION OF SET CULTURE YOGHURT AS A MADE-IN-TRANSIT PRODUCT

CHAPTER TEN : GENERAL DISCUSSION AND CONCLUSION

10.1 Development of set yoghurt as a MIT product

The feasibility of the made-in-transit (MIT) concept for manufacturing yoghurt was successfully investigated. It has been shown that extending the fermentation time from several hours to several days (168 h) is achievable by modification of starter culture composition, inoculum size and fermentation temperature, proving the thesis hypothesis. Impedance studies (Chapter 4) with three combinations of starter bacteria (*S. thermophilus* in co-culture with *L. delbrueckii* subsp. *bulgaricus* (STLB), with *L. acidophilus* (STLA) and with *L. casei* (STLC)) at various fermentation temperatures and inoculum sizes showed that all three combinations could grow at low temperature. This indicated that yoghurt could feasibly be made using an extended fermentation time at temperatures much lower than the conventional fermentation temperature of about 42°C.

Feasibility was confirmed by making yoghurts with varying starter bacteria combinations, inoculum sizes and fermentation temperatures (Chapter 4). It was found that the combination STLA, an inoculum size of 0.2% (v/v) and a fermentation temperature of 25°C was the most suitable in terms of extending the fermentation time. However, the final pH at 168 h was, at 4.77, somewhat higher than that desirable for yoghurt (pH 4.2-4.6). Further, gelation failed to occur in spite of the heat treatment (UHT sterilization) to which the milk base was subjected; heat treatment is expected to cause interactions between whey proteins and casein micelles which in turn is expected to result in the formation of a stronger gel, with gelation starting at a pH higher than 4.6 (Lucey & Singh, 1998).

Nevertheless, the set of conditions stated above was deemed to have the potential to be used for producing an acceptable yoghurt in 168 h of fermentation. UHT sterilization of the milk base was considered crucial as this ensures that competitors of starter bacteria are absent during the long fermentation (168 h).

The next step in this research was aimed at improving yoghurt texture for the otherwise successful starter culture/inoculum size/fermentation temperature combination identified in Chapter 4 by increasing the concentration of skim milk powder (SMP) in the milk base (Chapter 5). As expected, this did improve yoghurt texture (in terms of measured firmness). However, it was

considered likely to be uneconomical to produce yoghurt with very high concentrations (up to 18 and 20% (w/v)) of SMP. This realization led to the work described in Chapter 6, where the effects of fortifying the yoghurt milk base with relatively small amounts of high protein dairy ingredients was investigated. The five dried dairy ingredients used were skim milk powder (SMP), buttermilk powder (BMP), whey protein concentrate (WPC), milk protein concentrate (MPC) and sodium caseinate (NaCN). As yoghurt gelation is mainly the consequence of the formation of a protein network, it was thought that the selection of fortifying materials with relatively high protein contents could assist in improving the yoghurt texture cost effectively. The 12% of SMP in the milk base studied provided a protein level of 4.0% (w/w). Then, this milk base was fortified with an extra 1% protein contributed by the fortifying material, giving a total protein level in the yoghurt milk base of 5.0% for each dried dairy ingredient used.

The effects of two different heat treatments, UHT sterilization alone and a combination of UHT sterilization and a high temperature long time heat treatment were investigated at the same time. This was done in order to see if the increased degree of whey protein denaturation expected with the double heat treatment would help to improve yoghurt texture; Krasaekoopt, Bhandari & Deeth (2004) found that the lower extent of whey protein denaturation in UHT milk than in the case of more conventionally heat treated milk (85°C for 30 min) resulted in a poorer yoghurt texture.

Overall, the work described in Chapter 6 showed that the effect of heat treatment was small compared with the effect of fortifying material, and that UHT sterilization was sufficient as the milk base heat treatment. Fortifying material had relatively large effects. MPC and NaCN were found to be the most promising in terms of improving yoghurt firmness, but they accelerated fermentation, leading to pHs lower than acceptable after 168 h of fermentation.

