Effect of Meat Addition on Microbiological, Physicochemical and Sensory Properties of Dairy Yoghurt

By

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Effect of Meat Addition on Microbiological, Physicochemical and Sensory Properties of Dairy Yoghurt

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Abstract

Addition to dairy yoghurt of minced cooked beef was carried out as a way to provide additional health benefits to probiotic yoghurt. The study aimed to fortify yoghurt with high nutritional quality protein from meat. This novel health food will suit the majority of the population, particularly geriatric people, since foods such as yoghurt enriched with proteins would be suitable to deliver their specific nutritional requirements. The objectives of this study were to develop a new protein-rich yoghurt and determine its microbiological, physicochemical and sensory properties. The main phase was the preparation of yoghurts, with added beef meat (5%, 7%, and 9%) such that the total solids content (around 20%) remained constant, and was followed by homogenisation. Yoghurt containing homogenised meat (HMY), yoghurt containing unhomogenised meat (UHMY) and control plain yoghurt were produced. The yoghurt mixtures were heated at 85°C for 30 minutes followed by inoculation and incubation at 42°C for 5 hours after which they were stored for 21 days at 4°C. The results showed that the production of acidity and microbial counts were not affected by the meat addition during a 21 day storage period at 4°C, compared to the control. The microbiological counts of total lactic acid bacteria after 1 day of storage in meat-fortified yoghurts (around 30 ×10E7 cfu/g) were not significantly different from numbers in the control yoghurt. However, the counts showed significant loss of viability during the period of storage, although the final viable numbers in the yoghurts were high enough (>10E7 cfu/g) for the products to be designated probiotic. Fortifying the yoghurt with meat did not stimulate the growth of contaminating coliforms, *Salmonella* and *Listeria*. The fat content decreased while the protein content increased significantly (P<0.05) with increased addition of meat. The fat content of yoghurts ranged between 2.2% (Control) and 1.41% (9% meat addition), hence the yoghurts can be considered as low- fat products. Apparent viscosity and water holding capacity (WHC) decreased significantly (P<0.05) with the addition of meat. The control had the highest viscosity and WHC values followed by 5% meat yoghurts. Colour was different for yoghurts containing different added meat and also in terms of homogenisation. The addition of meat changed the colour of yoghurts particularly in those containing higher meat content that had been homogenised. They were darker with a redder colour. Sensory results revealed that samples fortified with 5% meat received the second highest scores for flavour after

that of the control. Meat addition resulted in significant decrease (P<0.05) in the overall flavour quality. Meat addition improved the odour of yoghurts but decreased significantly (P<0.05) the overall scores for appearance and texture. Results showed that addition of 5% meat could be used to produce a meat-added yoghurt without significant adverse effects on the microbial, physicochemical or sensory properties. As expected, 9% meat yoghurt had the highest protein content (9.98 %) compared to the control (6.1%). Further studies are needed to improve the quality of meat yoghurts in terms of apparent viscosity, whey separation, and colour and also enhance the overall flavour.

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to be the best of my knowledge and belief, 'Effect of Meat Addition on Microbiological, Physicochemical and Sensory Properties of Dairy Yoghurt' contains no material previously published or written by another person (any help that I have received in my research has been acknowledged), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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Date……………………….

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Chapter. 1. **Introduction**

Nowadays, consumers are concerned about the nutritional values of the food they eat. In terms of healthier lifestyle, a varied selection of food is being produced which contains elements that deliver specific health benefits. These foods are defined as functional foods, and are claimed to have health-promoting aspects and disease-preventing properties as well as their nutritive value (Arihara, 2006). Functional foods originated in Japan in the early 1980s (Arihara, 2006), and the global market is rapidly increasing. The most common functional foods contain probiotic microorganisms (Hap, 2010), and the consumer interest in dairy yoghurts has been improved by the modern addition of probiotic cultures (lactic fermentative cultures) that are traditionally used in yoghurts (Drake & Chen, 2000).

Yoghurt is a fermented food commonly consumed around the world. It was originally made in the Middle East and Asia and it is considered to be one of the oldest fermented milk products (Desai, 2012). Yoghurt is described by the U.S Food and Drug administration as the food produced by culturing one or more of the optional dairy ingredients cream, milk, partially skimmed milk, used alone or in combination with a characterising bacterial culture that contains the lactic acid producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Desai, 2012). Nutritionists believe that yoghurt foods contain essential amounts of organic acids with other substances that may improve therapeutically illnesses such as diarrhea and hypercholesterolemia (Fernandez-Garcia & McGregor, 1997). Yoghurt consumption is considered to be one of the most popular conventional snack foods that presented economic growth in food industry in 2009 (Estrada, Boeneke, Bechtel, & Sathivel, 2011). Yoghurt production increased dramatically from 240 million kg in 1980 to reach 2 billion kg in 2010 (Desai, 2012). In the United States of America (USA), for example, yoghurt consumption has grown annually between 3% and 10% over the past few years (Isabelle Sodini, Montella, & Tong, 2005). This growth has been ascribed to the beneficial health properties of yoghurt and its subsequent consumer appeal (Estrada et al., 2011). Today, yoghurt production commonly involves milk fortification with dairy ingredients to improve the protein intensity (Isabelle Sodini et al., 2005).

A primary function of modern food technology is to produce new food with appealing structures and with characteristics that provide additional health benefits. Proteins are one of the major classes of molecule available to present textural qualities, and the combination of protein molecules has been mentioned as one of the most important mechanisms for engineering food structures with desirable properties (Gerrard, 2002). The aggregations of food proteins can impact many attributes of food, including texture, viscosity, acidity and gelling properties (Gerrard, 2002). Hence, fortified dairy yoghurts attract a wide variety of consumers and have enabled increased markets for the yoghurt industry (Estrada et al., 2011).

As the consumption of traditional dairy milk-based yoghurts continues to increase and consumer interest in dairy yoghurts has been improved by the modern addition of probiotic cultures, several studies have addressed the fortification of dairy yoghurts with food proteins such as soy protein and whey protein. Fortification of dairy yoghurts with soy protein (Drake & Chen, 2000) and whey proteins (Berber, 2011) has been studied as a way to provide additional health benefits. Drake and Chen (2000) used dairy yoghurts fortified with soy protein, not only as a vehicle to deliver beneficial bacteria, but also to provide a different complement of proteins to consumers. In the present study, meat was added to dairy-based yoghurts to improve their properties as well as to provide food proteins of higher nutritional quality to the consumer.

Thus, the study aimed to fortify yoghurt with high nutritional quality protein from meat. A good method to provide an additional vehicle for consumption of meat protein is to combine the benefits and consumer market for dairy milk-based yoghurts with the potential health benefits of meat protein. Although milk proteins have a good nutritional profile and functional attributes (Alu'datt et al., 2012), meat proteins have protein attributes that are not found in milk. A study has shown that the qualities of proteins from animal sources are superior to those from plant sources such as soy protein (Bender, 1992).

Meat and meat products are very important as they contain concentrated sources of high value protein and their amino acid composition usually compensates for shortcomings in other foods (Bender, 1992). These micronutrients are either not present in vegetable proteins or have a poor bioavailability (Biesalski, 2005). Meat is also a good source of fat and minerals such as iron and zinc and several B vitamins (Biesalski, 2005).

Furthermore, meat has a low carbohydrate content, which contributes to a low glycaemic index that is considered to be useful in the management of some conditions such as obesity, diabetes development and cancer. Hence, meat consumption is beneficial for maintenance of health, particularly in older people (Biesalski, 2005). The target of this product is the general public, particularly geriatric people. A study has shown that as individuals grow older, they need to obtain their protein from less food, but ones that contain high-quality protein (Bhayana, 2011). Foods such as yoghurt enriched with proteins would be suitable to deliver their specific nutritional requirements.

Aim of the Study

There is no published literature on the use of meat addition to dairy-based yoghurts. The aim of this study, therefore, was to investigate the development of novel protein-rich savoury probiotic yoghurt that is fortified with meat protein. Addition of meat will, however, change the properties of the yoghurt, hence the microbiological, physicochemical and sensory characteristics were determined and compared with non-supplemented yoghurt.

The hypothesis was that addition of meat will improve the nutritional value of yoghurt with possible improved microbiological, physicochemical and sensory properties. In this study, yoghurt was as a vehicle to deliver meat nutrition. This study was carried out to explore how meat particles interact with the macromolecules of milk powder that could affect the characteristics of yoghurt.

Chapter. 2. Literature review

2.1 Yoghurt

2.1.1 Probiotic as functional food – The historic background

Consumption of functional foods varies according to factors such as ethnicity, cultural, social, economic, geographical and political backgrounds (Jiménez-Colmenero, Carballo, & Cofrades, 2001). Japan has pioneered in developing functional foods and has established several food regulations to modulate their consumption (Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010). The beneficial effects of lactic acid bacteria incorporated in fermented milk were first recognised in the beginning of the 20th century by the Russian bacteriologist Elie Metchnikoff. The discovery was based on the fact that Bulgarians generally enjoy good health and longevity which was related to consumption of yoghurt, a type of fermented milk (Hughes & Hoover, 1995). Later, in 1908, the same bacteriologist proposed a 'longevity-without-aging' theory that hypothesised that lactic acid bacteria caused the displacement of toxin producing bacteria normally present in the intestine, resulting in longevity by eliminating those toxic substances from the body. In addition, Metchnikoff confirmed that lactic acid and other products manufactured by lactic acid bacteria in sour milk reduced the growth and toxicity of anaerobic, spore-forming bacteria in the large intestine (Hap, 2010). In 1994, the World Health Organization (WHO) deemed probiotics to be one of the most important component of the immune system in the future (Hap, 2010).

Although an accurate record of when yoghurt was first produced is absent, it is the most popular dairy product and has been commercialised in a variety of forms and names all around the world through many years (Tarakci & Kucukoner, 2003). The use of this probiotic goes back many centuries. According to legend, yoghurt was first produced by the ancient Turkish people in Asia (Hussain & Atkinson, 2009), and the word yoghurt is derived from the Turkish word Jugurt, which describes any fermented food with acidic taste (Hussain & Atkinson, 2009). Yoghurt production involves the use of specific symbiotic/mixed cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Hussain & Atkinson, 2009). These beneficial bacteria, when consumed in appropriate proportions, can protect the intestines from harmful bacteria. These beneficial effects

have been extensively recognised in the food industry and the acceptance of yoghurt as a probiotic food has greatly increased in the subsequent years, being now recognised as a healthy food (Lourens-Hattingh & Viljoen, 2001). Furthermore, yoghurt is commercialised in various forms, including drinkable (liquid) or solid, low fat or fat free, fruity or cereal flavoured. Also, it is a multipurpose, healthy and nutritious food that can be employed on different meal occasions and can please distinct palates (Mckinley, 2005).

2.1.2   Definition of yoghurt

Interest in healthy food is increasing and consumers are becoming more aware and interested in incorporating probiotics into their diet (Sharareh Hekmat & Reid, 2006). Yoghurt is one of the most popular, tasty and healthy dairy products produced through the fermentation of lactic acid bacteria (LAB) (Ranathunga & Rmusk, 2013). Indeed, lactic acid is the product of lactose fermentation by LAB and bacterial reaction with milk, where protein gives yoghurt its texture and its characteristic tangy flavour (Shima et al., 2012). During the fermentation process, milk protein is hydrolysed, pH reduces, chemical reactions cause increase in viscosity and the metabolites produced contribute to the taste and possibly to the health promoting properties of yoghurt (Farnworth et al., 2007).

The Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) set broader international standards for yoghurt published in the Codex Standard for Fermented Milks (2003). This document defines yoghurt as the product of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* of cultures of fermentation. Also, a minimum amount of 2.7% milk protein, less than 15% milk fats, and at least 0.6% titratable acidity are specified. In addition, yoghurt must contain at least 10E6 colony forming units (CFU) per gram (Codex Standard, 2003).

According to Food Standards Australia New Zealand (FSANZ) Standard 2.5.3, yoghurt is described as fermented milk where the microbiological fermentation results in lactic acid production (FSANZ, 2008). The composition of fermented milk and yoghurt must contain each component as shown in Table 1 below.

Table 1. FSANZ Standard 2.5.3 required yoghurt characteristic as a fermented milk product.

Component or parameter	Proportion
Protein (measured as crude protein)	Minimum 3.0% w/w
pH	Maximum 4.5
Microorganisms from added culture	Minimum 10^6 cfu/g

Food Standards Australia New Zealand (2008)

2.1.3 Probiotics

Yoghurt has wider nutritional benefits in comparison with unfermented milk, and it is a major source of protein, calcium, riboflavin, vitamin B6 and vitamin B12. Furthermore, yoghurt is mainly a probiotic carrier food that can be used in the treatment of a variety of gastrointestinal diseases and diarrhea (Ashraf & Shah, 2011). Probiotics are defined as "mono or mixed cultures of live microorganisms that brings beneficial effects to the host by improving the properties of the indigenous microflora" (Nogueira, Albano, Gibbs, & Teixeira, 1998). Adequate amounts of probiotics promote an optimum balance in the microbial population of the digestive tract and it is linked to nutrition and health (Farnworth et al., 2007). Probiotics are facultative anaerobes inhabiting the lower distal part of the human gut, usually separate from human faeces. Probiotics are tasteless and can be integrated with a wide variety of products (Rodgers, 2007).

According to the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition, probiotics consumption is proposed as low cost and low-risk protection from infection and disease (Sadler, 1999). A therapeutic dose of at least 10E7 cells or colony-forming units (CFU) per gram are required for the stimulation of the immune system (Simmering & Blaut, 2001). Microflora is typically destroyed during treatments in food processing such as sterilisation, pasteurisation, disinfection, irradiation, washing and peeling. Furthermore, fortifying food with probiotics can balance the loss that occurs during food processing (Rodgers, 2007).

Lactic acid bacteria (LAB) have been used for food conservation and in other areas of the food industry for several centuries. These bacteria are Gram-positive bacilli and cocci and are responsible for carbohydrate metabolism through a process referred to as fermentation, yielding acid lactic as the final product of this reaction (Salminen, Deighton, Gorbach, & Wright, 1993). Also, the characteristic flavour and aroma are

produced by lactic acid bacteria (Tamime, Saarela, Korslund, Mistry, & Shah, 2008). LAB are safe for human consumption due to their ubiquity on the surface of the human body and in the gut, and their long history of safe use in food products (Hap, 2010).

2.1.4 Fermentation products of yoghurt bacteria

Yoghurt is the final product of a controlled fermentation of high solids whole milk which is usually cultivated with a symbiotic mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, where in these bacteria degrade casein supplying peptides and amino acids to the weakly proteolytic streptococci. Consequently, the redox potential is lowered by *S. thermophilus* acidifying the milk and creating a satisfactory environment for the *L. bulgaricus* growth, further increasing milk acidity (Nogueira et al., 1998). Together, the two species ferment almost all the lactose to lactic acid and provide flavour to the yoghurt with diacetyl (*S. thermophilus*) and acetaldehyde (*L. bulgaricus*) (Nogueira et al., 1998).

2.1.5 Yoghurt health benefits

There are more than 500 different species of bacteria in the human intestinal tract acting in symbiosis to promote gut health. However, the number of these beneficial microorganisms decreases with age, while the proportion of potentially pathogenic microbes increases (Desai, 2012). Nonetheless, incorporating probiotic bacteria into the human diet may reverse or slow down this unbalanced process by replenishing the probiotic losses through defecation (Sharareh Hekmat & Reid, 2006).

Despite the fact that yoghurt bacteria cultures are not natural inhabitants of the human intestine, they induce health benefits (Table 2) such as augmented protein digestibility, improved lactose tolerance, enhanced mineral absorption, controlled intestinal health and repaired immunity (Donkor, Nilmini, Stolic, Vasiljevic, & Shah, 2007). In the face of the absence of a consensual opinion in regards to probiotic usage in medicine, they have been used in the treatment of various types of diarrhoea (Szymański et al., 2006), urogenital infections, and gastrointestinal diseases such as Crohn's disease and pouchitis (Farnworth et al., 2007).

Table 2. A summary of the health promoting characteristics found in yoghurt.
Sanabria (2012, p. 6)

Action/effect	Alleged health benefit	Established in humans[a,b]
In digestive tract	Active against Helicobacter pylori	
	Enhanced lactose digestion	✓
	Stimulation of intestinal immunity	
	Stabilization of Crohn's disease	
	Stimulation of intestinal peristalsis	
On intestinal microflora	Improves balance between microbial populations	Increase in faecal bifidobacteria
	Decrease in faecal enzyme activity	✓
	Colonization of intestinal tract	✓
	Reduced carrier time for Salmonella spp.	
On diarrhea	Prevention/treatment of acute diarrhea	✓
	Prevention/treatment of rotavirus diarrhea	✓
	Prevention of antibiotic-induced diarrhea	✓
Other effects	Improved immunity to disease	
	Suppression of some cancers	
	Reduction in serum cholesterol	
	Reduction in hypertension	

2.1.6 Market for functional and probiotic foods including yoghurt

Between the years 1988 and 1998, more than 1700 functional foods have been introduced to the Japanese market, resulting in 14 billion dollar sales in 1999 (Zhang et al., 2010). As yoghurt is an important source of probiotics, its production and consumption are continuously growing (Shima et al., 2012). Product quality and consumer satisfaction also are very important to promote the sales of various types of yoghurt products (Fernandez-Garcia & McGregor, 1997). Fermented dairy products enriched with probiotic bacteria are reflected in the most profitable categories of functional foods (Sleator & Hill, 2008). In 1997, functional food products had 65% share of the European functional food market and were valued at US$ 889 million (Hilliam, 1998). According to a Leatherhead Food Research Association study, countries such as UK, France, Spain, Belgium, Netherlands, Denmark, Finland, and Sweden were producing more than 250 million kilograms in 1997 (Hilliam, 1998).