To solve this problem, optimization experiments were carried out (Chapter 7) to find combinations of starter culture composition, inoculum size and fermentation temperature for these two fortifying materials that would result in an acceptable final pH. The best combinations identified were (1) 0.002% of STLA, fortified with NaCN and (2) 0.2% of STLA, fortified with MPC, both fermented at 25°C. These conditions gave acceptable final pHs and firmness while maintaining the extended fermentation time of 168 h.

In order to discover whether the yoghurts produced using these two sets of conditions were not only acceptable in term of final pH and firmness, but also in terms of sensory quality and storage stability, the studies reported in Chapter 8 were carried out. Yoghurt microstructure was also examined. Sensory evaluation was carried out using trained panelists (descriptive testing) and consumer participants (acceptance testing). Based on the descriptive testing, the two yoghurts produced as MIT products had quite similar attributes, but very different from the standard yoghurt in terms of some attributes. This was not surprising in view of the differences in terms of formulation and fermentation conditions. Acceptance testing showed that, overall, the two MIT yoghurts were equally acceptable, and more acceptable than standard yoghurt. The storage stability of the MIT yoghurts was satisfactory.

The studies described in Chapters 4-8 have shown that it is possible to make yoghurt using an extended fermentation time of 168 h that has a satisfactory final pH, acceptable sensory characteristics, and suitable storage stability, and which thus has potential as an MIT product.

10.2 Development of mathematical models for predicting the effects of time and temperature during MIT yoghurt manufacture

As the production of MIT yoghurt involves a relatively long, temperature dependent fermentation, it was considered useful to develop models for predicting the effects of both time and temperature on such dependent variables as starter bacteria count, pH, and firmness. As changes in these variables depend ultimately on starter bacteria activity, a predictive microbiology approach was taken. The MIT yoghurt made using the combination STLA/inoculum size 0.002% (v/v)/NaCN-fortified was used in the experiments carried out to generate the data required.

Three autonomous primary models were trialled: the modified logistic equation, the modified Gompertz equation and the Baranyi equation, all suitable for fitting sigmoidal curves. The time dependence at constant temperature of starter bacteria growth and pH decline was found to be well modelled by all three equations, with the modified Gompertz equation being best for starter bacteria growth, and the modified logistic equation for pH decline. The modified Gompertz equation described firmness development slightly better than did the modified logistic equation; in this case, the Baranyi equation could not be fitted to the experimental data.

The parameters of the best models were related to temperature using the square root (Ratkowsky) equation as a secondary model. Combination of the primary and secondary models provides means of making predictions of the effects both time and temperature simultaneously. Comparison of predictions with data generated in extrapolation and interpolation experiments proved the efficacy of the models.

Such models are useful in designing fermentation conditions, and in predicting the effects of temperature variation during fermentation.

10.3 Recommendations for future study

The following are recommendations for further work necessary for bringing the concept of MIT yoghurt to commercial realization.

Fluctuations in fermentation temperature

In order to check that the predictive models developed in this study are useful under real conditions, validation using data obtained under fluctuating fermentation temperature conditions should be attempted. While, strictly speaking, dynamic models should be used for fluctuating temperature conditions (van Boekel, 2009), it is expected that the models described in Chapter 8 would be adequate for situation where temperature fluctuations are slow.

Impact of transportation

It is important to understand the effects of transportation conditions such as vibration or mechanical agitation on the in-fermentation yoghurt, especially yoghurt firmness. The application of suitable cushioning could also be studied if necessary.

Production of fruit containing and flavoured yoghurt

The market of fruit containing and flavoured yoghurt is wider than that for plain yoghurt. Therefore, the production of MIT yoghurt with added fruit or flavour will increase marketing

CHAPTER TEN: GENERAL DISCUSSION AND CONCLUSION

possibilities provided the extended fermentation time can be maintained. This should be checked.

Mechanisms of extended fermentation

It would be interesting and potentially useful to investigate the mechanisms of pH decline, firmness development, etc, during low temperature long time fermentation. This could lead to the development of mechanistic and thus potentially better predictive models.

Alternative fermented foods as MIT products

Other fermented foods could be investigated as potential MIT products as well. For instance, yoghurt drink has big potential and should be easier to develop, especially in terms of texture.

10.4 References

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Appendix 5a – Statement of Contribution to Doctoral Thesis Containing Publications

DRC 16



MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Nor Khaizura Ab Rashid

Name/Title of Principal Supervisor: Assoc Prof Steve Flint

Name of Published Research Output and full reference:

Development of made-in-transit set culture yoghurt: effect of increasing the concentration of reconstituted skim milk as the milk base

In which Chapter is the Published Work: Chapter 5

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: **80%**
and / or
- Describe the contribution that the candidate has made to the Published Work:

Nor Khaizura Ab Rashid
Digitally signed by Nor Khaizura Ab Rashid
DN: cn=Nor Khaizura Ab Rashid, o=Massey
University, ou=GRS, email=nor.khaizura@massey.ac.nz,
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Candidate's Signature

07/01/2013

Date

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Date: 2013.01.07 12:01:45 +1300

Principal Supervisor's signature

07/01/2013

Date

Appendix 8a - Descriptive Test Panel Interest Survey Form

SENSORY EVALUATION PANEL INTEREST SURVEY

The aim of this survey is to gauge the interest of people involved in panels to assess the organoleptic qualities of set yoghurt.

Please complete the form below, placing a tick in the box that is applicable.

All information will remain confidential.

Title & Name: _____

Date: _____

Contact no.: _____

Email address: _____

1. Are you interested in being trained for taste panel work?

Yes

No

2. Will you be available for panel tasting for the following dates and time?

Available

Unavailable

Date	Time
*22nd June 2011 OR 23rd June 2011	1pm - 3pm
4th July 2011	9am - 11am
4th July 2011	1pm - 230pm
8th July 2011	9am - 11am
11th July 2011	9am - 11am

*please state which date you are available:

4. Gender:

- Male
- Female

5. Age:

- 21 - 30
- 31 - 40
- 41 - 50
- 51 - 60
- 61 & above

6. Occupation:

Thank you for your time.

Appendix 8b - Screening Test Taste Panel Information Sheet

TASTE PANEL INFORMATION SHEET

Please complete the form below, placing a tick in the box/boxes that apply.

All information will remain confidential.

Name: _____

Date: _____

HEALTH:

Do you have any of the following:

- Dentures
- Diabetes
- Oral or gum disease
- Hypoglycaemia
- Food Allergies (if yes, please specify: _____)
- Hypertension
- Frequent colds / sinus problems

Do you take any medication or have any condition which affect your senses, especially taste and smell?

- Yes (if yes, please specify: _____)
- No

Are you colour blind?

- Yes
- No

Do you know of any taste insensitivities / blindness that you may have?

Yes (if yes, please specify: _____)

No

Do you currently smoke?

Yes

No

If you smoke, how often do you smoke?

Do not smoke regularly

Smoke 1 to 10 per day

Smoke 11 to 20 per day

Smoke over 20 per day

FOOD HABITS:

Are you currently on a restricted diet? If yes, please explain:

Are there any foods that you cannot eat for medical and/or religious reasons? (List foods)

What are your favourite foods?

Do you have strong food dislike or preferences?

How often do you consume yoghurt?

- Never
- Daily
- Every 3 days
- Once weekly
- Once fortnightly
- Once monthly
- Others (please specify: _____)

Is your ability to distinguish smell and tastes:

	SMELL	TASTE
Better than average	<input type="checkbox"/>	<input type="checkbox"/>
Average	<input type="checkbox"/>	<input type="checkbox"/>
Worse than average	<input type="checkbox"/>	<input type="checkbox"/>

FLAVOUR QUIZ:

If a recipe calls for thyme and there is none available, what would you substitute?

What are some other foods that taste like yoghurt?

How would you describe the difference between flavour and aroma?

What is the best or two word description of grated Italian cheese?