The USA is the most dynamic market for functional foods, and market share of functional foods in the total food market was expected to be 4 - 6% in 2008 (Zhang et al., 2010). In 2005, the sale of probiotic foods reached $764 million in the USA, which was projected to reach $1.1 billion by the end of this decade (Rodgers, 2007). Yoghurt

holds 50% of the cultured product market sales in U.S. and the demand is increasing every year (Thompson et al., 2007). At the end of 2008, the market for fermented products increased from $15.9 billion worldwide, resulting in a compound annual growth of 7%, and was estimated to reach $22.4 billion in 2013 (BCCResearch, 2009). Furthermore, according to the USDA National Agricultural Statistics Service (2008), 739 million kg of yoghurts were prepared in the United States in 1998, having a marked increase (120%) in 2008 generating 1.62 billion kg (Lourens-Hattingh & Viljoen, 2001).

2.2 Fortification of yoghurt

Health-conscious consumers prefer yoghurt with different characteristics than being motivated by price, convenience, mood, or familiarity (Rognlien, Duncan, O'Keefe, & Eigel, 2012). Yoghurt has been widely fortified with nutrients such as calcium (Singh & Muthukumarappan, 2008), proteins (Berber, 2011), vitamins (Cueva & Aryana, 2008), fish oils (Rognlien et al., 2012) and prebiotics with addition of probiotic cultures that enhance its health benefits. Moreover, emerging technologies focus on processing conditions to maximise the effects of added ingredients (Berber, 2011). In recent years, many different food ingredients such as soy protein (Drake & Chen, 2000), iron (Hekmat & McMahon, 1997) and fibre (Fernandez-Garcia & McGregor, 1997) have been included in yoghurt formulations to improve their nutritional value.

2.2.1 Enhancement of nutritional value via fortification

2.2.1.1 β-cyclodextrin (β-CD)

The health benefits of yoghurt can be improved by decreasing the cholesterol amount in the composition of this product. Lee et al. (2007) reported that cholesterol from milk (the major ingredient for the manufacture of yoghurt) could be effectively removed by β-cyclodextrin (β-CD). The physicochemical and sensory properties of cholesterol-reduced yoghurt were not remarkably changed from those of the control.

2.2.1.2 Chitosan

When low fat yoghurt was fortified with nanopowdered chitosan (NPC) to improve the functionality of yoghurt, no significant adverse effects on the physicochemical,

microbial, or sensory properties were found during storage. Research has reported that concentrations (0.3 to 0.5%. vol/vol) of NPC could be used to produce an NPC-added yoghurt without significantly adverse effects on the above properties (Seo, Lee, Chang, & Kwak, 2009).

2.2.1.3 Soy protein

Drake and Chen (2000) investigated the effects of adding soy protein into yoghurt as a method to deliver additional health benefits. The composition of yoghurt was altered with total solids equal to non-fat dried milk. The results revealed that microbiological counts, fermentation time, and developed acidity at the end of manufacture were not affected by soy protein. Nonetheless, yoghurt with added soy protein concentrate had a higher protein content and viscosity, sensory thickness, soy aroma and soy flavour were enhanced by adding soy protein to yoghurt composition (Drake & Chen, 2000).

2.2.1.4 Fibre

Dietary fibre may play a significant role in the prevention of some illnesses such as intestinal and cardiovascular disease (Fernández-Garía, McGregor, & Traylor, 1998). Yoghurt has been fortified with fibres and these fibres have been added from different sources such as soy, rice, oats, corn and sugar beet. Hence, adding fibre into sweetened plain yoghurt prompted acceleration in the acidification rate of the fortified yoghurts, followed by an increase in the apparent viscosity. On other hand, a grainy flavour and a gritty texture were intense in all fibre-fortified yoghurts, except in those made with oat fibre. In addition, soy and sugar beet fibres caused a significant decrease in viscosity due to partial syneresis. Furthermore, the fibre addition led to lower overall flavour and texture scores (Fernandez-Garcia & McGregor, 1997). Interestingly, yoghurt fortified with oat fibre resulted in the highest overall quality products.

2.2.1.5 Calcium

Calcium can be added to plain low fat yoghurt in the form of calcium gluconate without substantially changing the chemical and sensory properties of yoghurt (Singh & Muthukumarappan, 2008). In another study done to analyse sensory properties of yoghurt, calcium-enriched mango yoghurt was prepared after fortification of pasteurised yoghurt mix with 50mg Ca/100ml as calcium lactate. Fortification of yoghurt with calcium lactate at this level significantly ($P < 0.05$) raised the water holding capacity

(WHC). However, apparent viscosity measurements at constant shear rate showed a significant (P<0.05) decrease in calcium fortified fruit yoghurt. Flavour, colour, body and texture scores in the control and calcium fortified fruit yoghurt did not show any significant difference (P>0.05) (Singh & Muthukumarappan, 2008).

2.2.1.6 Iron

Yoghurt is a major source of calcium and high quality protein, but as with the majority of dairy products, contains only small amounts of iron (Desai, 2012). Fortification of yoghurt with iron could be used to meet this nutritional need. When yoghurts were manufactured and fortified with 10, 20, and 40mg of iron/kg of yoghurt, only a very slight increase in oxidised flavour occurred owing to iron fortification. The growth of starter culture bacteria and non-starter culture bacteria, and yoghurt lipid oxidation, were monitored over 30 days of refrigerated storage. Addition of iron at these concentrations did not promote the growth of either *Pseudomonas fluorescens* or *Escherichia coli,* even when these bacteria were added at 10E5 cfu/ml of yoghurt mix. In conclusion, fortification of yoghurt with iron is technically feasible and there was just a slight increase in oxidised flavour caused by iron fortification which was not noticed by trained panelists (Hekmat & McMahon, 1997).

2.2.1.7 Heart healthy nutrients

Incorporation of heart healthy nutrients (Thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), folic acid (vitamin B9), manganese and magnesium) with yoghurt at the 30%, 60% and 90% recommended dietary allowance (RDA) was studied (Cueva & Aryana, 2008). Results showed a significantly decreased synersis, pH, L*, and a* values, but significantly increased b* value. Yoghurt viscosity was significantly increased by the incorporation of the nutrients at the 60% level. This incorporation had no significant effect on the flavour, appearance, body, texture or microbial counts of the yoghurt. The slight modifications in instrumental colour and viscosity could not be indicated with sensory evaluation (Cueva & Aryana, 2008).

2.2.1.8 Whey protein

In a study done to evaluate yoghurts prepared with a particular amount of whey protein replacing non-fat dry milk, the results showed that whey proteins had properties that improved the textural properties of yoghurt when they are used as an ingredient (Berber,

2011). Whey protein yoghurts had better water-holding capacities when measured against the control, and it had also presented an increase in hardness and viscosity. Moreover, sensory properties were also analysed through descriptive methods with hedonic scales and the results showed that whey protein yoghurts increased flavour. However, unfortified yoghurt showed a better texture (Berber, 2011). Overall, when whey protein was used as substitute to replace non-fat dry milk completely, the resulting yoghurt was equal to or of greater quality than normal yoghurt products.

2.2.1.9   Fish oils

Enrichment of yoghurt with fish oils has received growing interest due to its already known antioxidant properties (Rognlien et al., 2012). Due to the presence of high content of polyunsaturated fatty acids, yoghurt containing fish oil is very prone to oxidation that might lead to the development of undesirable fishy and rancid off-flavours (Estrada et al., 2011). However, adding fat and flavourings can help to mask the fishy taste in dairy products fortified with fish oil (Rognlien et al., 2012). Estrada et al. (2011) noticed that fishy flavour added to strawberry yoghurt from an algae source was masked by the strawberry fruit base.

2.3   Formulation and microbial analysis

2.3.1   Viability of probiotic bacteria

Food enriched with live microorganisms, in particular lactic acid bacteria (LAB), has been traditionally associated with beneficial health outcomes to the intestinal microbial balance by restoring it to a healthy balance (Donkor et al., 2007). In order to achieve the desired protective effects in gut health, the probiotic content of a lactic product has to be sufficiently high. Nonetheless, no general agreement has been reached on the recommended levels, and the suggested amount of probiotics frequently referred to as therapeutic doses ranges from 10E6 (Kurmann & Robinson, 1991) to over 10E7 or 10E8 cfu/ml (Lourens-Hattingh & Viljoen, 2001). Consequently, the amount of viable and active cells per g or mL of probiotic food products at the moment of consumption is the most critical value for these products, determining if they will or will not contribute to the expected therapeutic effects (Sohrabvandi & Mortazavian, 2012).

To achieve food safety requirements and consumers' expectations, it is important to ensure a high survival rate of the bacteria, both during production and storage, thus assuring a sufficiently long product shelf life (Rouhi, Sohrabvandi, & Mortazavian, 2013). The viability of probiotic microorganisms can be calculated by plate count methodology. This is a simple, available, and inexpensive and routine testing methodology, which makes it a common practice (Sohrabvandi & Mortazavian, 2012). Typical numbers are shown in Table 3.

Table 3. Indication of the numbers of starter bacteria that have been isolated from retail cartons of yoghurt and some suggested standards relating to both contaminants and desirable organisms. Tamime and Robinson (2007, p. 566)

Organism	Yoghurt		
	Natural	Strawberry	Blackcurrant
S. thermophilus $\times 10^6$ cfu ml^{-1}	10–820	35–1100 54–250	80–1850
L. delbrueckii subsp. *bulgaricus* $\times 10^6$ cfu ml^{-1}	11–680	5–360 <1–150	5–400
Suggested advisory standards:	Satisfactory	Doubtful	Unsatisfactory
S. thermophilus $\times 10^6$ cfu ml^{-1}	>100	100–10	<10
L. delbrueckii subsp. *bulgaricus* $\times 10^6$ cfu ml^{-1}	>100	100–10	<10
Coliforms ⎫	<1	1–10	>10
Yeasts ⎬ cfu ml^{-1}	<10	10–100	>100
Moulds ⎭	<1	1–10	>10

2.3.2 Food pathogens

Consumer concerns about food safety have largely increased in recent years because of the substantial outbreaks associated with a variety of food products (Hoyle et al., 2009). Pathogens detection in fresh food can be influenced by factors such as the time required to detect viable levels of bacteria, the product's short shelf life, the magnitude and diversity of product composition, life cycle handling, and geographic source (Magaña, Schlemmer, & Lim, 2014). Additionally, raw produce could also have been the subject of potentially harmful pathogens by contamination with ground soils and other environmental factors such as agricultural animals, wildlife, or insect grazing, irrigation source water and composition, human contact, transport and production facility equipment (Magaña, Schlemmer, & Lim, 2014).

According to the United States Centre for Disease Control and Prevention, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica* are three of the eight most common pathogens involved in the vast majority of reported food borne illness, hospitalisations, and deaths each year (Magaña et al., 2014). Salmonellosis is the most commonly reported bacterial food-borne illness leading to hospitalisation and death. Listeriosis accounts for the third highest reported incidence of death. These pathogens are also characterised as possessing extraordinary persistence once established in food processing environments. In addition, *E. coli* is the primary cause of bloody diarrhoea that can progress to fatal hemolytic uremic syndrome (Magaña et al., 2014).

Lactic acid bacteria (LAB) can be utilised to prevent *Escherichia coli* growth and this approach may also be useful for reducing *salmonellosis* outbreaks linked to contaminated beef products (Ruby & Ingham, 2009). Furthermore, harmful microorganisms can be lowered or eliminated from food utilising techniques such as pasteurisation, cooking, freezing, washing with chlorinated or other sanitary rinse solutions and treatment with novel anti-microbials, or irradiation. Pathogens also can be inactivated by lowering food pH (Mataragas & Drosinos, 2008). Nonetheless, no records of staphylococcal food poisoning being associated with the consumption of yoghurt in the United Kingdom were found and scientific evidence that a virulent strain of *Staphylococcus aureus* was inhibited during fermentations to justify that examination for staphylococci is not normally required for yoghurt (Tamime & Robinson, 2007).

2.3.3 Lactic acid bacteria (LAB) as inhibitor of food pathogens

Lactic acid bacteria (LAB) are widely used in food fermentation including dairy, meat, vegetable and baked products. LAB are known to produce different antibacterial substances that inhibit the growth of several undesirable Gram-positive bacteria in the genera *Bacillus, Enterococcus, Listeria, Clostridium* and *Staphylococcus* (Salminen et al., 1993). They also produce metabolites such as hydrogen peroxide (H_2O_2), lactic acid, other organic acids and ethanol, which may cause growth inhibition. Production of H_2O_2 is considered as favourable for food preservation and prevention of pathogen implantation and growth (Ruby & Ingham, 2009).

An examination of yoghurt for contaminant organisms is, as indicated earlier, concerned with protection of the consumer from any potentially pathogenic species and assurance

that the material will not undergo microbial spoilage during its anticipated shelf life (Hsieh, Liu & Hwang, 2010). Yoghurt remains a safe food, and other beneficial fermentative bacteria are often combined with yoghurt starters to enhance desirable characteristics related to health properties (Gulmez & Guven, 2003). Bachrouri et al. (2002) suggest that the presence of food pathogens in yoghurt is more likely to reflect post processing contamination rather than the survival of the microorganism through the yoghurt fermentation process.

2.3.4 Yoghurt production and processing

Yoghurt is a product of milk fermentation that is usually manufactured by allowing milk to sour at $40 - 45^{\circ}$C. Nowadays the production is a well-controlled process that uses ingredients consisting mainly of milk, such as milk powder, and others such as sugar, fruit, flavours, colouring, emulsifiers, and stabilisers. Specific pure cultures of lactic acid bacteria are also included in yoghurt manufacture to conduct the fermentation process (Lourens-Hattingh & Viljoen, 2001).

The manufacturing relies on the following processes: milk procurement, reception and storage, separation, mix preparation, pasteurisation, homogenisation, inoculation and incubation, cooling and packaging. Also, a straining step is usually added after the cooling process to produce strained yoghurt (Desai, 2012).

2.3.4.1 Milk procurement, storage, separation and mix preparation

The process of milk procurement from dairy to the processing plant is done in insulated tanks, this method avoids unnecessary agitation of milk preventing lipolytic deterioration of milk flavour. Quality control checks are performed on the milk and the storage temperature of milk should be below 7°C. Also, skim milk and cream should be separated, removing sedimentary matter, while milk should be standardised to the % fat level according to the type of yoghurt to be produced. When preparing the mix, ingredients should be blended together to attain a desired formulation with the help of an agitator (Desai, 2012).

2.3.4.2 Pasteurisation

Yoghurt mix is pasteurised either at the temperature and time intervals of $80 - 85^{\circ}$C for 30 min or $90 - 95^{\circ}$C for 10 min and this process aims to destroy pathogens. However, when a certain texture is required, the above legal time/temperature standards are

exceeded for texture development (Desai, 2012). For example, yoghurt viscosity is affected by heat treatment, due to physical changes in whey proteins (denaturation) (Dave & Shah, 1998). Whey protein denaturation in the range of 70 – 95 % improves water absorption capacity, creating smooth consistency, high viscosity and less whey separation. Whey protein denaturation also promotes enzyme and nonpathogenic organisms inactivation, and production of stimulatory or inhibitory factors for starter cultures (Tamime & Robinson, 1999). Furthermore, some substances formed during heat treatment of milk, such as cysteine, glutathione or thiogluconate can stimulate the growth of yoghurt starter bacteria (Tamime & Robinson, 2007). Again, heat treatment plays an important role in the textural properties of yoghurt. The holding time of milk at a temperature above 75 C causes 99 % denaturation of β-lactoglobulins which improves the characteristic yoghurt gel due to the aggregation of casein micelles which occurs as a result of the heat treatment (Desai, 2012).

2.3.4.3 Homogenisation

There are a few physical and chemical changes that occur in yoghurt due to homogenisation of the milk or the mix. One of these is adsorption, which is defined as a process where the newly formed fat globules binding onto the casein micelles increases, leading to an increase in the total volume of the suspended matter thereby making it more viscous. Another change is the enhancement of yoghurt consistency, providing greater stability against whey separation (Chandan, 2006). Finally, the homogenisation process reduces the size of the milk fat globules avoiding cluster formation and surface aggregation (Chandan, 2006). Due to the increased surface area of the fat globules, homogenisation induces interactions between milk proteins, most importantly between casein and fat (Cano-Ruiz & Richter, 1997).

2.3.4.4 Inoculation fermentation and gel formation

At the first step of the fermentation, *S. thermophilus* grows faster than *L.bulgaricus*, fermenting the lactose and producing lactic acid, this metabolite is also formed from other compounds present in the milk (Lourens-Hattingh & Viljoen, 2001). When the pH reaches 5, the growth of *S.thermophilus* population slows and *L.bulgaricus* grows at a quicker rate. Gel formation in yoghurt comes about as a series of chemical, biological and physical actions. Microbial growth decreases the mix pH from 6.8 to 5.0 culminating in colloidal generating soluble calcium ions (Donkor et al., 2007). A further

pH reduction from 5.0 – 4.6 incites physical aggregation of the casein micelles. At the isoelectric point of casein (pH = 4.6), the casein micelles charges are neutralised. However, rearrangement and aggregation of casein micelles leads to protein gel formation and a particle gel structure when the pH is below the isoelectric point (Tamime & Robinson, 1999). In addition, the properties of such acid casein gels are closely related to the casein concentration, enthalpic nature of the gel and to the extent of attraction between the casein particles and the gelation mechanism. In addition, the size and distribution of casein micelles and the range of protein contact points also influences the structure of gel (Lourens-Hattingh & Viljoen, 2001). The incubation process typically takes between 3 and 6 hours at a temperature of around 40 –45°C (Tamime & Robinson, 2007). The consequences of acidification during fermentation influence some casein properties, consequently affecting their gelation characteristics during the formation of cultured products (Berber, 2011).

2.3.4.5 Cooling and Storage

Yoghurt is cooled and stored at temperatures below 5°C to inhibit microbial activity resulting in an extended shelf life (Desai, 2012). Cooling is an important step to prevent post-acidification effects initiated by starter culture activity. In addition, when the cooling rate is slow, the final yoghurt product pH will keep decreasing and further acid production to pH < 4.2 will make the yoghurt product undesirable (Lourens-Hattingh & Viljoen, 2001).

2.3.4.6 Straining

After the milk is cultured and cooled, to achieve a thicker and creamier consistency, it is strained to remove whey. The straining phase improves the texture of the yoghurt and decreases syneresis that is often found in yoghurt types (Desai, 2012).

2.3.5 Milk powders

Milk powders are skim milk, whole milk or buttermilk with water extracted but maintaining all of their original constituents present in the same ratio. Removing only water from pasteurised skim milk results in skim milk powder (SMP) formation. SMP contains not more than 5% by weight moisture and not more than 1.5% by weight milk fat (Desai, 2012). Whole milk powder (WMP) is the final product obtained from water removal from pasteurised milk and the fat percentage ranges from 26% to 40% and not

more than 5% by weight of moisture. Furthermore, lactose, milk proteins, milk fat and milk minerals are found in the same proportions as in the milk from which it was made (Desai, 2012). Generally, SMP is used to enrich milk preceding the yoghurt fermentation step (Isabelle Sodini et al., 2005). Yoghurt production usually involves fortification of milk with dairy ingredients to improve the total solids content. Yoghurt can also be made solely from recombined dried dairy ingredients such as skim milk powder, which is used widely, and other dried dairy ingredients (Isleten & Karagul-Yuceer, 2006).

2.4   Meat

The Food Standards Australia New Zealand (FSANZ) Food Standards Code defines meat as 'the whole or part of the carcass of any buffalo, camel, cattle, deer, goat, hare, pig, poultry, rabbit or sheep, slaughtered other than in a wild state, but does not include eggs, or foetuses' (Williams, 2007, p. 1). In Australia and New Zealand, the term "red meat" is applied by the meat industry to refer to meat from cattle, sheep and goat (i.e. beef, veal, lamb, mutton and goat meat) (Williams, 2007).

2.4.1   Meat as a source of protein

Meat and meat products are effective sources of high quality protein and their amino acid composition usually compensates for shortcomings in the staple food (Bender, 1992). Raw red muscle meat contains around 20 - 25 g protein/100 g. Cooked red meat contains 28–36 % protein, due to water decrease making the nutrient percentage higher during cooking. When compared with vegetable proteins, meat protein is highly digestible, at an average of 94% while the digestibility of beans averages 78% and whole wheat 86% (Williams, 2007). Protein from meat provides all the essential amino acids including lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine and valine. Moreover, amino acids such as glutamic acid and glutamine are also present in beef in high amounts, followed by arginine, alanine and aspartic acid. Human requirements for protein have been thoroughly studied over the years and the recommended daily ingestion is estimated 55 g per day for adult man and 45 g for woman (FAO/WHO, 1985). It is important to notice that these amounts refer to protein "good quality" and highly digestible proteins, otherwise the amount ingested must be increased proportionately to compensate for lower quality and lower digestibility (Bender, 1992).

2.4.2 Protein quality

The quality of a protein is an allotment of its ability to satisfy human requirements for amino acids. Proteins, independently on their source (dietary or tissue proteins), consist of two groups of amino acids; essential amino acids that have to be provided in the diet, and non-essential amino acids that can be synthesised by humans (Bender, 1992). Proteins from animal sources have greater quality in comparison to those from plant sources, and the reason might be the ease of digestibility (Bender, 1992). Protein Digestibility Corrected Amino Acid Score (PDCAAS) is a method for evaluating the protein quality, with a maximum possible score of 1.0. Animal meat such as beef has a score of approximately 0.9, compared with values of 0.5–0.7 for most plant foods (FAO/WHO, 1991).

2.4.3 The importance of meat as a source for micronutrients

Iron bioavailability is higher in meat products as they have a higher amount of heme iron (bioavailable iron) than plant-derived products. Similarly, folic acid has approximately 10-fold higher bioavailability from meat than from vegetables. Consequently, low meat consumption is associated with a number of nutritional deficiencies (Biesalski, 2005). Meat also contains a good lipid proportion, including essential omega-3 and polyunsaturated fats.

Meat and meat products do not only supply absorbed iron but also enhance iron and zinc absorption from other sources. They are also a rich source of some of the B vitamins. Consequently, meat consumption can alleviate common nutritional deficiencies by supplying the above nutrients (Bender, 1992). Furthermore, these micronutrients are either absent in vegetables or have a poor bioavailability (Biesalski, 2005).

In addition, meat also contributes to a low glycaemic index that is essential for the prevention and control of obesity, diabetes and cancer (insulin resistance hypothesis) (Biesalski, 2005). The explanation for this matter consists on the fact that meat products are rich in protein and low carbohydrate being an important nutrient for human health and development. As an essential part of a mixed diet, meat ensures appropriate shipment of essential micronutrients and amino acids, being also involved in regulatory processes of energy metabolism (Biesalski, 2005).

It is important to note that the nutritional composition of meat will vary due to animal breed, feeding regimen, season and meat cut. Generally, lean red meat has a relatively low fat content, moderate cholesterol, and is a rich source of protein and many essential vitamins and minerals (Williams, 2007). In summary, beef is a particularly good source of protein, niacin, vitamin B6, vitamin B12, phosphorus, zinc and iron and provides more than 25% of recommended dietary intakes (RDI) of these nutrients per 100 g (National Health and Medical Research Council, 2006).

2.4.4 The importance of meat intake for elderly people

Current research agrees that although the digestive and absorptive capacity of the digestive tract in the elderly population retains its absorptive capacity, impaired micronutrients bioavailability is a common problem among the elderly, probably as a result of disease rather than ageing itself (Black, 2007). Furthermore, the common incidence of atrophic gastritis in the elderly, affecting vitamin B12 absorption, is one reason to increase the recommend meat intake in this population group (Biesalski, 2005).

Elderly people are generally considered as a population risk group for the incidence of vitamin and trace element deficiencies, especially regarding the vitamins A, D, E, and folate as well as iron and calcium (Biesalski, 2005). Furthermore, age is associated with a steady reduction in body protein content which is reflected by declining fat-free mass that is mainly attributed to skeletal muscle losses defined as sarcopenia (Walrand et al., 2008). Moreover, amino acids play an important role in translational regulation of protein synthesis. Hence, increased protein intake improves whole body and muscle protein synthesis enhancing muscle mitochondrial function in healthy younger and older people (Walrand et al., 2008).

A healthy way for elderly people to obtain the daily protein amount required is by following a diet characterised by low-density, low-calorie and high-quality protein foods. Nonetheless, meat could be tough for old people to chew and accordingly enriched protein foods such as yoghurt would be suitable to meet their specific nutritional requirements (Bhayana, 2011). In addition, another recent report suggests that increasing dietary protein may help to maintain bone and muscle mass in aging people (Gaffney-Stomberg, Insogna, Rodriguez, & Kerstetter, 2009)

2.4.5 Generation of peptides from meat proteins

Meat proteins are broken into peptides by endogenous enzymes during meat products fermentation. Since lactobacilli grown in fermented meat products have only weak proteolytic activity, protein degradation is not greatly affected by the bacteria. However, lactic acid bacteria influence protein degradation by causing a decrease in pH, resulting in higher activity of muscle proteases (Arihara, 2006).

2.4.6 Future prospects

Meat and meat products are not only used to provide necessary nutrients, they are also expected to have additional functions in disease prevention, mental health improvement, and enhancement of general wellbeing (Siro, Kapolna, Kapolna, & Lugasi, 2008). Hence, these demands provide great opportunities for the meat industry. Furthermore, strategies to fortify foods with functional compounds to increase the proportion of micronutrients and limit or eliminate undesirable constituents can be done by dietary supplementation at animal production level, treatments, handling of meat raw materials, and reformulation of meat products (Zhang et al., 2010).

Only a few studies have been done to analyse the possible health benefits of functional meat and meat products in humans (Zhang et al., 2010). Also, further studies are needed to provide reliable evidence on the positive health outcomes obtained from the intake of functional meat and meat products. Finally, the bioavailability of functional ingredients should be maintained during processing and commercial storage. However, many countries do not adopt legislative regulations with regards to functional meat and meat products. Consumers and even experts on food and nutrition cannot differentiate clearly between conventional and functional foods (Niva, 2007). Thus, there is a necessity to generate a safe and most efficient evaluation process for each proposed functional food for consumer's information (Zhang et al., 2010).

2.5 Protein analysis

Food proteins provide nitrogen primarily in the form of essential amino acids that provide nutritional value and functional properties to food. They impart texture and flavour which are organoleptic properties that influence food purchase and consumption (Moore, DeVries, Lipp, Griffiths, & Abernethy, 2010). Analysis of elemental CHN composition (for the determination of carbon, hydrogen, and nitrogen content) is an

excellent alternative to determine total N. This technique is widely used in different branches of science, such as chemistry (Riqueza, de Aguiar, de Aguiar, & de Santa Maria, 2007), food science (Tanizawa, Abe, & Yamada, 2007), environmental sciences (Anderson, 2005), and others. CHN elemental analysers normally use small amounts of solid samples and the samples are dispensed into small tin capsules, which are carbonised at high temperatures (>900°C) for a few minutes. The contents of C, H, and N are oxidised and converted into gaseous forms, which are registered by the integrator connected to the analyser (Barbarino & Lourenço, 2009).

CHN analysis is a quick and sensitive method that can determine low nitrogen concentrations. Despite being an expensive method, it is very reliable and practical (Barbarino & Lourenço, 2009).This method is also the most practical and accurate procedure to analyse nitrogen content of biological samples (Barbarino & Lourenço, 2009). The protein content in food is estimated by multiplying the nitrogen content by a nitrogen-to-protein conversion factor, usually set at 6.25. This factor assumes the nitrogen content of proteins to be 16% (Mariotti, Tomé, & Mirand, 2008).

2.6   Sensory evaluation

Yoghurt is the most common dairy product consumed around the world and its sensory attributes have a large effect on consumer acceptability (Saint-Eve, Lévy, Martin, & Souchon, 2006). Flavour is very important for product acceptance. Yoghurt flavoured by strawberry remains the most popular yoghurt, followed by other fruit flavours (Thompson, Lopetcharat, & Drake, 2007). Allgeyer, Miller and Lee (2010) concluded that for flavoured yoghurt, a medium level of sweetness and a high viscosity drive consumer liking.

The main reason for determining sensory evaluation is to perform tests that are valid and reliable and that generate data on which sound decisions about the product can be based. The main concern of the food industry is to please consumer desires, thus is critical for the food industry to explore and understand consumer preferences. Sensory analyses by consumers are vital to attain product development, set up new product and promote product improvement (Choi, Phillips, & Resurreccion, 2007).

Sensory quality of food products is defined as the acceptance for the sensory qualities of a product by consumers who are the regular users of the product type, or who comprise

the target market for the product (Galvez & Resurreccion, 1992). The consumers play a very important role in the success of a product in the market. Product developers and manufacturers are rapidly grasping that a high level of acceptance by targeting consumers is an essential prerequisite for successfully commercialising a product (Febriani, 2011).

2.6.1 Consumer testing

Consumer testing can provide the best and the most reliable information because only consumers can accurately indicate the degree of liking or preference for a product. Moreover, attributes such as consumer perceptions and product acceptance are critical elements for defining quality (Desai, 2012). Sensory analysis in product development is becoming more important because the value of sensory techniques has been widely recognised and the consumer industry increases its use of sensory evaluation. Also, hedonic scales are carried out by sensory analyses attempt to assess product sensory acceptability (Boutrolle, Arranz, Rogeaux, & Delarue, 2005). Hedonic scales is a common assessment mechanism used when decisions on market introductions are made (Hersleth, Ueland, Allain, & Næs, 2005). Information about consumers' preferences is extremely important for the food industry, and can be used in modifying or improving product quality. In addition, knowledge on customers' age, gender and demographics, is also important for developing a product more strategically or specifically targeted for a certain population.

Chapter. 3. **Materials and methods**

This research was conducted in the food and microbiology laboratories at WS Building of the Auckland University of Technology (AUT), Auckland.

3.1 Materials

3.1.1 Starters and ingredients:

The yoghurt starter used was YC-380 which is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp *bulgaricus,* and was obtained from Chr Hansen, Hamilton, New Zealand.

The other dairy ingredients, namely skim milk powder (Anchor), whole milk powder (Anchor) and Fresh UHT Milk Standard (Meadow) were purchased from a New Zealand supermarket.

3.1.2 Media, chemicals and other materials

Lactobacilli Difco MRS (de Man, Rogosa and Sharp) agar and Difco Oxford agar plates were purchased from Fort Richards Ltd, Auckland. Petroleum ether and containers (500mL polycarbonate screw cap) were purchased from Thermo Fisher Scientific (Biolab Limited), Auckland. Portion sample measure cups were purchased from Office Max, Auckland. Difco Peptone powder, Violet Red Bile Agar (Difco VRBA), xylose–lysine–sodium deoxycholate agar (Difco XLD), Difco Selenite Cystine Broth, Difco Listeria Selective Enrichment Broth, 0.1M sodium hydroxide (NaOH) and phenolphthalein were obtained from Fort Richards Ltd, Auckland.

3.2 Yoghurt processing

3.2.1 Meat preparation

Minced beef obtained from New Zealand dairy bulls (18 - 24 months old) was supplied by AgResearch Ltd. (Ruakura, New Zealand). Before adding to yoghurt, the mince was cooked in a skillet over medium heat with stirring to ensure cooking evenly at 75°C

(McCurdy, 2009). The heat was lowered and cooking was continued for 10 -15 minutes until the mince was completely cooked. The cooked meat was then stored at 4°C and used within the same day of the preparation.

3.2.1 Yoghurt culture preparation

The starter cultures used were donated by Chr Hansen (Hamilton, New Zealand). One small bag (50 units) of yoghurt culture YC-380 was added to 500 ml of UHT milk (Meadow, New Zealand) under aseptic conditions. Two ml of the prepared starter culture was inoculated into 1 liter of yoghurt milk according to the instructions provided by the manufacturer.

3.2.2 Formulation

Yoghurt containing homogenised meat (HMY), yoghurt containing unhomogenised meat (UHMY) and plain yoghurt without meat addition (used as a control) were produced following the formulations presented in Table 4. All equipment, implements, and containers were sterilised by autoclaving at 121°C for 45 min before use.

The basal yoghurts were made by adding whole and skimmed milk powders (Anchor, New Zealand) into cold water to obtain about 20% total solids content. Yoghurts with added meat (5%, 7%, 9% w/w) were made by replacing the amount of whole milk powder with meat such that the total solids content remained constant (Table 4). These preparations were made in 500 ml containers and the ingredients were mixed together with a hand blender. The homogenised meat additions (for HMY samples) were prepared using a homogeniser (L5M-A Laboratory Mixer, Silverson®) at 7000 rpm for 2 minutes.

The yoghurt mixtures were then placed in a water bath for pasteurisation by heating with constant stirring at 85 ± 1°C for 30 min. Then, the milk was cooled until the temperature dropped to 43 ± 1°C using an ice cold water bath. The starter culture preparation was added at 2 ml per 1 litre of yoghurt mixes. The yoghurt mixes were then incubated in a water bath for 5 hour at temperature 42 ± 0.1°C. The fermented yoghurts were then quickly cooled in an ice bath and manually stirred prior to refrigeration in order to breakdown the gels formed during incubation. The yoghurts

were refrigerated at 4°C until analysis. All yoghurt formulations were stored at 4°C for 21 days, and samples were taken for analysis as appropriate. The experiments were done in three different trials.

Table 4. Formulations for the control and developed meat yoghurt products based on 400g.

Samples	Minced cooked meat/g*	Skim Powder/g	Whole Powder/g	Water/ml	Homogenisation
5UHMY	50	40	36	370	No
5HMY	50	40	36	370	Yes
7UHMY	70	40	28	358	No
7HMY	70	40	28	358	Yes
9UHMY	90	40	20	346	No
9HMY	90	40	20	346	Yes
Control	0	40	56	400	-

Samples are expressed as 5HMY= 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY= 9% unhomogenised meat yoghurt.