Describe some of the noticeable flavours in mayonnaise

Describe some of the noticeable flavours in sausage

Describe some of the noticeable flavours in corn flakes

Describe some noticeable flavours in coke

☺ **THANK YOU FOR YOUR PARTICIPATION** ☺

Appendix 8c - Identification of Basic Taste Test

Name: _____

Date: _____

In front of you are samples containing solutions representing the basic taste sensations. One or more of these solutions may be a blank or repeat. Your task is to identify the perceived basic taste (sweet, salty, sour, bitter) and intensity (weak, mild or strong).

Sample each solution in the order provided, taking a mouthful (approximately 15 ml) of each.

Take approximately 30 seconds per sample, but do not go back to the previously sampled vessels. You may rinse out your mouth at the beginning and end of the sample set with the water provided.

Code number	Taste description (sweet, salty, sour, bitter or taste not identified)	Intensity (weak, mild, strong or no taste)

☺ **THANK YOU FOR YOUR PARTICIPATION** ☺

Appendix 8d - Ranking of Basic Taste Test

Name: _____

Date: _____

You are presented with 4 set of samples, please taste the following samples and **rank** them in order of **increasing** intensity (lowest to highest) for four different attributes: sweetness, sourness, saltiness and bitterness.

1. SWEETNESS:

692

540

298

816

Least Sweet _____

Most Sweet _____

2. SOURNESS:

312

825

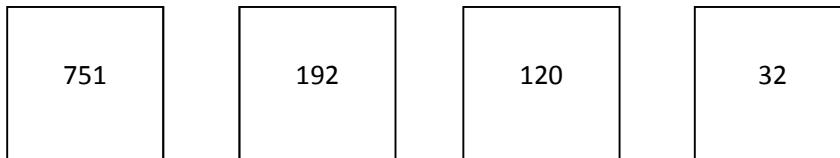
226

939

Least Sour _____

Most Sour _____

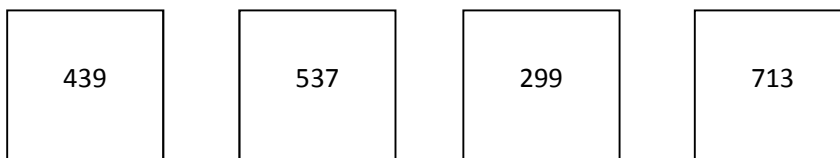
3. SALTINESS:



Least Salty _____

Most Salty _____

3. BITTERNESS:



Least Bitter _____

Most Bitter _____

☺ **THANK YOU FOR YOUR PARTICIPATION** ☺

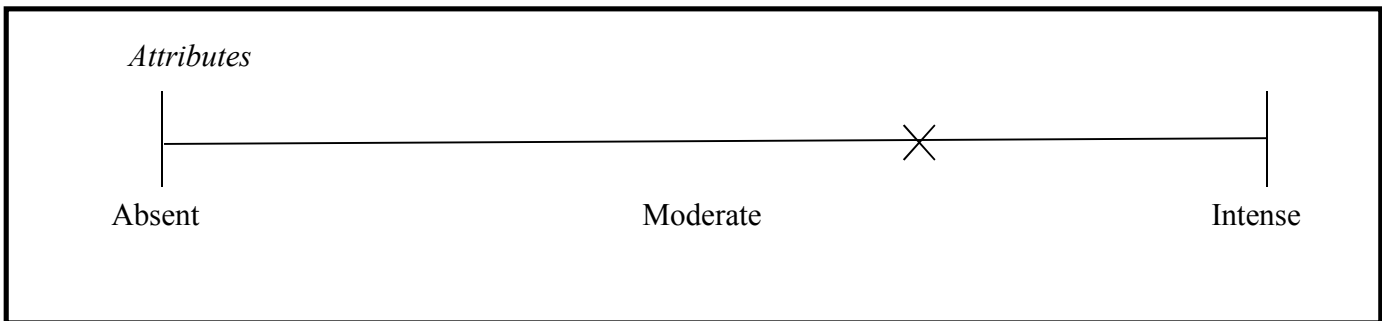
Appendix 8e - Descriptive Test Attribute Score Sheet