*The cooked mince meat contained 40% dry weight and 60% moisture weight, the yoghurts with added meat (5%, 7%, 9%w/w) were made by replacing amount of whole milk powder with the meat such that the total solids content remained constant at about 20%.

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┌─────────────────────────────────┐
│    Place milk powders in        │
│         containers              │
└─────────────────────────────────┘
                │
                ▼
┌─────────────────────────────┐        ┌──────────────────────────────────┐
│ Ingredients mix (meat added │ ─────► │ The homogenised meat at 7000 rpm │
│       in this step)         │        │          for 2 minutes           │
└─────────────────────────────┘        └──────────────────────────────────┘
                │                                         │
                ▼                                         │
┌─────────────────────────────┐        ◄──────────────────┘
│   Pasteurization: 85°C/30 min │
└─────────────────────────────┘
                │
                ▼
┌─────────────────────────────┐
│ Inoculation of lactic cultures (43°C) │
└─────────────────────────────┘
                │
                ▼
┌─────────────────────────────┐
│   Incubation for 5 h (42°C)  │
└─────────────────────────────┘
                │
                ▼
┌─────────────────────────────┐
│     Quick cooling and        │
│    refrigeration (4°C)       │
└─────────────────────────────┘
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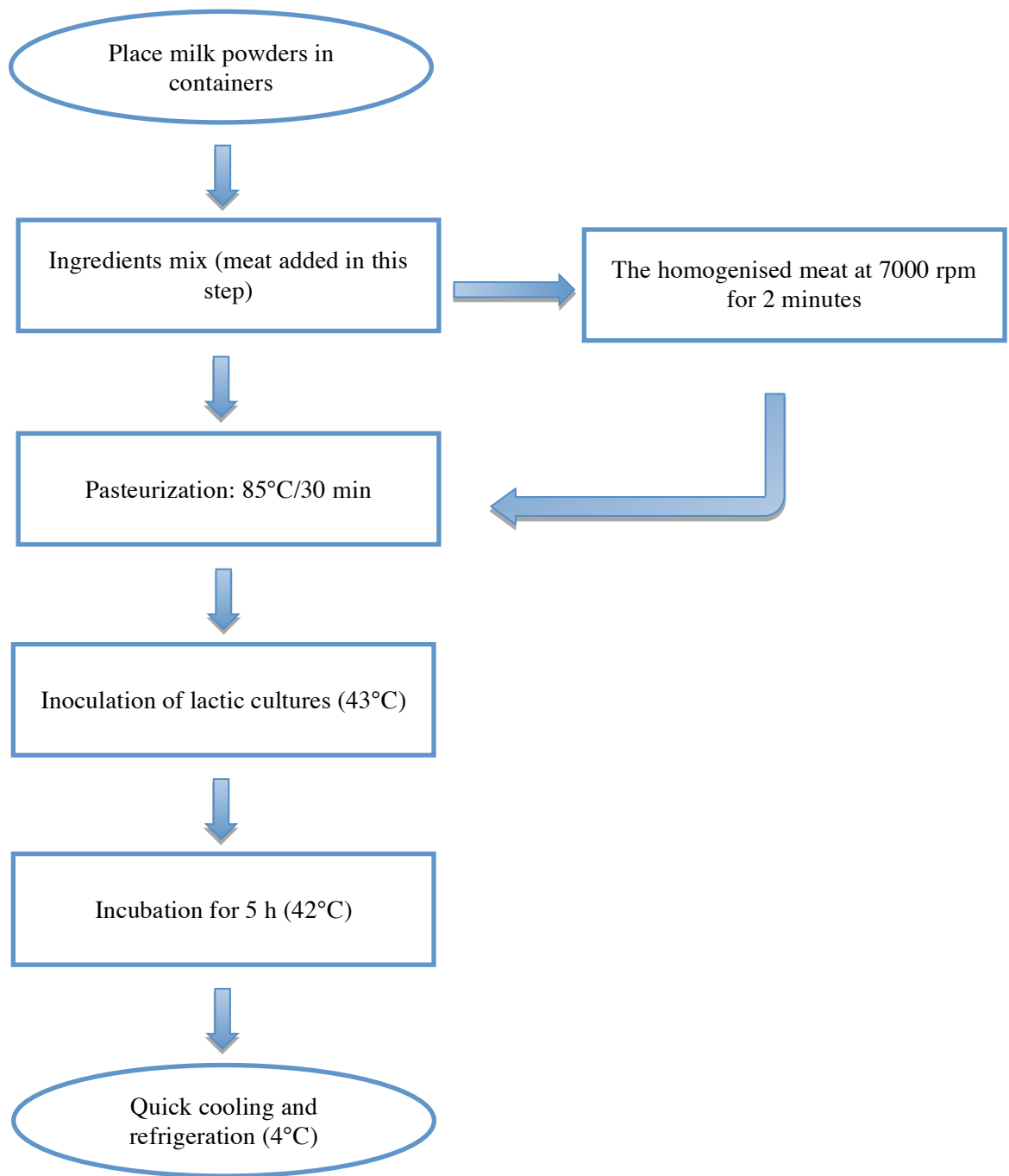
Figure 1. Yoghurt production process

3.3 Microbiology analysis

The procedures below were performed in three different trials.

3.3.1 Preparation of cultural media

MRS agar was prepared for LAB counts with 70 g of agar powder weighed and suspended into 1 L of distilled water. The medium was heated to boiling with agitation to completely dissolve the powder before autoclaving at 121°C for 15 min.

VRBA agar was prepared for coliform counts with 38.5 g of agar powder weighed and suspended into 1 L of distilled water. The medium was heated to boiling with agitation to completely dissolve the powder. No autoclaving was needed.

Selenite Cystine Broth was prepared for coliform counts with 23 g of powder weighed and suspended into 1 L of distilled water. The medium was heated to boiling with agitation to completely dissolve the powder. No autoclaving was needed.

XLD agar was prepared for *Salmonella* counts with 55 g of agar powder weighed and suspended into 1 L of distilled water. The medium was heated to boiling with agitation to completely dissolve the powder. No autoclaving was needed.

3.3.2 Lactic acid bacteria count

Total lactic acid bacteria (LAB) counts were determined weekly through 21 days of storage at 4°C by dilution pour plating using the methods of (Cueva & Aryana, 2008) and DeMan, Rogosa, Sharpe agar (MRS agar, Difco). The LAB numbers were counted at 0 (before fermentation), 1, 7, 14 and 21 days of storage.

Two 10-g samples of yoghurt were diluted with 90 ml of sterile 0.1% w/v peptone water. After uniform mixing, a 1-ml aliquot was mixed with 9 ml sterile peptone water (0.1 % w/v) to prepare serial decimal dilutions. A 1-ml aliquot from each dilution was pour plated using MRS agar. Triplicate plates of each dilution were prepared. The plates were incubated in anaerobic jars for 72 h at 37°C.

3.3.3 Detection of pathogens

Bacteriological methods for the detection of contaminating pathogens in milk and milk products using methods of "User Guide to Standard 1.6.1" have been reported by ANZFA (2001), and were adopted at days 1 and 21 of storage of yoghurt products stored at 4°C.

Coliform counts were determined by plating 1 ml of the diluted (10^{-1}) yoghurt samples on Violet Red Bile Agar (VRBA, Oxoid) in triplicate. For each dilution, two spread plates were prepared using a reusable alcohol spreader, plates were labeled and incubated at 35°C for 48 h.

Detection of *Salmonella* was performed using Xylose–Lysine–sodium Desoxycholate agar (XLD, Difco). Firstly, the yoghurt samples were inoculated into Selenite Cystine enrichment broth (Difco) at 35°C for 24 hours. The enrichment cultures were then serially diluted and spread plated in XLD agar. Triplicate plates were prepared which were then incubated at 35°C for 24 hours. The plates were examined for the presence of typical *Salmonella* colonies which are yellow to red with black centers (Ruby & Ingham, 2009).

Listeria counts were performed by spread plating in triplicate in the selective medium Oxford agar (Fort Richards Ltd, Auckland). For the enrichment step, 25 g of the samples were placed in 225 ml of the *Listeria* Selective Enrichment Broth (Difco) followed by homogenisation. The enrichment cultures were incubated at 35°C for 72 h. A serial dilution of these cultures was prepared and spread plated in Oxford agar in triplicates. The Oxford plates were incubated at 35°C for 48 hours. Typical *Listeria* colonies were observed as brown to black colonies.

3.4 Nutritional composition analyses:

All trials for nutritional composition analyses were carried out on the different yoghurts (control, 5MY, 7MY and 9MY) stored at 4°C on day 7. Each analysis was replicated three times.

3.4.1 Total solids

Total solid compositions were determined as described by AOAC (2000) based on the principle of drying to constant weight, following the formula below:

% Total solids (wt/wt) = wt. of dry sample/ wt. of wet sample* 100.

3.4.2 Fat analysis

Fat content was determined by AOAC (2000) procedure using the Soxhlet extraction method. The samples were firstly dried and ground into fine powder. Dry samples (10 g) were accurately weighed into the extraction thimble and plugged with glass wool. The weight of a pre-dried boiling flask was recorded. Petroleum ether (120 ml) was then added into the boiling flask. The boiling flask, Soxhlet flask, and condenser were assembled. Lipid was extracted using a Soxhlet extractor at a rate of five or six drops per second by condensation for approximately 4-5 hours by heating the solvent in a boiling flask. Finally, the flask was removed and the solvent was distilled off in the fume hood using the water bath. The boiling point of the petroleum ether is 40°C to 60°C, so the water bath was maintained at around 80°C. The flask was weighed when completely dried. The crude fat content in the samples was assumed to be the difference of weight before and after the sample had been refluxed. Fat was determined (on wet weight basis) by using the formula:

% Fat = g of fat in dry sample/g fat of in wet sample* 100.

3.4.3 Protein analysis

Total nitrogen content of the meat yoghurt samples was analysed and quantified by the CHN elemental composition using the method described by Barbarino and Lourenço (2009). Finely ground oven-dried samples were combusted in a CHN elemental

analyser (CE-440 elemental analyser, Exeter Analytical, INC), and protein content was calculated from this value (N x 6.25). Protein was determined (on wet weight basis) by using the same formula for fat.

For the CHN analysis, samples between 1.0 – 3.0 mg were weighed in small tin capsules and subjected to combustion at 925°C for about 2 min in the combustion box of the elemental analyser. Carbonisation was carried out in the presence of ultrapure O_2, promoting the full oxidation of the organic matter. Ultrapure helium was used as a carrier gas. Carbon, hydrogen, and nitrogen present in the samples were converted into CO_2, H_2O, and N_2, respectively. The gases were homogenised, depressurised, and separated using analytical columns and quantified through changes in thermal conductivity of the products. The values were registered automatically by the recorder and integrator coupled to the analyser. Acetanilide (C 71.09%; N 10.36%; H 6.71%) was used for calibrating the instrument. Final concentrations of C, H, and N in the samples were stoichiometrically calculated, considering the percentage of the elements in CHN analysis and the total mass of the oven-dried samples. Six replicates of each sample were analysed by the CHN method ($n = 6$).

3.5   Physicochemical analyses

All trials for physicochemical analysis were carried out on the different yoghurts and each analysis was replicated three times. Theses analyses were measured on day 1,7,14 and 21 during storage at 4°C.

3.5.1   pH measurement

According to the method described by AOAC (2000) the pH values were determined using a pH meter (Meterlab$^@$ Instrument) with a glass electrode standardised at 25°C over the range pH 4.0 to pH 7.0. Five ml of distilled water were added into 25 g of sample. The electrode was immersed in the sample and the pH reading was taken after allowing the meter to stabilise.

3.5.2   Total titratable acidity

The titratable acidity in yoghurt samples was estimated by titration of a suspension of 20 g yoghurt in 20 ml distilled water (AOAC, 2000). The sample then was titrated with 0.1M sodium hydroxide (NaOH) to a pink colour using 1% phenolphthalein as indicator. The titratable acidity was calculated as percent lactic acid as follows:

% Lactic acid = ml of alkali x Normality of alkali x 9 / Weight of sample x 100

3.5.3   Water holding capacity (WHC)

The syneresis of yoghurt, expressed as water holding capacity, was determined using the centrifugation method according to Singh and Muthukumarappan (2008). Approximately 20 g of yoghurt (PY) samples were placed in a tube and centrifuged (Heraeus Instrument labofuge 400e) for 10 min at 3000 rpm at 20°C. The whey expelled (WE) was collected and weighed in grams. For each treatment, three replicates were carried out. The WHC was calculated as:

WHC (%) = (PY-WE)/ (PY) ×100

3.5.4 Viscosity

Apparent viscosity was determined using the method described by Fernandez-Garcia & McGregor (1997). Apparent viscosity of each yoghurt (about 50g) was measured at 10°C in a 250-ml beaker. Samples were tested using a LV spindle number 3 rotated at 1.5 rpm for 1 min with LVT viscometer (Brookfield Engineering, Stoughton, Massachusetts). Yoghurt was gently stirred for 20 s continuously before analysis and triplicate measurements were conducted. Results were recorded in mPa.s.

To convert the viscometer dial reading to a viscosity value in units (mPa.s), the reading noted on the viscometer dial was multiplied by the appropriate factor reported in the manual (Brookfield Engineering Laboratories, n.d.)

Viscosity (mPa.s) = Dial reading x Factor

3.5.5 Colour

A Lab Scan spectrophotometer (Hunter Lab, Colorflex) was used for colour measurement. The spectrophotometer was calibrated with black and white reference standard tiles that came with the instrument. The results were reported in L* (lightness), a* (greenness- redness), and b* (blueness- yellowness) values. Measurements were carried out on 3 replicates for each treatment.

3.6 Sensory analysis

3.6.1 Consumer testing

Consumer acceptance testing of yoghurts was conducted by university students (n = 54). Yoghurts were evaluated five days after production. All samples were removed from the refrigerator 10 min before the start of evaluation sessions. Serving temperature ranged between 10 to 12°C. Yoghurt samples (approximately 10 g each) were divided into portion cups (Office Max, Auckland) and sealed with lids. Yoghurts were randomly presented to the panelists using three digital random numbers, under normal light at room temperature in the AUT Sensory Laboratory.

Panelists consumed water and unsalted plain crackers in between tasting each sample to refresh the palate. Samples were scored by consumers using a nine-point hedonic scale (1 = dislike extremely to 9 = like extremely) to indicate their liking of the products. The panelists were asked to evaluate appearance, flavour, texture, odour and overall quality of the samples. Panelists were also asked to rank samples according to their acceptance. Panelists were served seven samples at a time. The questionnaire for consumer testing is attached in Appendix A.

3.7   Statistical analysis

Mean values from three independent experiments are reported with standard deviations (mean ± standard deviation). The statistical significance of differences observed among treatment means was evaluated by analysis of variance (ANOVA) (XLSTAT version 2012, Auckland, New Zealand), followed by post hoc Tukey's test. The statistical significance of differences observed among treatment means during storage time was determined using ANOVA models to analyse effect of time, treatment and the interaction between time*treatment effect. Significance was defined at the 5% level (95% confidence level).

Chapter. 4. **Results and discussion**

4.1 Microbial analysis, pH and titration

Abbreviations: 5HMY= 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY= 9% unhomogenised meat yoghurt

4.1.1 Lactic acid bacteria counts of yoghurts

The initial viable count (before fermentation) in all seven yoghurts was in the range 1x10E5 cfu/g. After 5 h of fermentation at 42 ± 0.1°C, the yoghurts were stored in a refrigerator for 24 h at 4°C and the count were taken after that as first day count. The counts showed that there had been substantial growth of the LAB during the fermentation period. The viable LAB counts during storage are shown in Table 5 and Figure 2. After 1 day of storage the mean viable counts of all the tested samples (around 30 ×10E7) were not significantly different from those of the control (P<0.05). During the 21 days of storage at 4°C, there was a significant decrease in the viable count in all the yoghurts, but, apart from the samples taken at day 7, there was no significant effect of the presence of meat in the yoghurts. However, the differences observed after 7 days of storage indicate that the viable counts decreased more rapidly in those yoghurts that contained meat and had been homogenised (HMY). After 1 day the viable counts were 35×10E7±4.35 (for 5HMY), 32 ×10E7±3 (for 7HMY) and 28.33×10E7±6.5 (for 9HMY) which decreased rapidly after 7 days to 1.33×10E7±0.15, 3.83×10E7±1.89 and 3.43×10E7±0.92, respectively.

Apart from the samples taken at day 7, there was no significant effect on LAB survival of the presence of meat in the yoghurts. After 21 days of storage LAB counts were still > 2.3 x 10E7 cfu/g in the control and all the unhomogenised meat yoghurts (5UHMY, 7UHMY and 9UHMY), In all the homogenised meat yoghurts (5HMY, 7HMY and 9HMY) the mean counts decreased significantly to 0.16 x 10E7 ± 0.12, 0.46 x 10E7 ±0.14 and 0.35 x 10E7 ± 0.12, respectively (P<0.05). The differences observed after 7 days of storage indicate that the viable counts decreased more rapidly in those yoghurts that contained meat and had been homogenised (HMY). Generally, the samples*days interaction was significant (F = 53.77, P < 0.05) while the meat additions (treatments)

were not significant (F = 0.56, P > 0.05). The storage time significantly (F = 175.17, P < 0.05) affected the microbial counts.

Table 5. Total lactic acid bacteria LAB viable counts, CFU ×10E7 /g in yoghurts during storage at 4°C.

| Samples | Days | | | | (F value) | | |
	1	7	14	21	Samples	Days	Sample*Days
5HMY	35±4.35 [A,a]	2.83±0.28 [B,b]	1.33±0.15 [B,b]	0.16± 0.12[B,b]			
5UHMY	33.66±3.21 [A,a]	21±3.6 [B,a]	6.16±2.25 [C,ab]	2.3±0.43 [C,a]			
7HMY	32±3[A,a]	5.5±0.5 [B,b]	3.83±1.89 [BC,ab]	0.46±0.14 [C,b]			
7UHMY	35±5 [A,a]	19.66±6.42 [B,a]	6.66±2.08 [C,a]	2.56±1.25 [C,a]	0.56	175.17*	53.77*
9HMY	28.33±6.5 [A,a]	4.8±1.75 [B,b]	3.43±0.92 [B,ab]	0.35± 0.12[B,b]			
9UHMY	33.33±7.23 [A,a]	7.16±1.04 [B,b]	5.9±1.01 [B,ab]	3± 0.91[B,a]			
Control	30.33±3.51 [A,a]	17.66±2.51 [B,a]	7.13± 2.5[C,a]	2.83±0.2 [C,a]			

[A-C]Means ± standard deviations in periodic samples. Different superscript uppercase letters are significantly different (*P* < 0.05).
[a-b]Means ± standard deviations in yoghurt treatments. Different superscript lowercase letters are significantly different (*P* < 0.05).
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.
* P value was significant (P < 0.05).

Figure 2. Total lactic acid bacteria LAB viable counts in yoghurts during storage at 4°C.
A-C Different letters represent significant differences between storage days (P < 0.05)
a-b Different letters represent significant differences between yoghurt treatments (P < 0.05)
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.

4.1.2 Pathogens

The viable pathogens counts after 1 and 21 days are shown in Table 6. Neither *Salmonella* nor coliforms were detected in the yoghurts indicating that the count was less than 100 cfu per g. Viable *Listeria* was not detected either in any samples. The presence of the meat had no effect when compared to the control.

Table 6. Viable count of pathogens after 1 and 21 days of storage at 4 $^{\circ}$C.

	Coliform		*Salmonella*		*Listeria*	
	1	21	1	21	1	21
5HMY	No Growth	No Growth	No Growth	No Growth	Not detected	Not detected
5UHMY	No Growth	No Growth	No Growth	No Growth	Not detected	Not detected
7HMY	No Growth	No Growth	No Growth	No Growth	Not detected	Not detected
7UHMY	No Growth	No Growth	No Growth	No Growth	Not detected	Not detected
9HMY	No Growth	No Growth	No Growth	No Growth	Not detected	Not detected
9UHMY	No Growth	No Growth	No Growth	No Growth	Not detected	Not detected
Control	No Growth	No Growth	No Growth	No Growth	Not detected	Not detected

Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.

4.1.3 pH

The pH values of the yoghurts during 21 days of storage are shown in Figure 3 and Table 7. Changes in pH values in yoghurts during storage at 4°C. The initial pH value of the cooked minced beef was 5.96, and for the milk pH 7.2. After fermentation at 42 ± 0.1°C, the pH values of all the yoghurts were similar and the presence of the meat had no effect on the incubation time required to reach pH 4.35. After 1 day of storage at 4 °C, there were no differences in the pH values of the yoghurts. Although the 5UHMY value was slightly higher in comparison with other samples, no significant difference was found (P > 0.05). During 21 days of storage, the pH values of all the yoghurts decreased significantly from the values observed at day 1, but there were no significant differences among the different yoghurts. The mean pH values of all control and fortified samples ranged around 4.31 ± 0.22 (after day 1) and 4.00 ± 0.06 (after day 21). The normal pH of commercial yoghurt products ranges from 4.0 to 4.4 (Sahan et al., 2008). Generally, the storage period had a significant effect on the pH of the yoghurts (F = 25.2, P < 0.05), and the samples*day interaction was also significant (F = 3.311, P

< 0.05). However, the differences between the samples were not significant (F = 1.611, P > 0.05).

Table 7. Changes in pH values in yoghurts during storage at 4°C

| | Days | | | | (F value) | | |
Samples	1	7	14	21	Sample	Days	Sample*Days
5HMY	4.2±0.15 [A,a]	4±0.03 [AB,a]	4.03±0.07 [AB,a]	3.9± 0.01[B,a]			
5UHMY	4.28±0.2 [A,a]	4.07±0.07 [BC,a]	4.09±0.1 [B,a]	3.97±0.04 [C,a]			
7HMY	4.24±0.15[A,a]	4.02±0.005 [AB,a]	4.05±0.15 [AB,a]	3.91±0.01 [B,a]			
7UHMY	4.3±0.23 [A,a]	4.1±0.08 [A,a]	4.12±0.17 [A,a]	3.99±0.09 [A,a]	1.611	25.204*	3.311*
9HMY	4.3±0.23 [A,a]	4.09±0.05 [A,a]	4.1±0.15 [A,a]	3.97± 0.06[A,a]			
9UHMY	4.39±0.29 [A,a]	4.15±0.13 [A,a]	4.07±0.13 [A,a]	4.03± 0.14[A,a]			
Control	4.38±0.23 [A,a]	4.12±0.1 [A,a]	4.18±0.15 [A,a]	4.1±0.09 [A,a]			

[A-C]Means ± standard deviations in periodic samples. Different superscript uppercase letters are significantly different ($P < 0.05$).

[a]Means ± standard deviations in yoghurt treatments. Different superscript lowercase letters are significantly different ($P < 0.05$).
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.
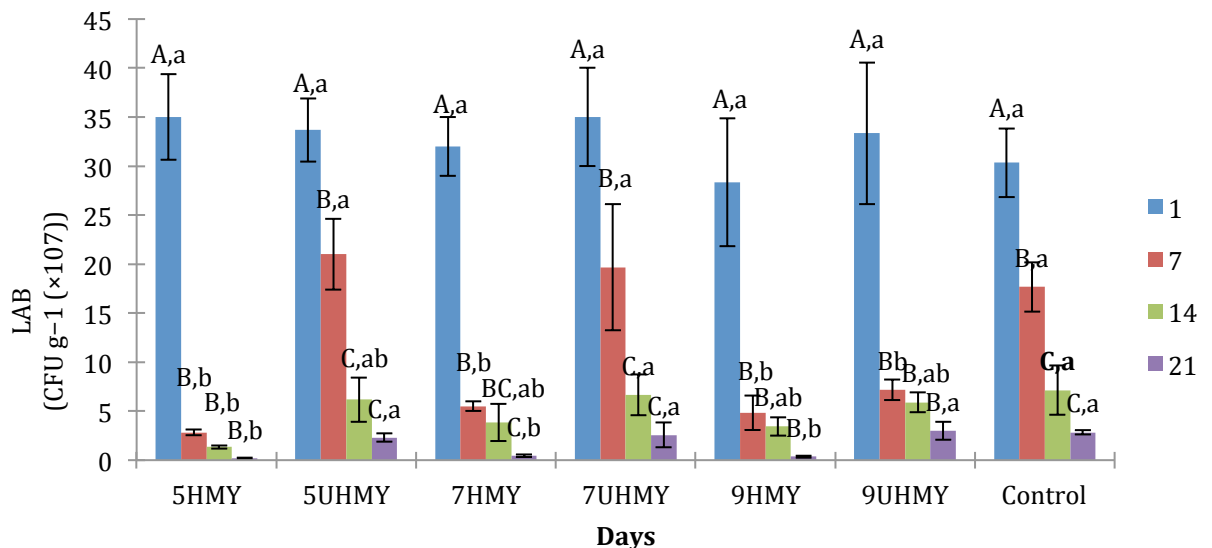* P value was significant (P < 0.05).

Figure 3. Changes in pH values in yoghurts during storage at 4°C.

A-C Different letters represent significant differences between storage days (P < 0.05)
a Different letters represent significant differences between yoghurt treatments (P < 0.05)
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.

4.1.4 Total acid concentration (by titration)

The results expressed as lactic acid (%) are shown in Table 8 and Figure 4. Overall, there was a slight decrease in the titratable acidity of all the yoghurts during 21 days of storage. The only significant differences (P<0.05) were observed at Day 21, where the addition of 5% and 7% of homogenised meat resulted in (1.66% LA) and (1.68% LA) which were higher concentrations compared to that of the 9% unhomogenised meat (1.49% LA) but was only slightly higher compared to the control (1.25% LA). Generally, the storage period did not have a significant effect on acidity of tested yoghurts (F = 1.743, P> 0.05). However, the samples*day interaction was significant (F = 1.728, P < 0.05). There were also significant differences in total acids produced in the different treatments (F = 8.021, P < 0.05).

Table 8. Changes in lactic acid concentration (%) in yoghurts during storage at 4°C.

Samples	Days				(F value)		
	1	7	14	21	Sample	Days	Sample*Days
5HMY	1.83±0.28 ^{A,a}	1.77±0.21 ^{A,a}	1.69±0.17 ^{A,a}	1.66± 0.15 ^{A,a}			
5UHMY	1.56±0.28 ^{A,a}	1.55±0.21 ^{A,a}	1.46±0.14 ^{A,a}	1.45±0.12 ^{A,ab}			
7HMY	1.84±0.27 ^{A,a}	1.81±0.15 ^{A,a}	1.71±0.15 ^{A,a}	1.68±0.13 ^{A,a}			
7UHMY	1.45±0.24 ^{A,a}	1.47±0.15 ^{A,a}	1.41±0.15 ^{A,a}	1.38±0.06 ^{A,ab}	8.021*	1.743	1.728*
9HMY	1.69±0.4 ^{A,a}	1.74±0.21 ^{A,a}	1.64±0.12 ^{A,a}	1.61± 0.19 ^{A,ab}			
9UHMY	1.45±0.22 ^{A,a}	1.37±0.16 ^{A,a}	1.28±0.1 ^{A,a}	1.25± 0.12 ^{A,b}			
Control	1.68±0.36 ^{A,a}	1.68±0.35 ^{A,a}	1.52±0.18 ^{A,a}	1.52±0.14 ^{A,ab}			

^{A}Means ± standard deviations in periodic samples. Different superscript uppercase letters are significantly different ($P < 0.05$).

$^{a-b}$Means ± standard deviations in yoghurt treatments. Different superscript lowercase letters are significantly different ($P < 0.05$).

Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.
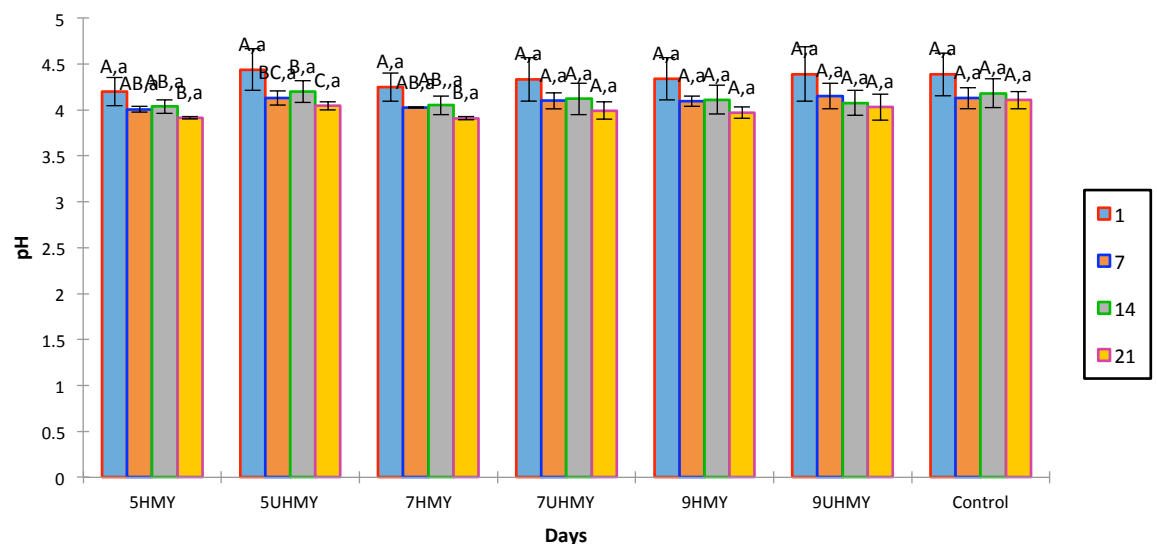
* P value was significant (P < 0.05).
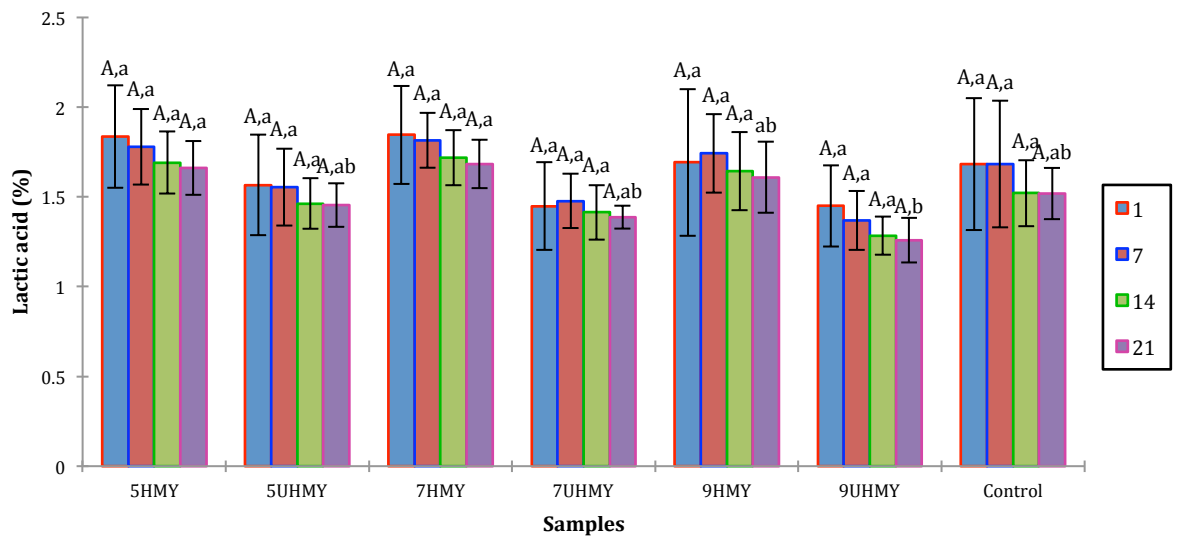
Figure 4. Changes in lactic acid concentration in yoghurts during storage at 4°C.

A Different letters represent significant differences between storage days (P < 0.05)
a-b Different letters represent significant differences between yoghurt treatments (P < 0.05)
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt;
9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7%
unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.

4.1.5 Discussion

The results showed that the addition of cooked minced beef to yoghurt had no significant effect on the viable counts of lactic acid bacteria during a 21 day storage period at 4°C, compared to the control. However, all the yoghurts, including the control, did show significant loss of viability during this period. Despite this, a concentration level of over 10E7 cfu/g in these yoghurts, particularly the meat yoghurts, would still qualify them as probiotic. The recommended amount of probiotics usually prescribed as therapeutic doses, ranges from 10E6 (Kurmann & Robinson, 1991) to up to 10E7 cfu/ml (Lourens-Hattingh & Viljoen, 2001). According to Food Standards Australia New Zealand (FSANZ) Standard 2.5.3, the present legislation states that the minimum viable quantity of probiotic culture should be not less than 10E6 colony/g of yoghurt during the period of storage and use (FSANZ, 2008). Furthermore, the Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) set broader international standards for yoghurt published in the Codex Standard for Fermented Milks. This document defines yoghurt as the product of starter cultures fermentation with a minimum amount of 2.7% milk protein, less than

15% milk fat, and at least 0.6% titratable acidity are specified. In addition, yoghurt must contain at least 10E7 colony forming units (CFU) per gram (Codex standard, 2003).

Interestingly, there was a significant indication that those yoghurts that contained homogenised meat showed a more rapid loss in viability than either the control or those yoghurts containing unhomogenised meat. This may be related to the slightly higher levels of acidity that were observed in these yoghurts, and may be associated with the small particle size of the homogenised meat.

The pH values of the yoghurts decreased during the 21 days storage period, but the addition of meat to the yoghurt had no significant effect compared to the control. In general, the pH was lower in yoghurts fortified with homogenised meat when compared with unhomogenised meat. However, the titratable acidity after 21 days of storage was higher in yoghurts containing 5% and 7% homogenised meat, but the difference was small compared to that in the control.

Thus, overall, the presence of meat in the yoghurts had no significant impact on the viable LAB counts, pH or titratable acidity of the yoghurts, except for a slightly more rapid viability decrease in the presence of homogenised meat, which also had slightly lower pH values. The survival of LAB in yoghurt during storage is important for the health properties of the product (Tamime & Robinson, 2007). The results in the present work indicate that the presence of meat had no adverse effect on the 21-day viable count, which remained in excess of 1 x10E7 cfu/g.