Name: _____

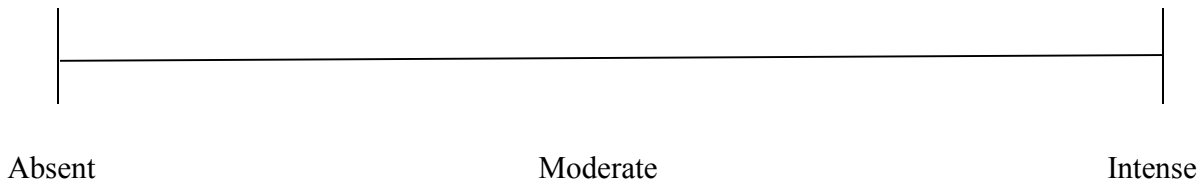
Sample code: ____

Instructions: Please rinse your mouth with the water provided before evaluating the sample. Please evaluate the sample using the attributes and scale below by marking a **cross** for the intensity you perceived on the line scale as shown below:

EXAMPLE:



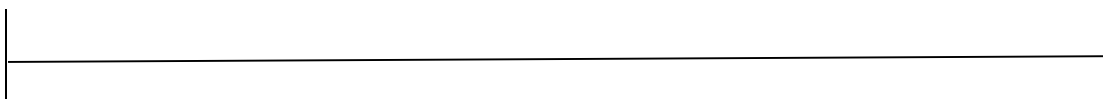
Appearance: Syneresis



Appearance: Smoothness



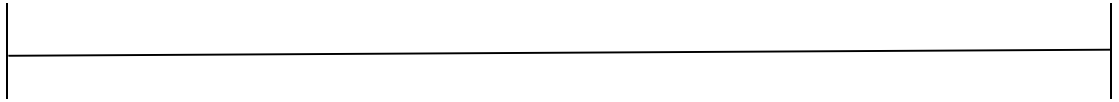
Appearance: Colour



White

Cream

Aroma: Sourness

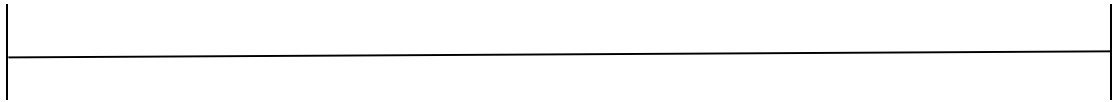


Absent

Moderate

Intense

Texture with *spoon* before stirring: Syneresis