In terms of maintenance of viable LAB counts, the present results are in agreement with those of Estrada et al. (2011) who added microencapsulated menhaden oil and salmon oil to yoghurt, and with those of Hekmat and McMahon (1997) who fortified the yoghurt with iron. Furthermore, the addition of purple rice bran oil did not affect the LAB counts compared to those of the controls during 6 weeks of frozen yoghurt storage at -22°C.

The presence of meat had no significant effect on the growth of potential pathogens in the yoghurts. Growth of pathogens in yoghurt is normally prevented by the low pH value of the product, but is always a risk when meat is added to this product. Low pH of the yoghurt is sufficient to inhibit any natural contaminations (Hekmat & McMahon,

1997). The present results showed no stimulation of selected pathogens when compared to the control, probably because the pH values were little affected by the meat. Thus, growth of any natural contaminants would have been inhibited. Bachrouri et al. (2002) reported that *E coli* does not survive during the yoghurt fermentation process, and any presence of this organism in the product would indicate post-processing contamination. Ruby and Ingham (2009) reported that presumptive *L. sakei* can inhibit the growth of both *E coli* and *Salmonella* in a beef broth medium and in fresh raw ground beef. Moreover, the study on addition of iron to yoghurt illustrated that there was no growth of *E. coli* in any of the yoghurts even when inoculated with 10E3 or 10E5 cfu/ml of this bacteria (Hekmat & McMahon, 1997). According to the Standard 1.6.1 - Microbiological Limits for Food, the limit for coliforms should be less than 100/g and for *Salmonella* and *Listeria* should not be detected in 25g throughout the shelf-life for specified dairy products and packaged cured or salted meat (ANZFA, 2001).

Detection of *Listeria* in some samples showed presumptive colonies fewer than 50 colonies per dilution after an enrichment step. This low plate count is below the statistical accuracy for viable count. To obtain such low concentration despite using an enrichment step meant that *Listeria* was considered absent in the samples.

A number of researchers have reported the use of LAB to control the growth of spoilage and pathogenic bacteria in a variety of foods (Salminen et al., 1993). A major benefit of some of these LAB is their ability to produce inhibitory compounds at refrigeration temperatures while not growing themselves. Moreover, H_2O_2 produced by these lactobacilli at refrigeration temperatures was reported as the principal cause for the inhibition of undesirable organisms in refrigerated foods (Ruby & Ingham, 2009).

4.2 Physicochemical characteristics

4.2.1 Nutritional composition

4.2.1.1 Total solids

Total solids include fat, protein, carbohydrate and minerals for yoghurt product. Total solids contents were similar in all the experimental yoghurts. However there were significant differences between levels of fat and protein in the samples. The total solids content of the yoghurt is shown in Table 9. The average total solids content of all yoghurts was around 19.5% with a standard deviation of 0.62. The moisture content of raw lean beef meat is 64%, while cooked lean beef meat was 60% and this decrease in water content was probably due to heating (Food Safety and Inspection Service, 2013). Skim milk powder (SMP) has a moisture content of <4% while for whole milk powder (WMP) it is 2.9% (Febriani, 2011). In this study, yoghurts with added meat (5%, 7%, 9%w/w) were made by replacing an amount of WMP with meat such that the total solids content remained constant to obtain about 20% total (Table 9).

Table 9. Nutritional composition of yoghurt samples containing meat.

Samples	Solid Content,%	Protein (% in weight basis)	Fat (% in weight basis)
Control	19.58 ± 0.1^a	6.1 ± 0.49^d	2.2 ± 0.02^a
5MY	19.52 ± 0.78^a	7.98 ± 0.44^c	1.7 ± 0.2^{ab}
7MY	19.53 ± 1^a	8.65 ± 0.24^b	1.57 ± 0.27^b
9MY	19.44 ± 0.54^a	9.98 ± 0.28^a	1.41 ± 0.23^b

[a-d]Means ± standard deviations in yoghurt treatments. Different superscript letters are significantly different ($P < 0.05$).
Samples expressed as 5MY= 5% meat yoghurt; 7HM = 7% meat yoghurt; 9MY = 9% meat yoghurt.

4.2.1.2 Fat

The fat content of meat yoghurts and control is shown in Table 9. The higher amount of fat content in the control was not significant (P>0.05) to 5MY but was significantly different with the 7MY and 9MY samples. The yoghurt samples in this study can be considered to be a low fat yoghurt as the results are in line with findings of Janhoj and Petersen (2006) who reported fat contents of low-fat stirred yoghurt ranging between 0.3 to 3.5%.

Samples containing more meat (7MY, 9MY) had significantly lower fat content (P<0.05) than the control. The fat content of lean beef meat is 2.8% (Williams, 2007). SMP has a fat content of <1%, while WMP is 28% (Febriani, 2011). It is clear that the low fat content of lean meat accounted for the decreasing amount of fat content in meat yoghurt samples. This is supported by the findings (Table 9) as samples containing increasing amount of meat addition (5%, 7% and 9%) had decreased fat content. During yoghurt making, the mix was homogenised and the fat becomes coated with casein, which causes the homogenised and size-reduced fat globules to behave as very large casein micelle-coated spheres. Thus, there is an increase in the consistency, and a decrease in syneresis (Berber, 2011).

4.2.1.3 Protein

One of the main objectives of this research was to develop a high protein yoghurt by increasing the protein content as much as possible with meat protein. Table 9 shows that the protein content was significantly different for all control and meat yoghurts. The protein content of control yoghurt (6.1 ± 0.49%) was in line with findings by Janhoj and Petersen (2006) who reported that the protein contents of low-fat stirred yoghurt ranged from 3.4 to 6.0%. As expected, the protein content increased dramatically in 5MY, 7MY and 9MY yoghurts (7.98 ± 0.44%, 8.65 ± 0.24% and 9.98 ± 0.28% respectively). There was a significant (p<0.05) increase of protein as more meat was added into the yoghurt.

Samples containing more meat addition had significantly higher protein content (P<0.05) than the sample containing lower meat. The protein content of cooked red meat is typically 28- 36g/100g (Williams, 2007). SMP has a protein content of 36% while WMP has 26% (Febriani, 2011). It is noticeable that the high protein content of meat was mainly responsible for the increasing amount of protein content in meat yoghurt samples. This is supported by the findings (Table 9) as samples containing increasing amount of meat addition (5%, 7% and 9%) had increased protein content.

By incorporating meat into yoghurt, a nutritious yoghurt product was obtained that had a higher protein content than normal yoghurt. A study reported the average protein content of probiotic yoghurt to be 5.4 % while the average protein content of natural yoghurt was 5.3% (Hussain & Atkinson, 2009). Drake and Chen (2000) further reported

that the addition of soy protein isolates to yoghurt resulted in higher protein contents due to soy protein concentrate composition, even though yoghurts were formulated with equivalent percent solids.

Different hydrocolloids, such as carrageenan and guar gum, are used in dairy formulations to physically stabilise dispersed materials and/or improve texture, resulting in high viscosity (Donkor et al., 2007). In this study, there were no hydrocolloids used in yoghurt formulations.

4.2.2 Viscosity of yoghurt

The viscosities of all meat yoghurts and the control are presented in Table 10 and Figure 5. The statistical analysis showed a significant (F = 87.212, P < 0.05) effect of meat additions (treatments) and the interaction between storage and meat additions (F = 49.296, P < 0.05) on the viscosity of yoghurt. However, the storage time did not affect (F = 0.906, P > 0.05) the viscosity values over 21 days.

The viscosity of the yoghurt containing the meat was significantly different from the control yoghurt sample (p<0.05). Generally, apparent viscosity decreased significantly (P<0.05) with the addition of meat. The plain yoghurt (control) had the highest viscosity value followed by 5UHMY and 5HMY and the difference was significant. The apparent viscosity of 5UHMY did not vary significantly (P<0.05) when compared to the control at day 21.

The addition of 5% meat resulted in a significantly (P<0.05) higher viscosity compared with yoghurt with other meat additions. As shown in Table 10, the initial viscosity values of control and yoghurt 5UHMY (16000±800 mPa.s and 11400±529 mPa.s respectively) were considerably higher than the other yoghurts (ranging between 7066±461 mPa.s for 5HMY and 4200±600 mPa.s for 9HMY). The viscosity of 5UHMY on day 21 (10400±400 mPa.s) was found to be statistically not significantly different from that of control yoghurt at (12853±1520 mPa.s).

Table 10. Viscosity values of yoghurts stored at 4°C.

Samples	Days				(F value)		
	1	7	14	21	Sample	Days	Sample*Days
5HMY	7066±461[A,c]	6720±811[A,c]	9866±2052 [A,bc]	8400± 1385[A,bc]			
5UHMY	11400±529 [A,b]	10733±702 [A,b]	11366±404 [A,b]	10400±400 [A,ab]			
7HMY	5000±200[C,de]	6026±280 [BC,cd]	8566±602 [A,bcd]	6240±634 [B,cd]			
7UHMY	5466±832 [A,cde]	6880±288 [A,c]	7466±1154 [A,cd]	6053±482 [A,cd]	87.212*	0.906	49.296*
9HMY	4200±600 [C,e]	5133±266 [BC,d]	6666±611 [A,d]	5746± 601[AB,d]			
9UHMY	6533±832 [A,cd]	4860±361 [B,d]	6400±0 [A,d]	4340±441[B,d]			
Control	16000±800 [A,a]	15133±808 [A,a]	14533±1514 [A,a]	12853±1520 [A,a]			

[A-C]Means ± standard deviations in periodic samples. Different superscript uppercase letters are significantly different ($P < 0.05$).

[a-d]Means ± standard deviations in yoghurt treatments. Different superscript lowercase letters are significantly different ($P < 0.05$).

Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.

* P value was significant (P < 0.05).
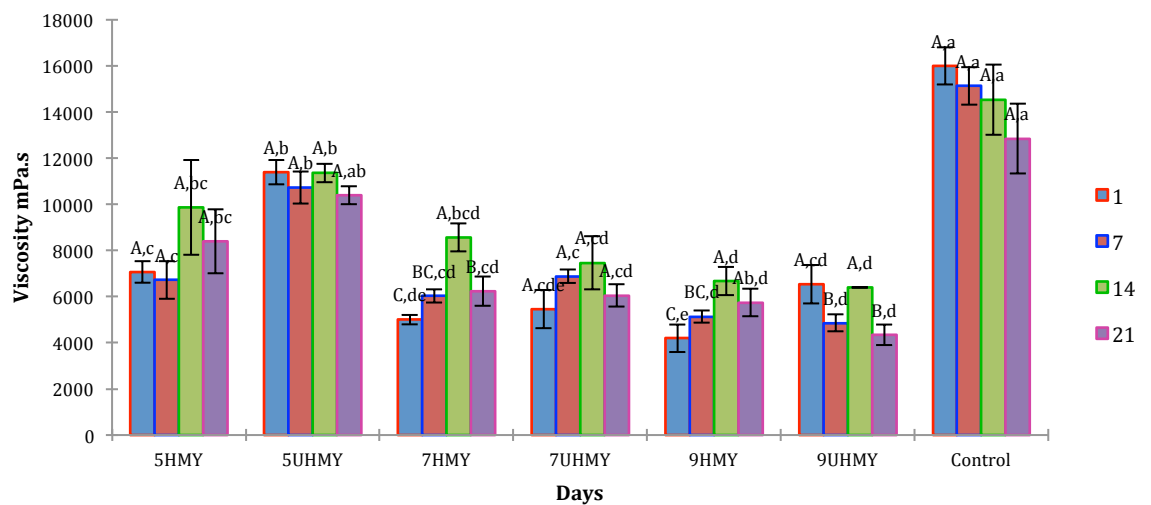
Figure 5. Viscosity values of yoghurts stored at 4°C.

A-C Different letters represent significant differences between storage days (P < 0.05).
a-d Different letters represent significant differences between yoghurt treatments (P < 0.05).
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.

4.2.2.1 Discussion

Yoghurt fortified with 5% meat had the second highest viscosity value after that of the control, whereas the 9% meat yoghurt had the lowest viscosity over the 21 days of storage. The findings showed that the more meat added, the lower the viscosity values. The increase in viscosity is related to increased protein-protein interactions and protein bonds that increase the elastic character of the gel matrix of the yoghurt (Damin et al., 2009).

The decrease in viscosity due to the addition of meat may be attributed to lack of interactions between meat molecules and dairy proteins. Aryana and Boeneke (2007) reported that apparent viscosities of yoghurts fortified with minerals such as chromium (Cr) and magnesium (Mg) were significantly lower than viscosities of control yoghurts. As yoghurt is a gel/matrix of casein micelles, Mn and Cr might induce a change in yoghurt gel microstructures making the matrix more open and loose, resulting in a lower viscosity (Achanta, Aryana & Boeneke, 2007). Sodini et al. (2005) reported different results, in which the addition of grains in yoghurt resulted in a coarse microstructure and low viscosity, which might be due to a lower degree of casein aggregation and a looser network. Sendra et al. (2010) observed that apple fibre fortification decreased yoghurt compression values, probably due to the formation of fibre aggregates that interfered with yoghurt structure. In the present study the addition of 5% meat had little influence on viscosity. However, there was more decrease in viscosity values in the 7% and 9% meat samples.

4.2.3 Water-holding capacity (WHC)

The syneresis of yoghurt, expressed as water-holding capacity, is an undesirable property. The water-holding capacity of all meat yoghurts and control are presented in Table 11 and Figure 6. Overall, there was a significant effect of meat additions (F = 38.717, P < 0.05) and the interaction between storage and meat additions (F = 13.249, P < 0.05) on the WHC of yoghurt. However, the storage time did not affect (F = 2.065, P > 0.05) the WHC values over 21 days storage.

The 5UHMY sample had the second highest water holding capacity among the samples, and was not significantly different (P>0.05) from the control (the highest value) throughout storage. On day 1, the WHC of 5UHMY was (78±3%) which was 6% lower

than the control yoghurt (84±2%). The WHC of 5UHMY on day 21 (70±2 %) was statistically lower than that of the control yoghurt at (79±3 %). Increased meat addition to the yoghurts led to a decrease in water holding capacity, and may be related to the lower viscosity and less stable structure (Cueva & Aryana, 2008).

Table 11. Values for WHC in yoghurts during storage at 4°C.

Sample	Days				(F value)		
	1	7	14	21	Sample	Days	Sample*Days
5HMY	69±5 [A,bc]	62±7 [A,ab]	66±4 [A,abc]	64± 4 [A,bc]			
5UHMY	78±3 [A,ab]	69±6 [A,ab]	70±7 [A,ab]	70±2 [A,b]			
7HMY	62±2 [A,cd]	58±3 [A,bc]	58±4 [A,bc]	57±0.5 [A,cd]			
7UHMY	67±3 [A,c]	65±3 [A,ab]	67±5 [A,abc]	65±3 [A,bc]	38.717*	2.065	13.249*
9HMY	55±1 [A,d]	49±0.5 [B,c]	54±1 [A,c]	55± 0.8 [A,d]			
9UHMY	71±3 [A,bc]	66±1 [A,ab]	72±4 [A,a]	68± 3 [A,b]			
Control	84±2 [A,a]	71±3 [B,a]	76±2 [AB,a]	79±3 [AB,a]			

[A-B]Means ± standard deviations in periodic samples. Different superscript uppercase letters are significantly different ($P < 0.05$).

[a-d]Means ± standard deviations in yoghurt treatments. Different superscript lowercase letters are significantly different ($P < 0.05$).

Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.

* P value was significant (P < 0.05).
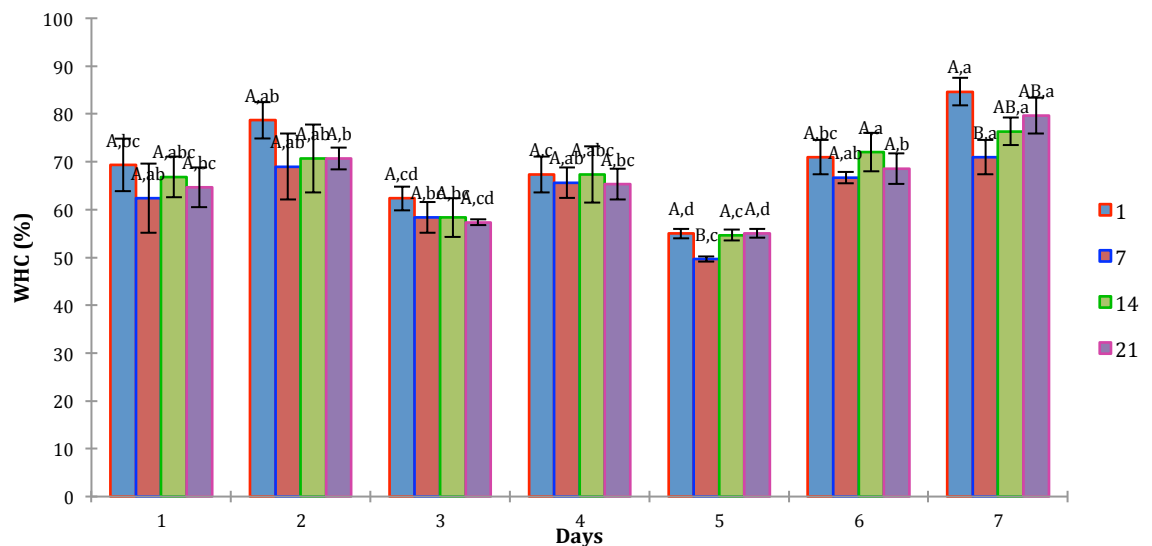
Figure 6. Values for WHC in yoghurts during storage at 4°C.