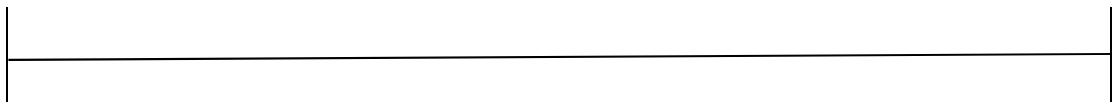


Absent

Moderate

Intense

Texture with *spoon* before stirring: Flowability

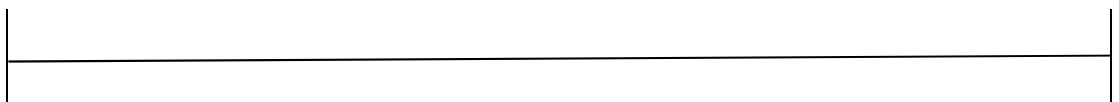


Absent

Moderate

Intense

Texture with *spoon* before stirring: Softness

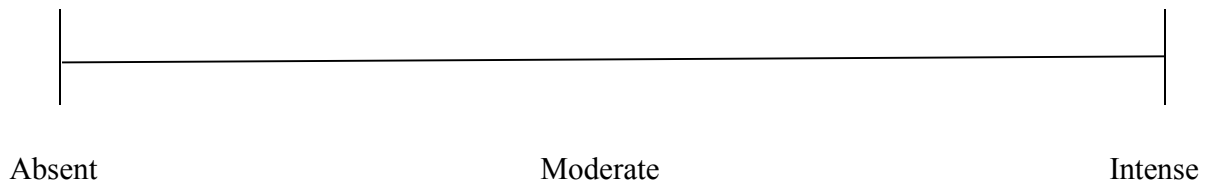


Hard

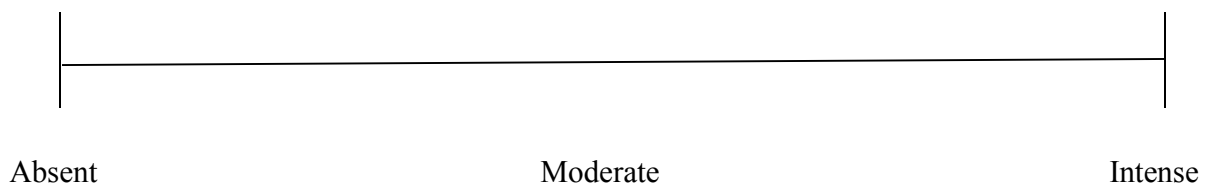
Moderate

Soft

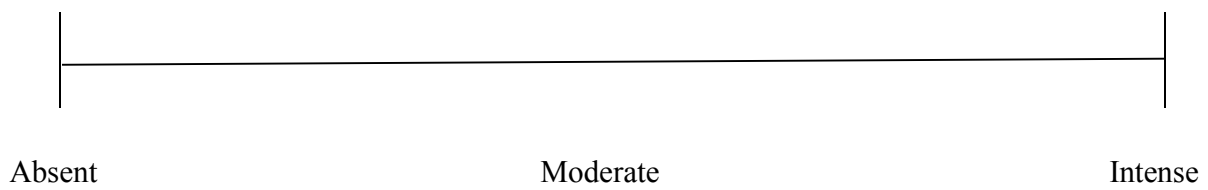
Texture in *mouth* before stirring: Thickness (mouth coating)



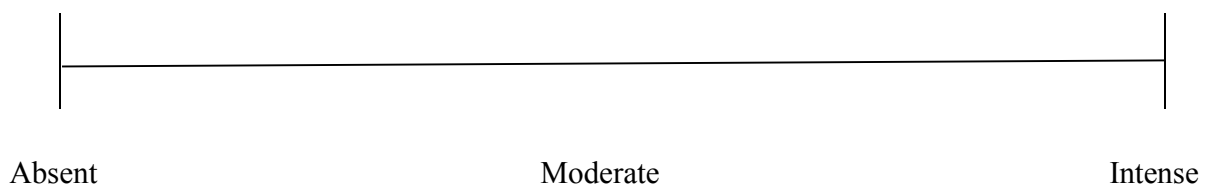
Texture in *mouth* before stirring: Stickiness



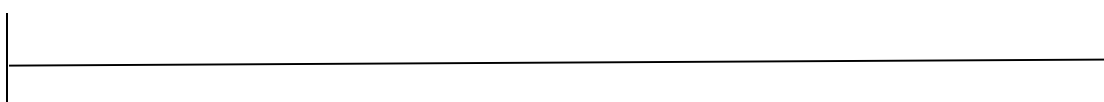
Texture in *mouth* before stirring: Graininess



Appearance after stirring: Lumpy



Texture with *spoon* after stirring: Syneresis

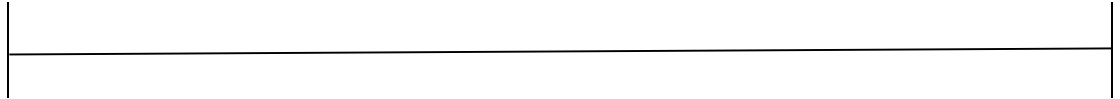


Absent

Moderate

Intense

Texture with *spoon* after stirring: Flowability

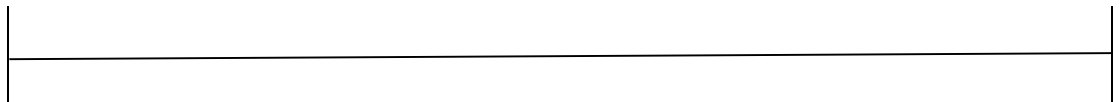


Absent

Moderate

Intense

Texture with *spoon* after stirring: Softness

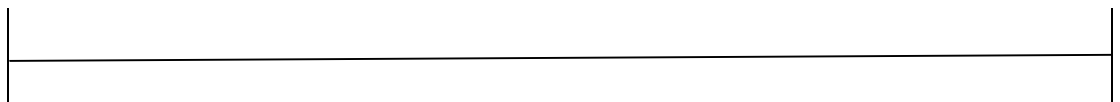


Hard

Moderate

Soft

Texture in *mouth* after stirring: Thickness (mouth coating)



Absent

Moderate

Intense

Texture in *mouth* after stirring: Stickiness

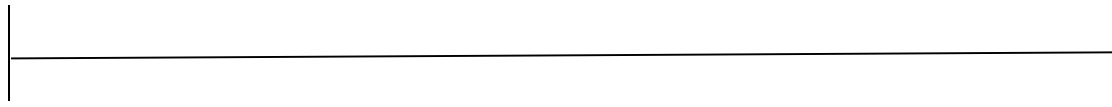


Absent

Moderate

Intense

Texture in *mouth* after stirring: Graininess

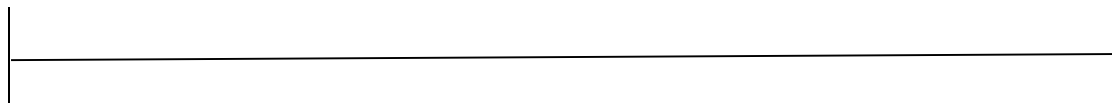


Absent

Moderate

Intense

Taste: Sourness

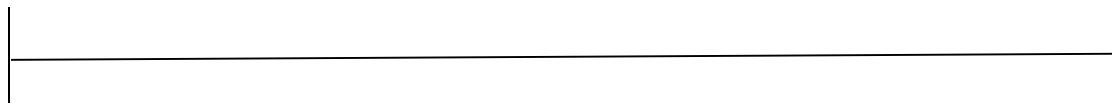


Absent

Moderate

Intense

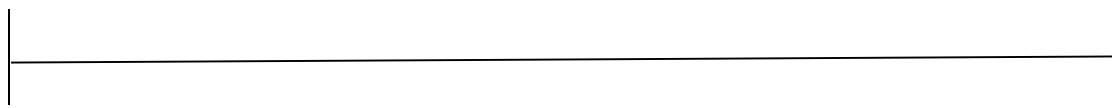
Taste: Astringent



Absent

Moderate

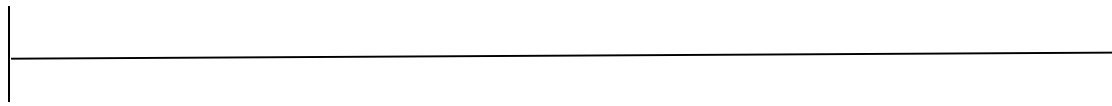
Intense



Absent

Moderate

Intense



Absent

Moderate

Intense



Absent

Moderate

Intense



Absent

Moderate

Intense

5. How much do you **LIKE** or **DISLIKE** the **TEXTURE (Thickness - mouth coating)** of this yoghurt?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

6. How much do you **LIKE** or **DISLIKE** the **TASTE (Sourness)** of this yoghurt?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

7. How much do you **LIKE** or **DISLIKE** this product **OVERALL**?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

7a. What, if anything, did you **LIKE** about this yoghurt?

7b. What, if anything, did you **DISLIKE** about this yoghurt?

GENERAL QUESTIONS / PERSONAL PARTICULARS

How often do you consume yoghurt?

- Never
- Daily
- Every 3 days
- Once weekly
- Once fortnightly
- Once Monthly

Which of the following groups includes your age?

- 21 - 30
- 31 - 40
- 41 - 50
- 51 - 60
- 60 & above

Gender:

- Male
- Female

Other comments:

THANK YOU FOR YOUR PARTICIPATION. :)