A-B Different letters represent significant differences between storage days (P < 0.05).
a-d Different letters represent significant differences between yoghurt treatments (P < 0.05).
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.

4.2.3.1 Discussion

WHC measurements showed significant differences between meat fortified and control yoghurt samples (Figure 6). The value of WHC in the control was not significantly to that of 5UHMY but was significantly higher than the other yoghurts. Yoghurts fortified with meat in this study had more syneresis. In previous studies it was always noted that reduction in whey separation corresponded with an increase in WHC (Nouri, Ezzatpanah, & Abbasi, 2011). However, HMY yoghurt with 7% and 9% meat addition exhibited the lowest water holding capacity ($p < 0.05$) which is possibly due to the lower viscosity. Meat addition negatively affected the structure and rheological properties of yoghurts in this study. Lower WHC or whey separation is related to an unstable gel network and excessive rearrangements of a weak gel network (Lucey, 2001). Hence, increased homogenised meat addition to the yoghurt resulted in decreased colloidal meat linkage between casein micelles and, hence, less intense network of the yoghurt gels. Singh and Muthukumarappan (2008) measured whey separation of yoghurt and observed a similar decrease in whey separation of calcium fortified plain yoghurt as compared to control. On the other hand, Nal et al. (2005) reported that the incorporation of inulin at more than 1 g/100 ml into yoghurt increased whey separation. The present results suggest that 5% meat addition to yoghurt is a suitable product as it caused only a limited effect on whey separation.

4.2.4 Correlation between viscosity, WHC, fat and protein

The presence of fat is important on both texture and flavour of yoghurt. Low-fat yoghurts had lower texture and sensory results than full fat yoghurts (Berber, 2011). This might explain the changes in texture in the experimental yoghurt products, which contained less fat. Other studies (Sandoval-Castilla et al., 2004) also reported that fat globules contributed to texture and flavour of yoghurt by enhancing its body and imparting richness to the flavour. In addition, the presence of fat supports the interactions of fat globules with protein molecules, which are important for the textural properties of the finished product (Berber, 2011). In this study, a relationship was found between fat content and viscosity. The higher the fat content, the more similar the meat yoghurts were to the control. During yoghurt making, the mix is homogenised and the fat becomes coated with casein, which causes the homogenised and size-reduced fat

globules to behave as very large casein micelle-coated spheres. Thus, there is an increase in the consistency, and a decrease in syneresis (Keogh & O'Kennedy, 1998).

A strong gel matrix is formed when emulsion formation occurs (Hui, 2012). Another important factor that affects the rheological properties of yoghurt is protein. Myofibrillar meat proteins produce a strong gel. Conversely, sarcoplasmic meat proteins do not contribute to the stabilisation of the product because the gel they produce is very weak (Hui, 2012). Therefore the addition of more beef meat resulted in a decrease in viscosity. Typically a decrease in viscosity would suggest a decrease in WHC probably because of less water being held within the product (Cueva & Aryana, 2008). Changing fibrous proteins into a viscous fluid is relatively easy with pork and chicken meat, but more difficult with beef and lamb (Hui, 2012). This is because different animal species may present a wide variety of protein characteristics, probably due to interaction effects (Zorba, 2006). These differences in functional properties can also derive from intrinsic factors such as protein structure, molecular mass, and amino acid composition (Liu et al., 2008).

Low-fat products have gained popularity because of increasing demands of consumers who seek healthy options across product categories. However, fat solids reduction in yoghurt has been associated with poor texture including viscosity and WHC. Therefore, production of low-fat and non-fat yoghurt demands careful control of texture and flavour attributes (Isleten & Karagul-Yuceer, 2006). In order to improve these low-fat meat yoghurts, the dispersed phase should be totally or partially replaced by other materials that can form a two-phase system similar to an emulsion. Fat substitutes such as starches, hydrocolloids, gums (pectin, carrageenan, gelan, xanthan), and plant proteins could be further added. These ingredients also provide gelling properties and texture, bind liquids, control syneresis, improve slicing, and increase product yield (Hui, 2012).

Figure 7. The image of the experimental yoghurt samples.

Samples expressed as 5%HMY= 5%homogenised meat yoghurt; 7%HMY = 7%homogenised meat yoghurt; 9%HMY = 9%homogenised meat yoghurt; 5%UMY = 5%unhomogenised meat yoghurt; 7%UMY= 7%unhomogenised meat yoghurt; 9%UMY= 9%unhomogenised meat yoghurt.

4.2.5   Colour of yoghurts

Colour is a very important aspect in food since it is usually the first property the consumer observes (Saenz et al., 1993). Changes in physical, chemical or microbiological parameters in yoghurt can influence shelf life and cause colour deterioration (Coggins et al., 2010). Colour is also an indicator of quality, freshness, conservation state, flavour expectation and commercial value (Fradique et al., 2010).

Colour varied with the homogenised meat yoghurts. The homogenised meat yoghurts were darker with a redder colour as shown in Figure 7. This was consistent with the Hunter L*, a*, and b* values shown in Table 12, Table 13 and Table 14. The L* and b* values for all the samples did not change significantly during storage. However, a* values for the HMY samples decreased from positive to negative values during storage. All samples had significantly lower L* values during storage compared to the control. a* values of HMY samples significantly decreased during storage, while the control and

UHMY had steady negative a* values. The b* values for the yoghurt samples were not significantly affected by the addition of 5% meat compared to the control.

4.2.5.1 The L* (Lightness) values

The L* value changed over 21 days of storage as seen in Figure 8. There was a significant interaction (F = 69.514, P < 0.05) between meat addition (treatments) and storage time. Storage time alone (F = 0.124, P > 0.05) had no effect on L* values. Meat addition (F = 309, P < 0.05) had a significant effect on L* values. The control sample had significantly higher L*, which indicated a higher lightness compared to other yoghurt samples. Lightness decreased with increasing amount of meat. In addition, L* had significantly lower values in yoghurts that contained homogenised meat (HMY). Lee and others (1990) also reported decreased lightness (L*) values with soy yoghurts compared to dairy yoghurts, as soy protein concentrate was a brown-coloured powder. In this study the decrease in lightness was most probably due to the brown colour of the cooked meat. With an increase in meat content there was a decrease in lightness.

Table 12. Values for L* in yoghurts during storage at 4 °C.

	Days				(F value)		
Sample	1	7	14	21	Sample	Days	Sample*Days
5HMY	80.4±1.8 [A,c]	79.4±1.3 [A,c]	78.6±1.5 [A,cd]	79± 1 [A,cd]			
5UHMY	86.4±1.7 [A,b]	84.5±0.5 [A,b]	83±1.9 [A,b]	85±2.1 [A,b]			
7HMY	76±1 [A,de]	75.9±1 [A,d]	75.3±3 [A,de]	75.6±0.5 [A,e]			
7UHMY	82.6±2 [A,bc]	82.4±1.7 [A,b]	82.1±2.1 [A,bc]	81.6±1.1 [A,c]	309*	0.124	69.514*
9HMY	72.9±0.05 [A,e]	71.5±0.5 [B,e]	71.5±0.5 [B,e]	72.3± 0.4 [AB,f]			
9UHMY	79.6±2.5 [A,cd]	78.7±0.26 [A,c]	78.5±0.6 [A,cd]	78.5± 0.4 [A,de]			
Control	91.8±0.1 [A,a]	92.3±0.5 [A,a]	92.8±0.1 [A,a]	92.4±0.5 [A,a]			

[A-B] Means ± standard deviations in periodic samples. Different superscript uppercase letters are significantly different ($P < 0.05$).
[a-f] Means ± standard deviations in yoghurt treatments. Different superscript lowercase letters are significantly different ($P < 0.05$).
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.
* P value was significant (P < 0.05).

Figure 8. Values for L* in yoghurts during storage at 4°C.

A-B Different letters represent significant differences between storage days (P < 0.05).
a-f Different letters represent significant differences between yoghurt treatments (P < 0.05).
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt;
9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7%
unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.

4.2.5.2   The a* (red–green axis) values

The a* values of yoghurts changed over the 21 days of storage as shown in Figure 9. The treatment*day interaction (F = 28.054, P < 0.05), day effect (F = 3.249, P < 0.05) and treatment effect (F = 32.187, P < 0.05) were all significant. The control had significantly lower a* values (red colour) compared to all yoghurts except for 5UHMY. The highest a* values of yoghurts were the homogenised yoghurts at 7% and 9% which were not significantly different from each other except at the end of storage. The a* values of yoghurt containing homogenised meat decreased significantly from day 7 to day 21 of storage. The higher redness value in samples with meat is caused by the colour of myoglobin in meat. Meat colour turned to dark brown during heat treatment due to the denaturation of myoglobin (Mancini & Hunt, 2005). Therefore the redness increased with increasing amount of meat. These changes were noticeable to sensory consumers and were noted by an increase in darkness or brown colour resulting in lower scores of appearance.

Table 13. Values for a* in yoghurts during storage at 4°C.

| Sample | Days | | | | (F value) | | |
	1	7	14	21	Sample	Days	Sample*Days
5HMY	0.22±0.02 [A,bc]	0.05±0.3 [A,bc]	-0.3±0.7 [A,abc]	-0.7± 0.3[A,b]			
5UHMY	-1.09±0.6 [A,de]	-1.1±0.2 [A,de]	-1.27±0.2 [A,cd]	-1.34±0.03 [A,cd]			
7HMY	0.94±0.2[A,ab]	0.88±0.4 [A,ab]	0.14±0.2 [A,ab]	-0.06±0.3 [B,a]			
7UHMY	-0.76±0.16 [A,cd]	-0.75±0.2 [A,cd]	-1.08±0.3 [A,cd]	-1.1±0.1 [A,bc]	32.187*	3.249*	28.054*
9HMY	1.5±0.04 [A,a]	1.3±0.19 [A,a]	0.4±0.19 [B,a]	-1.06± 0.09 [C,bc]			
9UHMY	-0.48±0.5 [A,cd]	-0.5±0.3 [A,cd]	-0.74±0.2 [A,bc]	-0.89± 0.19[A,bc]			
Control	-1.8±0.29 [A,e]	-1.9±0.3 [A,e]	-2±0.1 [A,d]	-1.8±0.08 [A,d]			

[A-C]Means ± standard deviations in periodic samples. Different superscript uppercase letters are significantly different ($P < 0.05$).

[a-e]Means ± standard deviations in yoghurt treatments. Different superscript lowercase letters are significantly different ($P < 0.05$).

Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.
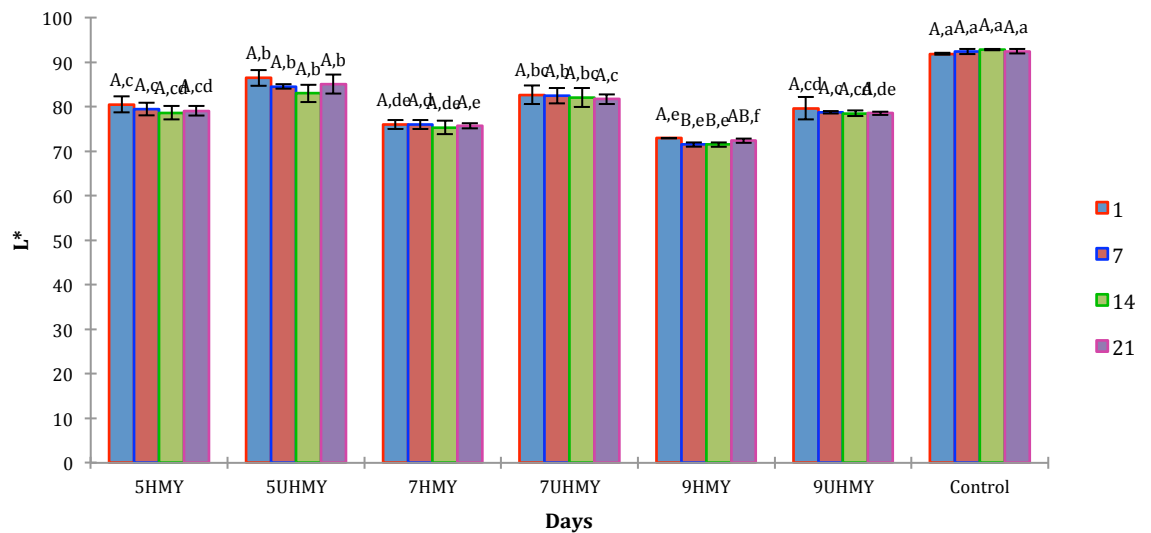
* P value was significant (P < 0.05).

Figure 9. Values for a* in yoghurts during storage at 4 °C.
A-C Different letters represent significant differences between storage days (P < 0.05).
a-e Different letters represent significant differences between yoghurt treatments (P < 0.05).
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.

4.2.6 The b* (yellow–blue axis) values

The b* values of yoghurts changed over 21 days of storage as shown in Figure 10 .The treatment*day interaction was significant (F = 86.059, P < 0.05). Storage time did not significantly (F = 0.18, P > 0.05) affect the b* values. However meat addition treatments were significant (F = 323, P < 0.05). The yoghurt with homogenised meat fortified at 9% and 7% had the highest b* values which indicated yellowness. The control sample exhibited significantly lower yellowness than 9HMY and 7HMY but not with 5HMY. The colour difference between the HMY and control sample may have resulted from the whey separation that occurred more in homogenised meat yoghurts. In a study on fortification of frozen yoghurt with purple rice bran oil there was significantly higher yellowness compared to the control at week 6 of storage which was related to the release of whey over time (Sanabria, 2012). However, the control sample exhibited significantly higher yellowness than the UHMY samples.

Table 14. Values for b* in yoghurts during storage at 4 $^{\circ}$C.

Samples	Days				(F value)		
	1	7	14	21	Sample	Days	Sample*Days
5HMY	15.3±0.8 ^{A,b}	15.7±0.7 ^{A,bc}	15.8±0.4 ^{A,bc}	15.3± 0.4^{A,bc}			
5UHMY	11.1±0.9 ^{A,c}	9.9±1 ^{A,d}	10±0.7 ^{A,d}	9.7±0.4 ^{A,d}			
7HMY	16±0.3 ^{C,ab}	16.9±0.1 ^{A,ab}	16.7±0.2 ^{AB,ab}	16.2±0.1 ^{BC,ab}			
7UHMY	9.7±0.9 ^{A,c}	9.8±0.7 ^{A,d}	10.1±0.6 ^{A,d}	9±0.5 ^{A,d}	323*	0.18	86.059*
9HMY	17.8±0.1 ^{A,a}	17.9±0.1 ^{A,a}	17.7±0.09 ^{A,a}	17.2± 0.2^{B,a}			
9UHMY	10±0.5 ^{A,c}	10.2±0.7 ^{A,d}	9.7±0.3 ^{A,d}	9.1± 0.3^{A,d}			
Control	15.2±0.4 ^{A,b}	14.4±1 ^{A,c}	15.2±0.09 ^{A,c}	14.3±1.1 ^{A,c}			

$^{A-C}$Means ± standard deviations in periodic samples. Different superscript uppercase letters are significantly different (*P* < 0.05).

$^{a-d}$Means ± standard deviations in yoghurt treatments. Different superscript lowercase letters are significantly different (*P* < 0.05).

Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.
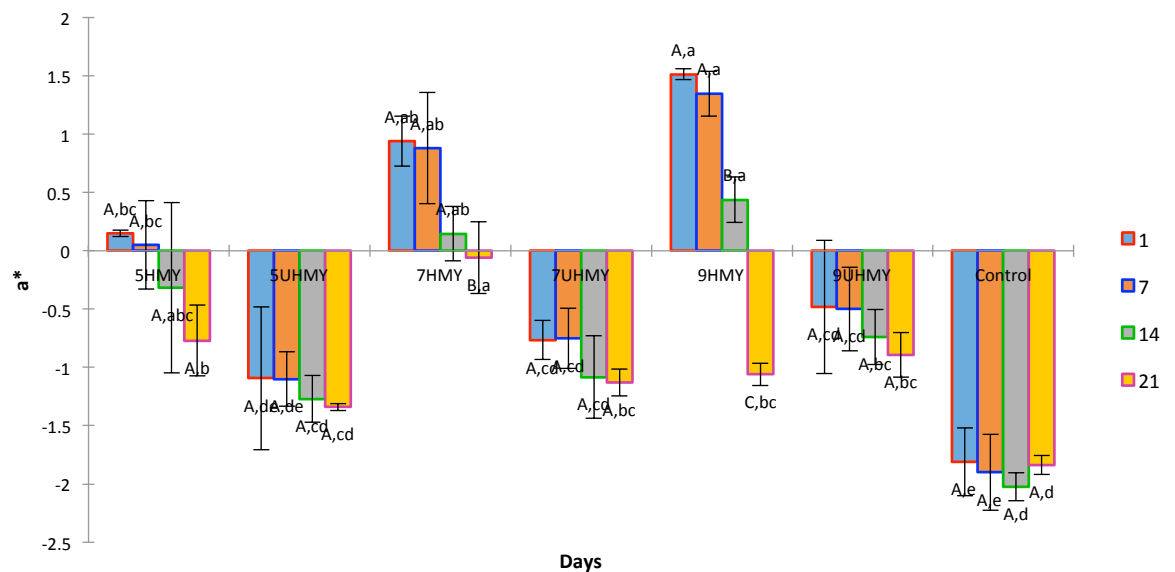
* P value was significant (P < 0.05).
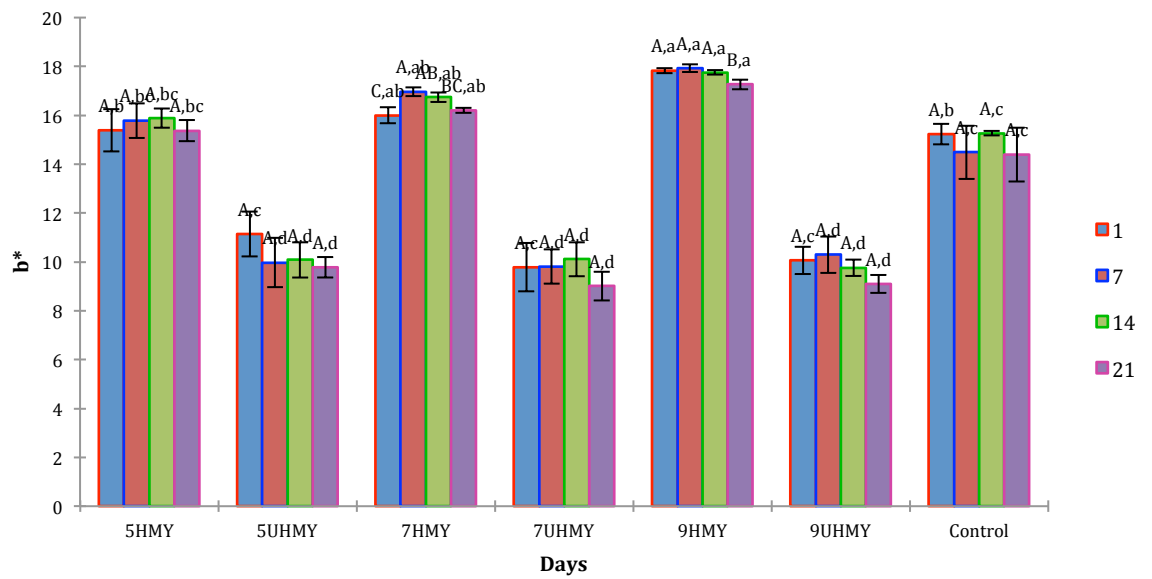
Figure 10. Values for b* in yoghurts during storage at 4°C.

A-C Different letters represent significant differences between storage days (P < 0.05).
a-d Different letters represent significant differences between yoghurt treatments (P < 0.05).
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.

4.3 Sensory

4.3.1 Consumer acceptance

Table 15. Consumer liking of yoghurt products containing meat.

Samples	Overall Liking	Flavour	Appearance	Texture	Odour
Control	5.7±1.8^a	5.6±2^a	6.07±2^a	6.05±1.9^a	5.7±1.7^a
5UHMY	4.7±1.7^{ab}	4.7±1.9^{ab}	4.8±1.7^b	4.6±1.8^b	4.9±1.9^{ab}
5HMY	4.6±2^b	4.4±2.2^b	4.8±2^b	4.5±2.1^{bc}	5±1.9^{ab}
7UHMY	4.4±2^b	4.3±2.1^b	4.1±1.8^{bc}	4.2±2^{bc}	4.8±1.8^{bc}
7HMY	4.4±1.8^b	3.9±2^b	4.2±1.9^{bc}	4.16±1.9^{bc}	4.6±1.9^b
9UHMY	4.1±1.8^b	4.1±1.8^b	4±1.7^{bc}	4.12±1.9^{bc}	4.33±1.8^b
9HMY	3.7±1.8^b	3.74±1.9^b	3.6±1.6^c	3.42±1.9^c	4.22±1.8^b

$^{a-c}$Means ± standard deviations in yoghurt treatments. Different superscript letters are significantly different ($P < 0.05$).
Samples expressed as 5HMY = 5% homogenised meat yoghurt; 7HMY = 7% homogenised meat yoghurt; 9HMY = 9% homogenised meat yoghurt; 5UHMY = 5% unhomogenised meat yoghurt; 7UHMY= 7% unhomogenised meat yoghurt; 9UHMY = 9% unhomogenised meat yoghurt.
9 Hedonic line scale with left end represents 1 (extremely dislike), right end represents 9 extremely like, and middle represents 5 (neither like nor dislike).

Fifty-four untrained consumers participated in this study and the data were analysed using analysis of variance as presented in Table 15. According to the consumer acceptance test, the control sample had the highest liking scores for all attributes. In terms of overall Liking, flavour and odour, the mean scores of 5UHMY were not significantly different from that of the control (P>0.05). However 5UHMY had lower scores with regard to appearance and texture, which was probably due to the low viscosity and high redness values. Sample 5HMY was not significantly different in liking compared to 5UHMY. However it was significantly lower in liking compared to the control for all attributes except for odour (p > 0.05). Most yoghurt samples with 5% meat addition had liking scores of around 4.5-5, which indicated that they were acceptable to the consumers. Based on all attributes acceptance, 9% meat-added yoghurts were the least preferred yoghurt by the consumers. There were no significant effects in terms of homogenisation (UHMY and HMY) on the acceptability of yoghurts.

4.3.1.1 Discussion

Yoghurt is a popular food in New Zealand and commercial yoghurt is often flavoured with fruits. Consumers in this study are probably unfamiliar with a new yoghurt product with meat. Yoghurt with low level of meat addition (5%) scored higher acceptability than high meat levels (7% and 9%). The low acceptance of yoghurts with more meat was probably due to the low viscosity and WHC as well as high redness. Overall acceptance, and flavour and odour acceptance of sample 5UHMY was, however, not significantly different from the control yoghurt. The difference was significant with regard to appearance and texture and for this reason, these attributes could probably be further improved to increase consumer acceptance of the meat yoghurt.

The overall acceptability, including appearance, flavour, texture and odour, is the important factor which determines the acceptance or rejection of a food article (Hussain & Atkinson, 2009). The overall acceptability of the control and 5UHMY were the highest. This suggests that 5% meat-fortified yoghurt was the most suitable product. Fermented dairy flavour is a mild, delicate flavour and is easily overpowered by strong flavours (Drake & Chen, 2000). The control and 5UHMY yoghurts were rated above average (4.5) on the 9-point hedonic scale and were liked by the panelists while the other samples were rated around 4. The present results are in agreement with those of Hekmat and McMahon (1997) who added iron to yoghurt and showed that the small increase in oxidised flavour that result from iron fortification had a negligible effect on the acceptance of yoghurt.

Flavour problems in soymilk yoghurts also have been described as off-flavours, beany, grassy, and lack of fermented dairy flavour (Lee et al., 1990). In the present study, yoghurts with added meat may retain some fermented dairy aroma and flavour, but these attributes decreased with increasing meat addition. Yoghurts with addition of more meat contained less dried milk powder and thus a lower concentration of lactose. Although lactose does not have a high sweetness intensity, the decrease in lactose concentration may also contribute to a decrease in sensory perception (Drake & Chen, 2000).

Colour, as one aspect of appearance, plays a major role in the acceptability of a food product. There were significant differences (P<0.05) between samples with regard to

appearance scores, which were affected by addition of meat that resulted in a darker and redder colour. However, Hekmat and McMahon (1997) have reported that consumer panels did not observe significant differences in the appearance or overall quality among yoghurts fortified with iron chloride, casein-chelated iron, or whey protein-chelated iron.

The sensory results are supported and related to protein and fat content, as well as viscosity and water holding capacity (WHC). The high fat content yoghurts include the control and 5MY that had better viscosity and WHC values. Lower fat yoghurts (7% and 9%) had lower texture and sensory scores. Fat globules contribute to texture and flavour of yoghurt by enhancing its body and imparting richness to the flavour (Sandoval-Castilla et al., 2004). In addition, some meat proteins like sarcoplasmic protein do not support the stabilisation of the high meat yoghurt products because the gel they produce is very weak (Hui, 2012). The lower sensory acceptance in these experimental 7% and 9% meat yoghurts, however, can be enhanced by addition of hydrocolloids and some flavours such as mint that may provide better texture and flavour and as a result increase product acceptance.

Chapter. 5. **Conclusion**

Meat and meat products are important sources for protein, fat, essential amino acids, minerals and vitamin and other nutrients. Adequate intake ensures a normal function of general healthy system especially in risk groups such as elderly or growing children. Meat should be consequently recommended (Biesalski, 2005). There is a big market for food products with added nutraceutical compounds that help improve health. The production of a functional yoghurt containing meat is a new alternative to broaden the yoghurt market to health conscious consumers. A good method to provide additional vehicles for consumption of meat is to combine the benefits of and consumer market for probiotic yoghurts with the potential health benefits of meat. The aim of this study was to investigate the development of novel protein-rich savoury probiotic yoghurt that is produced with meat addition.

The specific objectives of the study were to determine the effect of meat fortification on the microbiological, physicochemical and sensory characteristics of yoghurt during 21 days of refrigerator storage. The microbiology results showed that in comparison to the control, the only adverse effect of meat addition to yoghurt is a more rapid loss in viable LAB counts during storage at 4°C. However, after 21 days of storage, there were no significant differences in the viable counts, pH or total acidity and the count in the yoghurts were still high for the products to be considered probiotic. In addition, there was no apparent stimulation of growth of pathogens caused by the meat in yoghurt. Since food safety is a critical aspect of food quality, efforts should be directed to ensure that the new functional dairy products are safe. Without proof of product safety, most consumers would hesitate to adopt new foods in their diet.

Fortification of yoghurt with 5% meat is technically feasible, but 7% and 9% meat addition resulted in a decrease in rheological properties. Although supplementation with 5% meat showed higher viscosity and suffered less syneresis than the other meat yoghurts, it was relatively lower than the plain yoghurt (control). Although total solids were kept constant compared to the control in this study, the slight decrease of fat content in the 5% addition of meat probably could be responsible for the improved viscosity and whey separation to make the yoghurts stable. Addition of meat significantly increased the redness of 7% and 9% meat yoghurts, and decreased their

colour lightness. Sensory tests also showed that 5% meat yoghurts had better scores than 7% and 9% meat yoghurts for flavour, odour and overall liking after that of the control. On the other hand, appearance and texture scores for all the meat yoghurts were lower than those of the control.

This study used meat to enrich a dairy food. It is the first investigation of yoghurt made from a combination of milk and red meat. Total solids content was not significantly different for control and meat yoghurts since it had been adjusted at the formulation step. The addition of meat increased the protein but decreased the fat content. Samples containing increased amount of meat had increased protein content which would accordingly benefit the particular people who demand more nutritional food for their health.

Generally, results showed that addition of 5% meat could be used to produce a meat-added yoghurt without significant adverse effects on the microbiological, physicochemical and sensory properties. The negative effect on the characteristics at 7% and 9% meat added, as seen in the present study, makes meat fortification of dairy foods a particular challenge. Adding sweetener or mint flavour may aid in enhancing existing dairy flavours. The addition of hydrocolloids may also improve the lower rheological properties. The fortification of dairy yoghurts with small, but dietarily significant amounts of meat may provide an acceptable way to introduce a novel yoghurt product to the market. To determine the potential for commercialisation of yoghurt fortified with meat, further research is suggested to improve its sensory qualities and physicochemical characteristics.

Chapter. 6. **References**

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Chapter. 7. **Appendices**

Appendix A. Instruction and questionnaire for consumer testing

Questionnaire

Sensory Evaluation of Yoghurt

You will be given a tray with 4 yoghurt samples and three yoghurt dips. Each product is marked with a 3-digit code. Please answer the short questionnaire. Taste each sample and rate the acceptability of each product. Please taste the samples in the order presented.

Gender: Male □   Female □

Age: 18 - 20 □   21-30 □

                         31-40 □   older than 40 □

1. How often do you consume yoghurt?

          Everyday □   More than once a week □

2 -4 times a month □   Less than once a month (occasionally) □

2. How do you use yoghurt?

Alone (consume as is) □   Dip □

  Dressing / condiment □   In a smoothie □

Other, please specify …………………………………………………

3. Please indicate if you are allergic to meat?

(If you are, please do not proceed with the test)

Yes □   No □

 4. Are you a vegetarian or culturally sensitive to the presence of meat in yoghurt?

(If you are, please do not proceed with the test)

Yes □   No □

Instructions:

Please taste this product and indicate your **Overall Liking** of this product by putting a check mark in the appropriate box

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Taste the product as often as you need and indicate your evaluation of the following characteristics:

➢ Flavour

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

➢ Appearance

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

➢ Texture (Mouthfeel)

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

➢ Odour

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Appendix B. Statistical Analysis of variance of Lactic acid bacteria (LAB)

Days and sample interaction

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	27	13448.927	498.108	53.775	< 0.0001
Error	56	518.721	9.263		
Corrected Total	83	13967.648			

Days

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	12122.319	4040.773	175.178	< 0.0001
Error	80	1845.329	23.067		
Corrected Total	83	13967.648			

Samples

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	6	585.100	97.517	0.561	0.760
Error	77	13382.548	173.799		
Corrected Total	83	13967.648			

Appendix C. Statistical Analysis of variance of titration

Days and sample interaction

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	27	2.181	0.081	1.728	0.042
Error	56	2.618	0.047		
Corrected Total	83	4.799			

Days

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	0.294	0.098	1.743	0.165
Error	80	4.504	0.056		
Corrected Total	83	4.799			

Samples

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	6	1.846	0.308	8.021	< 0.0001
Error	77	2.953	0.038		
Corrected Total	83	4.799			

Appendix D. Statistical Analysis of variance of pH

Days and sample interaction

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	27	1.632	0.060	3.311	< 0.0001
Error	56	1.023	0.018		
Corrected Total	83	2.655			

Days

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	1.290	0.430	25.204	< 0.0001
Error	80	1.365	0.017		
Corrected Total	83	2.655			

Samples

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	6	0.296	0.049	1.611	0.155
Error	77	2.359	0.031		
Corrected Total	83	2.655			

Appendix E. Statistical Analysis of variance of WHC

Days and sample interaction

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	27	5453.749	201.991	13.249	< 0.0001
Error	56	853.773	15.246		
Corrected Total	83	6307.522			

Days

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	453.434	151.145	2.065	0.111
Error	80	5854.089	73.176		
Corrected Total	83	6307.522			

Samples

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	6	4737.284	789.547	38.717	< 0.0001
Error	77	1570.238	20.393		
Corrected Total	83	6307.522			

Appendix F. Statistical Analysis of variance of viscosity

Days and sample interaction

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	27	922898514.286	34181426.455	49.296	< 0.0001
Error	56	38829866.667	693390.476		
Corrected Total	83	961728380.952			

Days

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	31585485.714	10528495.238	0.906	0.442
Error	80	930142895.238	11626786.190		
Corrected Total	83	961728380.952			

Samples

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	6	838362047.619	139727007.937	87.212	< 0.0001
Error	77	123366333.333	1602160.173		
Corrected Total	83	961728380.952			

Appendix G. Statistical Analysis of variance of colour (L* values)

Days and sample interaction

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	27	3146.461	116.536	69.514	< 0.0001
Error	56	93.880	1.676		
Corrected Total	83	3240.340			

Days

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	14.953	4.984	0.124	0.946
Error	80	3225.387	40.317		
Corrected Total	83	3240.340			

Samples

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	6	3111.356	518.559	309.564	< 0.0001
Error	77	128.985	1.675		
Corrected Total	83	3240.340			

Appendix H. Statistical Analysis of variance of colour (a* values)

Days and sample interaction

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	27	74.630	2.764	28.054	< 0.0001
Error	56	5.518	0.099		
Corrected Total	83	80.148			

Days

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	8.704	2.901	3.249	0.026
Error	80	71.444	0.893		
Corrected Total	83	80.148			

Samples

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	6	57.301	9.550	32.187	< 0.0001
Error	77	22.846	0.297		
Corrected Total	83	80.148			

Appendix I. Statistical Analysis of variance of colour (b* values)

Days and sample interaction

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	27	872.510	32.315	86.059	< 0.0001
Error	56	21.028	0.375		
Corrected Total	83	893.538			

Days

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	6.001	2.000	0.180	0.909
Error	80	887.537	11.094		
Corrected Total	83	893.538			

Samples

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	6	859.455	143.242	323.609	< 0.0001
Error	77	34.083	0.443		
Corrected Total	83	893.538			

Appendix J. Statistical Analysis of variance of Fat

Samples

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	1.055	0.352	7.728	0.009
Error	8	0.364	0.046		
Corrected Total	11	1.420			

Appendix K. Statistical Analysis of variance of protein

Samples

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	64.209	21.403	162.615	< 0.0001
Error	38	5.001	0.132		
Corrected Total	41	69.210			

Appendix L. Statistical Analysis of variance of solid contents

Samples

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	0.041	0.014	0.026	0.994
Error	12	6.245	0.520		
Corrected Total	15	6.286			

Appendix M. Statistical Analysis of variance of consumer testing

Samples

	Overall Liking	Flavour	Appearance	Texture	Odour
R^2	0.092	0.077	0.140	0.126	0.058
F	6.283	5.178	10.091	8.939	3.823
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.001